# Selection and characterization of human recombinant antibodies against Orthopoxviruses from an immunoglobulin library and mapping of functional epitopes of Vaccinia virus surface proteins

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## TABLE OF CONTENTS

## LIST OF FIGURES



## LIST OF TABLES

# 1 GENERAL INTRODUCTION

## 1.1 TAXONOMY OF ORTHOPOXVIRUSES

Poxviruses are double-stranded DNA (dsDNA) viruses with large genomes (130-380 kb) (Lefkowitz et al., 2006). They are widely distributed and can be classified into the following two subfamilies: *Entomopoxvirinae*, infecting insects; and *Chordopoxvirinae*, infecting vertebrates (Van Regenmortel et al., 2000). The latter includes ten genera: *Orthopox*-, *Parapox*-, *Avipox*-, *Capripox*-, *Leporipox*-, *Suipox*-, *Molluscipox*-, *Crocodylipox*-, *Cervidpox*- and *Yatapoxviruses* (Haller et al., 2014). Genetic analysis revealed that at least 91 genes are conserved in *Chordopoxvirinae* (Upton et al., 2003). However, the AT- and GC-content of their genomes differ. Therefore, the majority of poxviruses contain an AT-rich genome, whereas *Crocodylipox*-, *Molluscipox*- and *Parapoxviruses* contain a rather GC-rich genome (Roychoudhury et al., 2011). The genus *Orthopoxvirus* (OPXV) consists of the following 10 species: variola-, vaccinia-, cow-, monkey-, mouse-(ectromelia virus), camel-, racoon-, vole-, tatera- and skunkpox viruses (https://talk.ictvonline.org). Members of the OPXVs are morphologically identical and antigenically closely related. For these reasons, they induce cross-reactive immune-responses and cross-protection due to the stimulation of the specific and the non-specific immune system (Czerny and Mahnel, 1990; Essbauer et al., 2010; Fenner et al., 1989). Variola virus (VARV), which is the causative agent of smallpox, is the most popular member of OPXVs. It supposedly caused more fatalities than all other human infectious diseases taken together (McFadden, 2005). However, since 1979, VARV is officially eradicated due to strict and highly successful vaccination programs. Moreover, VARV has no known natural reservoir in the environment and is not zoonotic (Fenner et al., 1988). There are various OPXVs with zoonotic potential like vaccinia virus (VACV), cowpox virus (CPXV), buffalopox virus (BPXV), monkeypox virus (MPXV) and camelpox virus

(CMLV) (Becker et al., 2009; Bera et al., 2011; Brown and Leggat, 2016; Campe et al., 2009; Kurth et al., 2008; Pauli et al., 2010; Singh et al., 2012; Singh et al., 2007; Vorou et al., 2008). VACV for instance was used as a naturally attenuated live vaccine for the smallpox eradication (Fenner et al., 1988). BPXV, a variant of VACV, was first discovered in India (Ramkrishnan and Ananthapadmanabham, 1957), while CPXV is considered to be the most ancient poxvirus with the widest host range including different heterogeneous strains (Dabrowski et al., 2013; Meyer et al., 1999). Besides cases in cattle, CPXV infections could be detected in cats, in rats and in humans as well as in rodents, which seem to be their natural reservoir (Baxby, 1977; Chantrey et al., 1999). In healthy humans, an infection with CPXV is usually non-lethal and self-limiting (Wolfs et al., 2002). MPXV infections on the other hand are more severe and may result in death (Frey and Belshe, 2004; Likos et al., 2005). Rodents, especially squirrels, seem to be their natural reservoir (Haller et al., 2014). Moreover, rodents were also found to be vulnerable to ectromelia virus (ECTV). The first ECTV case was discovered in 1930, when laboratory mice, wild mice and other rodents were infected (Marchal, 1930). Because of the genetic similarity to VARV, ECTV infection of laboratory mice is an interesting model for poxvirus pathogenesis and antibody (Ab) neutralization studies (Esteban and Buller, 2005). Taterapox virus (TaPXV) also infects rodents, as it is similar to VARV and VACV. In fact, it is considered to be the closest phylogenic relative based on restriction mapping, genome sequence and comparisons of conserved genes (Parker et al., 2017). Therefore, it has been assumed that VARV was transferred to humans from African rodents (Esposito et al., 2006). In phylogenetic analysis, camelpox virus (CMLV) was shown to be in this clade as well by clustering with VARV and TaPXV (Hughes et al., 2010).

The following study will focus on VACV, as it is the best characterized poxvirus (Moss, 2007).

## 1.2 STRUCTURE OF VACCINIA VIRUS

VACV, the prototype of OPXV, has a large DNA genome, possessing a GC-content of 36% and replicating in the cytoplasm (Condit et al., 2006; Goebel et al., 1990; Moss, 2007). The genome size varies between the different strains from 150 to 300 kb (Esposito and Knight, 1985; Esposito et al., 2006; Lefkowitz et al., 2006), encoding approximately 200 polypeptides (Goebel et al., 1990; Moss, 2007). Around half of them are highly conserved and involved in cell entry, genome replication, transcription and virion assembly (Upton et al., 2003).

The brick-shaped OPXV virions are present in enveloped as well as non-enveloped form (Appleyard et al., 1971; Condit et al., 2006; Payne, 1986) with a size of 250 x 350 nm (Moss, 2006). The so-called ellipsoidal shaped lateral bodies (LB), which consist of proteins, are found on both sides of the biconcave DNA-genome-containing core (Cyrklaff et al., 2005; Ichihashi et al., 1984) (figure 1).

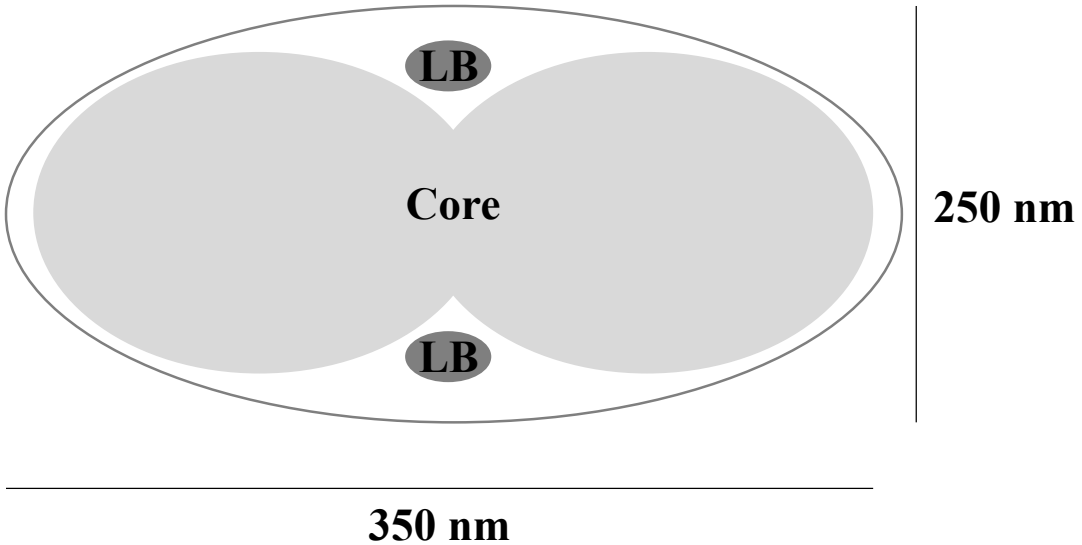


**FIG 1** Schematic IMV virion of VACV. The brick-shaped virion has a size of 250 x 350 nm. The dumbbell-shaped core is flanked by two lateral bodies (LB). (Modified according to (Condit et al., 2006).)

The virions possess a variety of conserved genes as well as various factors and enzymes for gene expression which are located within the center of the linear dsDNA genome (Haller et al., 2014; Moss, 2013b; Van Vliet et al., 2009). Two hairpin loop termini are

attached next to the center, consisting of inverted terminal repeats (ITRs) (Baroudy et al., 1982) which are highly diverse and are associated to host range or immune evasion (Haller et al., 2014; Moss, 2013b; Van Vliet et al., 2009). The majority of these genes are essential in virus replication and morphogenesis (Van Vliet et al., 2009). For instance, in VACV Western reserve (WR) 118 early, 53 intermediate and 38 late genes can be found (Yang et al., 2011).

## 1.3 MORPHOLOGY

VACV produces different forms of infectious particles during its replication cycle (Smith et al., 2002) (figure 2).

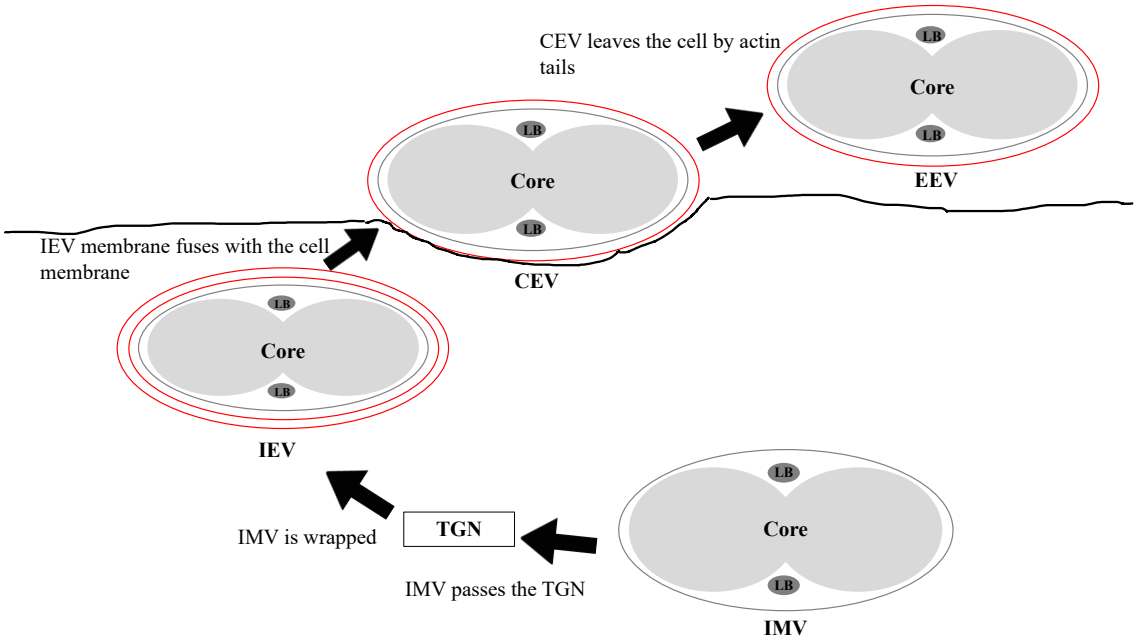


**FIG 2** Schematic morphology overview. IMV passes a trans-Golgi network to receive a double membrane in order to develop into IEV. IEV is then transported to the cell surface where it fuses with the cell membrane to exit the cell. CEV particles are retained on the surface membrane, whereas EEV particles are released by actin tails. (Modified according to (Smith et al., 2002).)

Intracellular mature virus (IMV), the most abundant infectious form (>90%), is released upon cell lysis (Moss, 2012; Smith et al., 2003). The infectious IMV particles are built within cytoplasmic factories from non-infectious crescent precursor cells (Smith et al.,

2002). IMV is composed of a dumbbell-shaped core, including the dsDNA genome, structural proteins as well as transcription enzymes, LBs and a lipoprotein membrane (Hiller and Weber, 1985; Schmelz et al., 1994; Tooze et al., 1993). Up to 25-40% of the total IMVs are able to leave the factory and become wrapped by a double layer of membrane, which is either derived from early endosomes or from the *trans*-Golgi network (Hiller and Weber, 1985; Payne, 1979). The envelopment of the virion is dependent on different proteins (A26, A33, A34, A36, B5 and F13) (Blasco and Moss, 1991; McIntosh and Smith, 1996; Parkinson and Smith, 1994; Rodriguez and Smith, 1990; Roper et al., 1998; Wolffe et al., 1993). The enveloped virion form is known as intracellular enveloped virus (IEV) (Hiller and Weber, 1985). IEV fuses with the plasma membrane at the surface of the cell, in order to exit it by exocytosis (Geada et al., 2001). If the particles are retained on the surface membrane, they will termed cell-associated enveloped virus (CEV) (Payne, 1980; Smith et al., 2002). A small proportion of particles known as extracellular enveloped virus (EEV), is released by actin tails, which are induced by CEVs (Smith et al., 2002).

In the case of VACV WR, only 0.5% of the total strain is present as EEV, in contrast to VACV IHD-J, where 8-27% are available as EEV depending on the cell type (Payne, 1979). This difference in EEV formation is due to one aa difference in the A34 protein, being responsible for wrapping (Blasco et al., 1993). CEVs and EEVs are indistinguishable and are built like IMVs, apart from having an additional outer membrane (Blasco and Moss, 1992). IMVs and EEVs differ in their envelope proteins (Benhnia et al., 2009b; Davies et al., 2005; Hsiao et al., 1999; Ichihashi and Oie, 1996; Kaever et al., 2016; Matho et al., 2017, 2018; Matho et al., 2015; Moss, 2006, 2011; Rodriguez et al., 1985; Smith et al., 2003; Smith et al., 2002; Ulaeto et al., 1996; Wolffe et al., 1995). CEVs and EEVs are important for rapid cell-to-cell spread due to their actin tail formation (Blasco and

Moss, 1992; Moss, 2012; Payne, 1980; Roper et al., 1998; Smith et al., 2002), whereas IMVs mediate host-to-host transmission between hosts (Moss, 2012; Smith et al., 2003).

## 1.4 ENTRY

The replication cycle starts with the electrostatic attachment of virions to the cell surface of the host cell (Moss, 2016). Because of the above-mentioned differences between IMVs and EEVs, diverse attachment factors exist comprising five IMV proteins (Schmidt et al., 2013). While the proteins A27 and H3 bind to glycosaminoglycans (GAGs) heparan sulfate, the D8 protein binds to GAGs chondroitin sulfate (Chung et al., 1998; Hsiao et al., 1999; Lin et al., 2000; Moss, 2016). A26 protein detects laminin within the extracellular matrix (Chiu et al., 2007; Howard et al., 2008; Moss, 2016), whereas L1 protein binds to an unknown protein of the cell surface to block the virus entry (Foo et al., 2009).

After attachment, IEVs either fuse with the plasma membrane or with the membrane of an endocytic vesicle to enter the cell (Doms et al., 1990; Earp et al., 2005; Geada et al., 2001; Mercer and Helenius, 2008, 2009; Smith et al., 2002). The virus entry thereby depends on the environmental pH value, on the cell type as well as on the used virus strain (Bengali et al., 2012; Chang et al., 2010; Whitbeck et al., 2009). While low pH values (pH 4.5-5.0) trigger endocytosis, membrane fusion requires a neutral pH value (Bengali et al., 2012; Chang et al., 2012). Moreover, a complex of conserved proteins is needed for membrane fusion (Moss, 2012). This so called entry fusion complex (EFC) consists of nine integral proteins (A16, A21, A28, G3, G9, H2, J5, L5 and O3) as well as two EFC-associated proteins (L1 and F9) (Bisht et al., 2008; Brown et al., 2006; Diesterbeck et al., 2018; Laliberte et al., 2011; Moss, 2012, 2016; Nichols et al., 2008; Satheshkumar and Moss, 2009; Senkevich et al., 2005). If any one of the integral EFC proteins is missing, the EFC is severely destabilized (Moss, 2016). Other investigations showed, that besides the EFC, proteins A27 and A17 also play a role in entry fusion (Gong et al., 1990;

Kochan et al., 2008; Rodriguez and Esteban, 1987; Rodriguez et al., 1987). However, there are no genetic evidences demonstrating the fusion participation (Moss, 2013a). As mentioned above, CEVs and EEVs are important for rapid cell-to-cell spread. However, cell-to-cell spread was considerably reduced after a loss of A33, A34 or A36 proteins during conditional lethal mutant assay by the prevention of actin tail formation (Rottger et al., 1999; Wolffe et al., 1997).

At low pH, both IMVs and EEVs are taken up by macropinocytosis, which is a form of endocytosis (Schmidt et al., 2011; Townsley et al., 2006). Thereby, virus particles are engulfed with fluid and transported deeper into the cytoplasm in endocytotic vesicles. In contrast to the fusion at neutral pH, macropinocytosis requires intense actin dynamics and is dependent on cell signaling pathways including epidermal growth factor receptor (EGFR) signaling (Mercer and Helenius, 2008, 2009; Schmidt et al., 2012). Externalization of the virion core into the cytoplasm occurs through identical fusion mechanisms involving the EFC as described for fusion with the plasma membrane (Schmidt et al., 2012). Despite the more complex mechanism of macropinocytosis, it is suggested to have various advantages compared to fusion at the cell membrane. The virus particle is transported deeper into the cell by the endocytic vesicle and the release takes place directly at its final destination, therefore evading cytoskeletal barriers altogether. Furthermore, macropinocytosis prevents leaving residues of the viral membrane at the plasma membrane, therefore preventing a detection by the immune system (Schmidt et al., 2012).

The entry mechanism of VACV not only differs with respect to the pH value, it is also dependent on the VACV strain (Mercer et al., 2010; Whitbeck et al., 2009). For instance, VACV Western Reserve strain (WR) mainly uses the endocytotic pathway, whereas MVA enters HeLa cells via cell membrane fusion (Chang et al., 2010). However, investigations on MV and EEV entry in CPXV and other OPXV revealed macropinocytosis as the predominant entry pathway (Bengali et al., 2012). It was concluded that low pH

macropinocytosis is the original entry pathway of OPXV and that the neutral pH plasma fusion pathway of some VACV strains was gained during extensive laboratory passages *in vitro* (Bengali et al., 2012). Additionally, preference of the entry route was shown to be correlated with the absence or presence of A26. VACV strains expressing A26 prefer the endocytotic pathway, while strains lacking A26 enter by plasma fusion (Bengali et al., 2012; Chang et al., 2010). A26 forms a complex with A25, A27 and A17 and interacts with the EFC (Howard et al., 2008) . Since A25 and A26 have to be inactivated for successful plasma membrane fusion, they are referred to as fusion suppressors (Chang et al., 2010).

## 1.5 PROTEINS OF VACCINIA VIRUS

The 190 kb genome of VACV encodes more than 200 viral proteins (Goebel et al., 1990). Each protein is encoded by an open reading frame (ORF), which is labelled threefold: Firstly, it contains a capital letter (A-O), indicating the Hind III DNA fragment, secondly, an additional Arabic number, specifying the position within the fragment, and lastly an L or R indicating the transcription direction from left to the right or vice versa. The corresponding protein is labelled in the same way but without coding the transcription direction (L or R) (Earley et al., 2008; Yoder et al., 2006). Protein expression differs and can be divided into three different classes: early class proteins, which are expressed 15 min after cell-entry, intermediate class proteins from 2 to 4 hours after cell-entry as well as late class proteins from 4 h after entry to cell death (Grosenbach and Hruby, 1998). Several structural proteins of immunological relevance have been detected in the envelope of IMV (Essani et al., 1982; Hiller and Weber, 1982; Ichihashi and Oie, 1980, 1988; Stern and Dales, 1976) and EEV particles (Bell et al., 2004; Benhnia et al., 2009b; Putz et al., 2006) (figure 3).

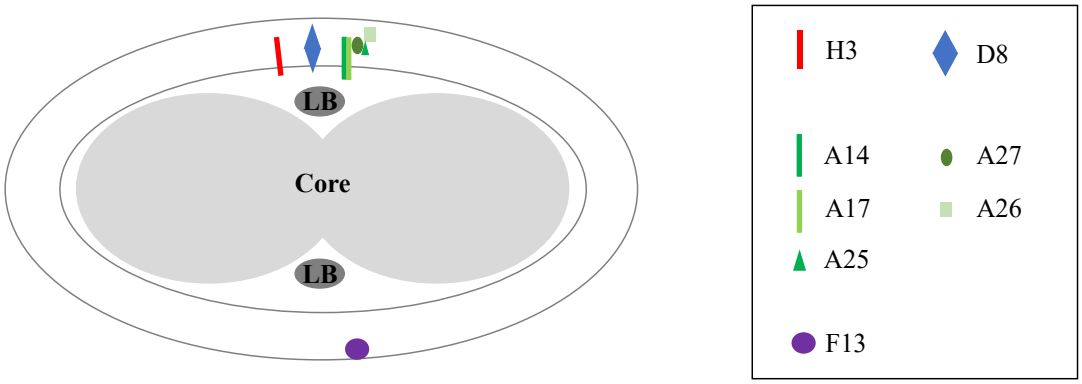


**FIG 3** Schematic representation of a VACV extracellular enveloped virion (EEV) showing the distribution of immunogenic envelope proteins. The envelope membranes are resized to enhance the clarity.

Importantly, some of these proteins lead to the formation of neutralizing antibodies (Ichihashi and Oie, 1988; Oie and Ichihashi, 1987; Rodriguez et al., 1985) and induce protective immunity *in vivo* (Benhnia et al., 2009b; Czerny et al., 1994; Czerny and Mahnel, 1990; Galmiche et al., 1999; Hooper et al., 2000; Hsiao et al., 1999; Ichihashi and Oie, 1996; Kaever et al., 2016; Lai et al., 1991; Matho et al., 2012; Matho et al., 2015; McCausland et al., 2010; Pulford et al., 2004; Ramirez et al., 2002; Reeman et al., 2017; Rodriguez et al., 1985). Vaccination stimulates the specific proliferation of B-cells and T-cells, hence eliminating the virus during infection (Novembre et al., 1989). Therefore, it will be necessary to develop vaccines, which will also stimulate B- and T-cell response in order to achieve a powerful level of efficacy. Furthermore, the complement system, which is predominantly studied for EEV defense, is an important protection mechanism as well. It is involved in the destruction of the EEV membrane, leading to the successful access of IMV neutralizing antibodies. Moreover, it is involved in opsonization, which is responsible for lysis of infected cells. It is also known, that poxviruses encode a complement regulatory protein to attenuate the hosts defense response (Moss, 2011). Therefore, it is important to identify the major neutralizing targets of virus particles in order to develop safer subunit-based vaccines. In this regard, immunogenic envelope proteins have

been studied in IMV and EEV. So far, 13 IMV proteins were shown to be immunogenic (A10, A13, A14, A17, A25, A26, A27, A28, C3, D8, D13, H3 and L1 (Davies et al., 2005; Hsiao et al., 1999; Kaever et al., 2016; Nelson et al., 2008; Rodriguez et al., 1985; Wolffe et al., 1995). Immunogenic EEV envelope proteins are A33, A56 and B5 (Galmiche1999; (Bell et al., 2004; Benhnia et al., 2009b; Putz et al., 2006).

In the following, this thesis will focus on the immunogenic VACV IMV A27, D8 and H3 proteins as well as F13, which is incorporated in the EEV envelope. These four chosen proteins all have important functions within the VACV replication cycle (figure 3, table 1).

**Table 1** Protein functions

| **Protein** | **Involvement in replication cycle** | **Protein** | **Involvement in replication cycle** |
|---|---|---|---|
| A14 | Crescent formation, morphogenesis | A26 | Attachment |
| A17 | Entry, crescent formation, maturation | H3 | Attachment, morphogenesis |
| A27 | Entry, egress, wrapping | D8 | Attachment |
| A25 | Fusion suppression; ATI-formation | F13 | Wrapping |

### 1.5.1 A25- A26- A27- A17- A14- Complex

There is a complex located at the inner side of the IMV membrane composed of A25, A26, A27, A17 and A14 (figure 4).

The 84 kDa A25 protein of VACV is a truncated, apparently non-functional homolog of the CPXV A-type inclusion (ATI) matrix protein (Amegadzie et al., 1992; Patel and Pickup, 1987; Patel et al., 1986). The A25 orthologues of CPXV and ECTV both have masses of 160 kDa. An important function of the A25 is fusion suppression, therefore

avoiding fusion among MV particles to reserve virion infectivity (Chang et al., 2010). The ATI formation is dependent on a full-length ortholog of the truncated A25 protein as well as on the A26 protein, which is involved in the embedding of the virions within it (McKelvey et al., 2002). So, there is an interaction between the A25 and the A26 proteins.

The 500 aa long A26 protein (4c) of OPXV is one of the structural IMV proteins of VACV which was first identified (Katz and Moss, 1970; Ulaeto et al., 1996). A26 (58 kDa) and A27 (12.6 kDa) form a disulfide-bonded complex resulting in a band of about 90 kDa in SDS-PAGE (Ichihashi, 1981) and hence representing the relationship between these proteins. This intermolecular disulfide bond requires the C-terminal cysteines 441 and 442 of A26 as well as 71 and 72 of A27 (Ching et al., 2009). It is noticeable that around 44% of the C-terminal regions of A26 (aa residues 443-472) and A27 (aa residues 43-85) are similar to each other (McKelvey et al., 2002). Without A27, A26 is shown to be unstable (Howard et al., 2008). Moreover, on the membrane surface of A26, there is an intramolecular disulfide bond between cysteines 43 and 342, which makes it topologically impossible to act as a transmembrane region (Ching et al., 2009). A26 is highly conserved in most poxviruses, including OPXVs producing ATIs, such as CPXV, ECTV and RCNV, as well as non ATI-producing poxviruses like VARV, VACV, MPXV and CMLV (91-95% aa identity) (McKelvey et al., 2002; Shchelkunov et al., 2001). Therefore, genes direct the inclusion of IMVs within the ATIs (McKelvey et al., 2002). This inclusion is important in order to protect the virions and therefore promote the host-to-host-spread (Howard et al., 2008). Moreover, it is involved in the virus attachment, by binding to the cell surfaces extracellular matrix protein laminin (Chiu et al., 2007). A26 plays an important role in increasing the MV production (Ulaeto et al., 1996). Other studies have confirmed this by showing an increase of the retrograde transport of MVs (McKelvey et al., 2002).

The conserved 14 kDa A27 protein is encoded by ORF A27L (Goebel et al., 1990; Kaever et al., 2016) and is one of the best characterized IMV envelope proteins (Gong et al., 1989; Gong et al., 1990; Lai et al., 1990; Rodriguez and Esteban, 1987; Rodriguez et al., 1985). It is involved in attachment by binding to the glycosaminoglycan heparan sulphate on the surface of mammalian cells, which is mediated through a turn-like structure, formed by a KKPE segment (Shih et al., 2009). Because the A27 protein has no trans-membrane domain, it builds a complex with four other membrane proteins (A14, A17, A25 and A26) (Howard et al., 2008; Rodriguez et al., 1993; Unger et al., 2013; Vazquez et al., 1998). The 110 amino acids (aa) of A27 protein can be divided into four functional areas: a N-terminal signal peptide, a Lys/Arg-rich heparin binding domain (HBD), an α-helical coiled-coil domain (CCD) and a C-terminal leucine zipper motif (LZD) (Vazquez et al., 1998). The HBD (aa 21-34), including the KKPE segment (aa 26-29), is essential for binding heparan sulphate (Chung et al., 1998; Shih et al., 2009). The CCD (aa 43-84) possesses the cysteine residues 71 and 72, which are responsible for forming disulfide bonds with the fusion suppressor A26 protein (Ching et al., 2009). Moreover, the CCD is responsible to stabilize the protein structure (Wang et al., 2014). The LZD (aa 85-110) is considered to be the binding region of the A17 transmembrane protein (Vazquez et al., 1998). A27 is very important during the virus replication cycle as it regulates cell entry and virus egress (Chung et al., 1998; Hsiao et al., 1998; Rodriguez and Smith, 1990). Without A27, normal amounts of IMV would be produced, however there is a loss of wrapped virions (Rodriguez and Smith, 1990). Moreover, it is an immunogenic protein, because anti-A27 antibodies can prevent a VACV infection (He et al., 2007). Interest-ingly, six epitope regions recognized by different anti-A27 monoclonal antibodies (mAbs) were identified and could be mapped by SPOT synthesis: (epitope #1A: aa 32-39, #1B: aa 28-33, #1C: aa 26-31, #1D: 28-34, #4: aa 9-14, and #5: aa 68-71) (Ahsendorf

et al., 2019; Czerny et al., 1994).

The 203 aa long conserved A17 transmembrane protein comprises a mass of 21 kD (Rodriguez et al., 1993; Unger et al., 2013; Wang et al., 2014). It is synthesized in the rough endoplasmic reticulum (Husain et al., 2006; Rodriguez et al., 1998). The integral membrane protein contains two internal hydrophobic domains, which are typical for transmembrane proteins (Rodriguez et al., 1993). The N-terminus is exposed to the outside, whereas the C-terminus is incorporated into the IMV membrane (Wang et al., 2014). The transmembrane regions are located at aa residues 66-86 and 139-159 (https://www.uniprot.org/uniprot/P68593). The C-terminus of A17 is phosphorylated by viral F10 (Unger et al., 2013). A17 is involved in cell entry (Moss, 2012), crescent formation (Unger et al., 2013), IMV assembly (Rodriguez et al., 1995; Wang et al., 2014) and virion maturation (Unger et al., 2013). During crescent formation, it anchors D13 to the nascent membrane and mediates dissociation of D13 during maturation due to proteolytic processing (Erlandson et al., 2014; Unger et al., 2013). Without A17, the virions are surrounded with membrane vesicles, but crescents are not formed (Rodriguez et al., 1995; Rodriguez et al., 1996; Wolffe et al., 1996). Besides the interaction to A27, A17 also forms a complex with the IMV protein A14, as they are both synthesized in the endoplasmic reticulum and incorporated as dimers into the IMV envelope membrane, however, in opposite polarity (Husain et al., 2006; Rodriguez et al., 1998; Unger et al., 2013). It is suggested that they interact via their hydrophobic transmembrane domains, but detailed mechanism of the interaction remain unknown (Unger et al., 2013).

The 15 kDa A14 integral membrane protein consists of 90 aa (Rodriguez et al., 1998; Unger et al., 2013). As well as the A17, it is synthesized in the rough endoplasmic reticulum and in the endoplasmic reticulum-Golgi intermediate compartment (Husain et al.,

2006; Rodriguez et al., 1998). During infection, A14 is myristilated and phosphorylated on Ser85 residue (Mercer and Traktman, 2003; Rodriguez et al., 1997). However, a deletion of this aa does not disturb the virion morphogenesis (Mercer and Traktman, 2003). Moreover, it generally appears as a disulfide-linked dimer (Rodriguez et al., 1997), which is formed by a disulfide bond on Cys71 residue (Mercer and Traktman, 2003). It is a transmembrane protein, which anchors the complex to the viral membrane by the aa residues 13-31 (hydrophobic region) and 45-64 (hydrophilic region) (Mercer and Traktman, 2003). A14 is essential for virus replication and is additionally interfering in crescent formation and morphogenesis. Without A14, there is a reduced virus yield and a lesser plaque formation (Rodriguez et al., 1998). It is part of the A14-A17-A27 protein complex (Rodriguez et al., 1997), however the exact interaction of A14 and A17 is not yet known. Conjectures exist, saying that they are linked by their transmembrane regions (Unger et al., 2013).

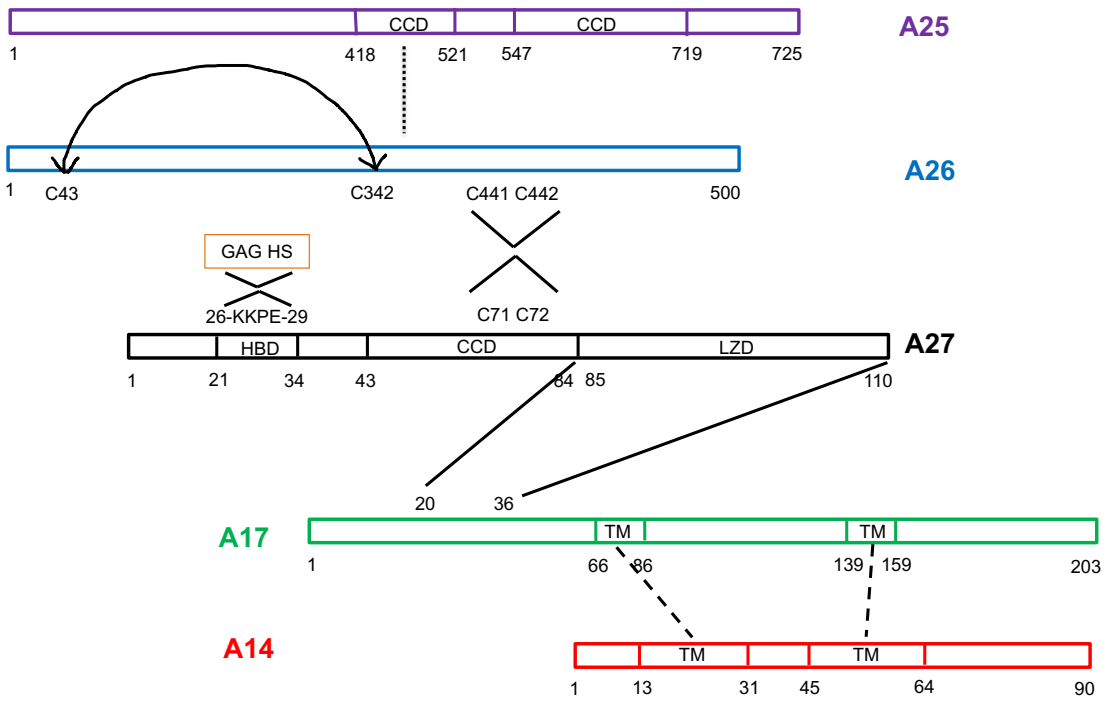


**FIG 4** Schematic representation of the A25- A26- A27- A17- A14- complex. The exact linkage between A25 and A26 is not clear up to now. A26 and A27 are connected by disulfide bonds. A27 is anchored to the viral membrane by binding to the

transmembrane protein A17 in the marked area. The exact linkage between A17 and A14 is unknown, but it is suggested that they are connected by their transmembrane regions.

### 1.5.2 D8

The D8L-gene is highly conserved in OPXVs (Goebel et al., 1990). It encodes for a 32-kDa protein (Goebel et al., 1990; Niles and Seto, 1988), which is a type 1 membrane protein (Hsiao et al., 1999) composed of 304 aa (Matho et al., 2012). VACV D8 protein consists of one N-terminally ectodomain encompassing a carbonic anhydrase (CAH) domain (residues 1-234), a disordered stalk region of unknown function (residues 235-273), a helical transmembrane domain (residues 274-294) and an intra-virion tail (residues 295-304) (Matho et al., 2012). The CAH domain is important for chondroitin sulfate (CS) binding, which is essential for the absorption of the virus to the mammalian host cell surface (Hsiao et al., 1999; Maa et al., 1990). There are different anti-D8 antibodies targeted at the CAH domain rather than the stalk region (Matho et al., 2014; Matho et al., 2012). Besides its function in virus attachment, D8 is an immunodominant VACV protein that induces a strong antibody response (Sakhatskyy et al., 2006). Antibody pools targeting D8, H3 and A27 are able to block the viral adhesion to the host cell and therefor protect from infection (Benhnia et al., 2008). However, most anti-D8 antibodies neutralized VACV in *vitro* only in the presence of a complement (Matho et al., 2012; Matho et al., 2017).

### 1.5.3 H3

The 35 kDa protein, encoded by the open reading frame (ORF) H3L on the HindIII H-fragment of VACV, was identified as a part of the viral membrane (Chertov et al., 1991; Lin et al., 2000; Stern and Dales, 1976; Takahashi et al., 1994; Zinoviev et al., 1994). It consists of 324 amino acids (aa) and seems to be highly conserved (Davies et al., 2005;

Lin et al., 2000). C-terminally, two hydrophobic domains were found to be a putative membrane anchor (Chertov et al., 1991; Goebel et al., 1990; Rosel et al., 1986; Takahashi et al., 1994). This transmembrane region consists of 21 aa (Lin et al., 2000). The N-terminal aa is modified, passively protecting the protein against aminopeptidases (Chertov et al., 1991; Zinoviev et al., 1994). H3 binds to the glycosaminoglycan heparan sulfate on the surface of mammalian cells and thereby promotes the attachment to the target cell (Davies et al., 2005). If H3L is inactivated, the virions are intensely attenuated and the infectivity is reduced up to six fold (Lin et al., 2000). H3 is a very crucial IMV envelope protein since it is highly immunogenic (Davies et al., 2005). After vaccination with Dryvax, the widely used live vaccine against smallpox, anti-H3 was found as the main component in the sera of human vaccines. Neutralization capability of anti-H3 has been demonstrated *in vitro* as well as *in vivo* in mouse models. Mice that were vaccinated with recombinant H3 protein also gained a high titer of anti-H3 antibodies (Davies et al., 2005). Summing up, recombinant H3 seems to be an essential component for the development of subunit-based smallpox vaccines.

### 1.5.4 F13

The major envelope protein of EEV particles is the not glycosylated 37 kDa F13 membrane protein (Hiller et al., 1981; Hirt et al., 1986), which is encoded by the ORF F13L gene and consists of 372 aa (Grosenbach and Hruby, 1998; Hirt et al., 1986). It is highly conserved in poxviruses, which leads to the assumption that it plays an important role in the life cycle (Blake et al., 1991). It belongs to the late class proteins, so it is expressed 6-8 h post infection (Hirt et al., 1986). The F13 protein has no transmembrane domain. Instead, it is palmitoylated at the motif TMDX1-12AAC(C)A and the cysteine residues 185 and 186 (Grosenbach et al., 1997), which are located within the TGN membrane (Hiller and Weber, 1985; Schmutz et al., 1995). Interestingly, nonpalmitylated variants

do not produce EEVs and are therefore less effective in cell-to-cell spread. Moreover, without palmitic acid, F13 is not able to anchor the virion membrane (Grosenbach and Hruby, 1998). The 37 kDa protein is anchored to the outer side of the TGN membrane, so that it is located on the inner side of the EEV membrane and the outer side of the IEV membrane after wrapping (Husain and Moss, 2003), existing predominantly as a monomer (Schmutz et al., 1995; Schmutz and Wittek, 1995). The conserved histidine-lysine-aspartate (HKD) phospholipase motif, which contains two 16 aa motifs, is responsible for membrane wrapping of IMV (Roper and Moss, 1999). Aa mutations at the K and D position result in a reduced plaque size (Roper and Moss, 1999), which in turn can cause reductions in quality and infectivity, the amount of EEV and a failed actin tail formation (Blasco and Moss, 1992; McIntosh and Smith, 1996; Roper et al., 1998). So, the F13 plays an important role in the membrane association, the virion wrapping progress and the EEV production (Borrego et al., 1999; Husain and Moss, 2001). Another interesting region is the conserved YPPL motif (aa residues 153-156), which is conserved in all strains of OPXVs and is also essential for EEV formation (Honeychurch et al., 2007). Moreover, F13 is also involved in cell fusion at low pH (Blasco and Moss, 1991). Interestingly, two antiviral anti-OPXVs-drugs have been identified, targeting the F13 protein. Whereas $N_1$-isonicotinoly-$N_2$-3-methyl-4-chlorobenzoylhydrazine (IMCBH) is active *in vitro*, ST-246 (Tecovirimat®) is also active *in vivo* (Yang et al., 2005). Both drugs aim to inhibit plaque- and EEV formation and therefore also stop the spread of various OPXVs (Yang et al., 2005). ST-246 is an orally applied, small nontoxic low-molecular-weight molecule (Duraffour et al., 2015; Yang et al., 2005), which is also able to cause changes in the intracellular localization of F13L (Yang et al., 2005). Currently, treatment with ST-246 comprises 14 days of application, but even after only 5 days of treatment, a 80% protection from lethal infection was revealed in mice. Surviving mice were resistant to reinfection, suggesting that protective immunity was elicited (Yang et al., 2005). So, ST-

246 is an effective tool in controlling OPXV infections (Grosenbach et al., 2010).

## 1.6 AIM OF THIS THESIS

The major scope of this thesis was to map epitopes on immune-protective proteins to enlighten OPXV evolution and inform the development of safer vaccines and antivirals. It is known, that VARV caused more fatalities than all other human infectious diseases together. Although VARV was eradicated in 1979, public health concerns remain, as many poxviruses such as MPXV, CPXV, VACV (Cantagalo), BPXV and CMLV have significant zoonotic potential. Moreover, there is a fear of using poxviruses as bio-terroristic weapons, making research on poxvirus replication a top priority. So, the accurate mapping of relevant epitopes on immuno-protective proteins is of high interest. This knowledge enables phylogenetic studies and insights into OPXV evolution. Data on virus species-specific epitope variations will inform about future development of safer vaccines or antivirals. It will also enable a target directed screening of human immunoglobulin libraries for the detection of protective recombinant antibodies against OPXV.

# 2 STUDIES PERFORMED

## 2.1 CHAPTER I

Paper published

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### Species-Specific Conservation of Linear Antigenic Sites on VACV A27 Protein Homologues of Orthopoxviruses

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**ABSTRACT**

The vaccinia virus (VACV) A27 protein and its homologs, which are found in a large number of members of the genus *Orthopoxvirus* (OPXV), are targets of viral neutralization by host antibodies. We have mapped six binding sites (epitopes #1A: aa 32–39, #1B: aa 28–33, #1C: aa 26–31, #1D: 28–34, #4: aa 9–14, and #5: aa 68–71) of A27 specific monoclonal antibodies (mAbs) using peptide arrays. MAbs recognizing epitopes #1A–D and #4 neutralized VACV Elstree in a complement dependent way (50% plaque-reduction: 12.5–200 µg/mL). Fusion of VACV at low pH was blocked through inhibition of epitope #1A. To determine the sequence variability of the six antigenic sites, 391 sequences of A27 protein homologs available were compared. Epitopes #4 and #5 were conserved among most of the OPXVs, while the sequential epitope complex #1A–D was more variable and, therefore, responsible for species-specific epitope characteristics. The accurate and reliable mapping of defined epitopes on immuno-protective proteins such as the A27 of VACV enables phylogenetic studies and insights into OPXV evolution as well as to pave the way to the development of safer vaccines and chemical or biological antivirals.

**KEYWORDS**

Vaccinia virus A27 protein homologs, epitope mapping, phylogenetic epitope variation, neutralizing antibodies

## 1. INTRODUCTION

The genus *Orthopoxvirus* (OPXV) contains a group of large and closely related DNA viruses within the family Poxviridae, encompassing viruses that replicate in the cytoplasm of vertebrate or invertebrate cells (Condit et al., 2006; Kurth et al., 2008). Vaccinia virus (VACV), the prototype of the genus, was applied as the vaccine against the related Variola virus (VARV). This vaccination campaign led to the eradication of

smallpox (Becker et al., 2009; Fenner et al., 1988). Immunization with VACV elicits potent B- and T-cell mediated immune responses, which provide cross protection against all the other OPXVs (Novembre et al., 1989). Currently, the majority of humans worldwide have no longer a protective immunity against poxviruses because of the termination of the vaccination campaign four decades ago. Therefore, there is considerable concern about the use of VARV and monkeypox virus (MPXV) as potential biological weapons (Henderson, 1999; Rimoin et al., 2010), particularly after recent outbreaks of MPXV in the Democratic Republic of Congo, the United States of America, Nigeria and the United Kingdom (Ladnyj et al., 1972; Reed et al., 2004; Vaughan et al., 2018) and as well as being reported by the World Health Organization (WHO, 2017). Moreover, reservoirs for other closely related OPXVs, e.g. cowpox virus (CPXV), exist in the environment and may also endanger human health under certain circumstances (Becker et al., 2009; Campe et al., 2009; Howard et al., 2008; Kurth et al., 2008; Vogel et al., 2012; Vorou et al., 2008), especially in immuno-compromised humans (Czerny et al., 1991; Eis-Hubinger et al., 1990; Fassbender et al., 2016; Kinnunen et al., 2015; Redfield et al., 1987). Therefore, it is crucial to join forces in the development of safer vaccines, antiviral agents, and protective human recombinant antibodies for passive immunization.

VACV contains a double-stranded DNA genome of approximately 194,000 nt, depending on the strain, encoding more than 200 polypeptides (Moss, 2006). Morphogenesis results in two distinct infectious forms of virus particles (Smith et al., 2002; Ulaeto et al., 1996). The majority consists of the fully functioning intracellular mature virus (IMV) with a single envelope, as well as a small proportion of extracellular enveloped virus (EEV), which is surrounded by an additional Golgi-derived envelope. IMV is the predominant infectious form remaining within the infected cell and mediating host-to-host transmission, whereas EEVs, on the other hand, are important for direct cell-to-cell transmission inside the host (Appleyard et al., 1971; Blasco and Moss, 1992; Boulter and

Appleyard, 1973; Isaacs et al., 1992; Payne, 1978; Payne, 1980; Roper et al., 1998; Smith et al., 2003). Viral particles linked to the outer surface of the cell have been visualized by electron microscopy and were named cell-associated enveloped virus (CEV) (Blasco and Moss, 1992; Smith et al., 2002).

Vaccination results in the induction of neutralizing antibodies against several VACV envelope proteins. Structural proteins of immunological relevance containing targets for neutralizing antibodies were identified on both IMV (including A27, D8, H3, A17, and L1), and EEV/CEV (including A33 and B5) (Aldaz-Carroll et al., 2005; Hsiao et al., 1998, 1999; Matho et al., 2015; Moss, 2012, 2016; Rodriguez et al., 1985; Vogel et al., 2012). Most importantly, these proteins led to the induction of protective immunity in vivo (Benhnia et al., 2009b; Czerny et al., 1994; Czerny and Mahnel, 1990; Galmiche et al., 1999; Hooper et al., 2000; Hsiao et al., 1999; Ichihashi and Oie, 1996; Kaever et al., 2016; Lai et al., 1991; Matho et al., 2012; Matho et al., 2015; McCausland et al., 2010; Pulford et al., 2004; Ramirez et al., 2002; Reeman et al., 2017; Rodriguez et al., 1985). One of the best characterized and intensively studied IMV envelope proteins is the A27 protein (Gong et al., 1989; Gong et al., 1990; Lai et al., 1990; Rodriguez and Esteban, 1987; Rodriguez et al., 1985), encoded by a gene corresponding to the VACV Copenhagen open reading frame (ORF) A27L (Goebel et al., 1990; Kaever et al., 2016). This protein is present in all members of OPXVs, forms a trimeric structure on the surface of IMVs, and binds to the glycosaminoglycan (GAG) heparan sulfate on the surface of mammalian cells (Campe et al., 2009; Howard et al., 2008) by a turn-like structure, which is formed by a KKPE segment (Shih et al., 2009). Additionally, the A27 protein builds a complex together with four other membrane proteins (A14, A17, A25 and A26). Because A27 lacks its own trans-membrane domain, its association with A17 mediates the anchorage within the envelope of IMVs (Howard et al., 2008; Rodriguez et al., 1993; Unger et al., 2013; Vazquez et al., 1998). The 110 amino acids of the A27 protein can be divided into four

functional areas: an N-terminal signal peptide, a Lys/Arg-rich heparin binding domain (HBD), an α-helical coiled-coil domain (CCD), and a C-terminal leucine zipper motif (LZD) (Campe et al., 2009; Chang et al., 2013; Vazquez et al., 1998). The HBD (aa 21-34) including the KKPE segment (aa 26-29), is essential for binding to heparan sulfate (Chang et al., 2013; Chung et al., 1998; Hsiao et al., 1998; Shih et al., 2009). The CCD (aa 43-84) possesses the two cysteine residues 71 and 72, which are responsible for forming disulfide bonds with the A26 protein (Chang et al., 2013; Ching et al., 2009). The LZD (aa 85-110) is considered to be the binding region of A17 (Campe et al., 2009; Chang et al., 2013; Kochan et al., 2008; Wang et al., 2014). A27 is important for virus replication, as it regulates cell entry and virus egress. Conditional lethal mutant independent assays like isopropyl-o-thiogalactoside (IPTG)-induced expression of the A27 protein during infection restores the interaction of IMV with Golgi-derived membranes leading to EEV formation. Thus, the A27 protein is essential for the envelopment of IMV by Golgi membrane and for their subsequent egress from the cell (Rodriguez and Smith, 1990). The A27 protein was designated as the fusion protein, because monoclonal antibodies binding to this protein of 14 kDa were able to block fusion (Gong et al., 1990; Rodriguez and Esteban, 1987). However, more recent evidence suggests it is more likely that a complex of at least 11 envelope proteins is responsible for fusion (Moss, 2012, 2016). The A27 protein, however, is not integrated within this complex.

Here, we have identified six linear epitopes recognized by A27 mAbs (Czerny et al., 1994) using SPOT synthesis on cellulose membranes and peptide microarray technology. Affinities were investigated and neutralization capabilities of the mAbs were improved after the addition of human complement. The identified epitopes toward the far ends of A27 were conserved among OPXV upon screening all A27 sequences available in the GenBank, while the centrally located epitopes were species-specific.

## 2. MATERIAL AND METHODS

### 2.1. Cells and viruses

The permanent monkey kidney cell line MA-104 cultured in minimum essential medium (MEM) (PAN-BIOTECH, Aidenbach, Germany) and supplemented with 7% fetal calf serum (FCS), was used to propagate the VACV strains Bern, CVA, Elstree, IHD-J, Copenhagen wild type (WT), Copenhagen host range (HR), R325, TT, the neuro-vaccinia virus strains Hagen, Levaditi and Munich 1, as well as the OPXV strains camelpox virus (CMLV) CP1, CPXV KR2 Brighton, mousepox virus (ectromelia; ECTV) Munich 1, and MPXV Copenhagen (for references see (Czerny and Mahnel, 1990)). For virus propagation FCS was reduced to 2%. Infectivity titers were determined on 24-well plates (Nunc, Wiesbaden, Germany) and calculated as plaque forming units (pfu/ml). For plaque reduction tests, Vero cells cultured in MEM, supplemented with 5% FCS were used and maintained in the same way as MA-104. For syncytium formation and fusion experiments, BS-C-1 cells cultured in MEM, supplemented with 10% FCS were used to propagate the VACV strain Western Reserve (WR). Virus multiplication was carried out in MEM with 2.5% FCS as described before (Roper, 2006; Roper et al., 1998).

The Modified VACV Ankara (MVA) was grown in primary embryonic chicken fibroblast cells (CEF). Due to its micro-plaque generation, infectivity titer was calculated as $TCID_{50}$/ml after titration in 96-well microplates. The culture medium was MEM containing 2.5% FCS.

All virus preparations were purified and concentrated by sucrose gradient centrifugation as previously described (Czerny et al., 1994; Joklik, 1962). The purified preparations consisted of intracellular mature virus (IMV). Protein contents of the samples were determined according to the method of Lowry et al. (Lowry et al., 1951).

### 2.2. Polyclonal and monoclonal antibodies

Polyclonal rabbit hyperimmune sera and monoclonal BALB/c-mouse antibodies against purified VACV MVA, VACV Munich 1, CPXV KR2 Brighton, ECTV Munich 1, and MPXV Copenhagen were prepared as described elsewhere (Czerny et al., 1994; Czerny and Mahnel, 1990). The monoclonal antibodies (mAbs) were cross-reactive against other OPXVs in a species-specific manner. For this study, the cross-reactive but A27-specific mAbs anti-VACV 5B4/F2 (epitope #1A), anti-VACV 2C11/1B4 (epitope #1B), anti-CPXV 3F5/2D5 (epitope #1C), anti-CPXV 1D5/1E10 (epitope #1D), anti-ECTV 2G8/1E4 (epitope #4), and anti-ECTV 5B1/2G6 (epitope #5) were used. Monoclonal antibodies from cell culture supernatants or polyclonal hyper-immune sera were purified on Protein G sepharose columns (HiTrap™ 5 ml Protein G HP, Sigma Aldrich, Taufkirchen, Germany), dialyzed against phosphate-buffered saline (PBS) and sterilized by centrifugation at 20,238 x g. Protein contents of the antibody preparations were determined according to the method of Lowry et al. (Lowry et al., 1951).

### 2.3. Plaque Reduction Test

The neutralization potency of six A27-specific mAbs was tested by plaque reduction test (PRT) against VACV Elstree as reference strain. Purified antibodies were diluted with MEM (PAN-BIOTECH, Aidenbach, Germany) and adjusted to a concentration of 400 µg/ml. A volume of 125 µl of the antibody preparations was titrated in two-fold serial dilutions on 96-well microplates containing 125 µl/well MEM supplemented with 2.5% FCS to avoid antibody coating. After antibody titration, one dilution series received 1% sterile human complement (SIGMA Aldrich, Taufkirchen, Germany) per well, the other remained free of complement. Then, 100 pfu (125 µl) of VACV Elstree was added to each well. As plaque-forming control, 250 µl MEM/well with or without 0.5% human

complement, containing 100 pfu VACV Elstree was used. The virus negative control was 250 µl MEM/well alone with or without 0.5% human complement. After incubation of the 96-well microplates at 37°C for one hour, the mixtures were transferred to 24-well plates containing a confluent monolayer of Vero cells. After incubation at 37°C for one hour, the supernatants were poured out and replaced by 0.5 ml MEM containing 2.5% FCS and 0.5% methyl cellulose (Sigma Aldrich, Taufkirchen, Germany). The plates were then incubated at 37°C for 48 hours, before the cells being fixed and stained with a solution containing 25% formaldehyde, 8.5% ethanol and 1.5% crystal violet. The plaques were counted by visual inspection while illuminated. Neutralization was determined as ≥50% plaque reduction compared to the virus control. Each PRT was performed in triplicates.

**2.4. Inhibition of cell fusion and syncytium formation**

Cell fusion experiments were performed as described before (Roper, 2006; Roper et al., 1998; Wolffe et al., 1993). Confluent BS-C-1 monolayers cultured in MEM with 2.5% FCS in 24-well plates (1 ml/well) were infected with 100 pfu/well VACV WR for 1 h at 37°C, washed twice and incubated either with warm medium alone or with warm MEM containing purified mAbs (200 µg/ml). Then, 24 h post infection, the cells were incubated for 3 min at 37°C at pH 4.8 with warm fusion buffer [phosphate-buffered saline with 10 mM 2-(N-morpholino)ethanesulfonic acid and 10 mM HEPES]. The cells were washed twice with warm MEM (treated for two min at 37°C). Afterwards, warm medium (MEM + 1% FCS), with or without mAbs (200 µg/ml), was added again. The cells were incubated for 4 h at 37°C and then observed by phase-contrast microscopy. An indicator for cell fusion was the formation of syncytia, which are large, structure-less, fused cell areas (Gong et al., 1990).

### 2.5. Binding affinities of the mAbs in indirect ELISAs

For quantification of the binding affinities of mAbs to different OPXVs, an indirect ELISA was applied. 96-well microplates were coated with 1 µg/ml of the VACV strains Bern, CVA, Elstree, IHD-J, Copenhagen wild type (WT), Copenhagen host range (HR), R325, TT, the neuro-vaccinia virus strains Hagen, Levaditi and Munich1, the modified VACV Ankara (MVA) as well as the OPXV strains camelpox virus (CMLV) CP1, cowpox virus (CPXV) KR2 Brighton, mousepox virus (ectromelia; ECTV) Munich 1, and MPXV Copenhagen in carbonate/bicarbonate buffer (pH 9.6; 100 µl/well). After blocking with 2% skimmed milk and 10% fetal calf serum in PBS, purified mAbs adjusted to a concentration of 50 µg/ml were titrated in two-fold serial dilutions (100 µl/well). Incubation was performed at 37°C for 1 h. After five washing steps with PBS, the peroxidase conjugated goat anti-mouse IgG (whole molecule; Sigma Aldrich, Taufkirchen, Germany) was added to the 96-well microplate in a working dilution of 1:2,000 (100 µl/well) and incubated at 37°C for 1 h. Thereafter, the plate was washed five times with PBS again, before the developing solution (3, 3', 5, 5' tetramethylbenzidine; Abcam, Cambridge, UK) was added (100 µl/well). The reaction was stopped by 1 N hydrochloric acid (50 µl/well). The OD-values were measured by a photometric plate reader (TECAN Sunrise plate reader with the Magellan complete software, Männedorf, Switzerland) at a wavelength of 450 nm. Affinity was calculated from the average absorption of the triplicates using Michaelis-Menten kinetics (Michaelis and Menten, 1913; Michaelis et al., 2011) and the program GraphPad Prism version 7.00 for Mac (La Jolla California, USA).

### 2.6. Epitope mapping by SPOT synthesis on cellulose membranes

The whole A27 protein of VACV Copenhagen (Goebel et al., 1990; Rodriguez and

Esteban, 1987) representing 110 amino acids (Goebel et al., 1990), was directly synthesized stepwise on derivatized cellulose membranes through 101 decapeptides with an offset of one aa (9 aa overlap). The synthesis on derivatized cellulose membranes using Fmoc-protected amino acid pentafluorophenyl or /V-hyroxyoxo-dihydro-benzotriazine esters and the screening were performed according to the method described before (Frank, 1992) and the manufacturer of the SPOTs kit (Cambridge-Research Biochemicals, ICI, representative in Germany IC-Chemikalien, Carl-Zeiss-Ring 15, Ismaning).

The reactivity of the generated peptides with mAbs was tested using β-galactosidase-labeled goat anti-mouse immunoglobulins (Abcam, Cambridge, UK) as secondary antibodies. The color development of the peptide spots occurred after treatment with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, the substrate for the β-galactosidase-labeled secondary antibodies.

**2.7. Epitope mapping by microarray scanning chips**

An OPXV microarray chip was designed as depicted in Fig. S1. 15-mer peptides overlapping by 12 amino acids (3 aa offset) were synthesized via SPOT synthesis on a cellulose membrane (Frank, 1992), passed through the $SC^2$ process (Dikmans et al., 2006) and spotted onto microscope glass slides. The chip contained eight identical arrays of 521 peptides each (Fig. S1A). A total of 475 of those overlapping peptides represented the entire amino acid sequences of A27, D8, H3, L1, A33, and B5 proteins of VACV Western Reserve (Fig. S1B, GenBank accession number: AY243312.1). Forty-six peptides were amino acid variations of VACV A27 and D8 proteins to the corresponding homologs of other OPXVs (Table S1). In addition, ten cellulose-conjugated biotin spots served as a positive control and orientation for the SPOT Calling Program. The OPXV microarray chip was designed to screen four samples simultaneously. Therefore, each peptide was

printed eight times to obtain technical replicates, which could be divided into 4 identical sub-arrays using an adhesive chamber (SecureSeal, Sigma-Aldrich Co. LLC, USA). In order to obtain equal antibody concentrations of 2µg/µl per chamber, protein concentrations were measured using a NanoDrop ND-1000 Spectrophotometer. The screening procedure with the microarray chip was performed as previously reported (Hotop et al., 2014).

### 2.8. DataBase analysis of A27 protein sequences and OPXV phylogeny

All A27 protein sequences of different OPXV strains available until 31st August 2018 were downloaded from the NCBI GenBank database (Altschul et al., 1990; Pruitt et al., 2005, 2007). So, a total of 391 complete and partial A27 sequences were aligned by the Clustal W (Thompson et al., 1994) option in the Lasergene MegAlign 12 software (DNAStar, Madison, WI, USA). The used data included sequences from Old World species such as VARV, VACV, buffalopox virus (BPXV), rabbitpox virus (RPXV), horsepox virus (HSPV), MPXV, CPXV, CMLV, ECTV, and taterapox virus (TaPXV) as well as the New World species raccoonpox virus (RCNV), volepox virus (VPXV), and skunkpox virus (SkPXV). A phylogenic tree was created with Geneious software (version 9.1.6, Biomatters Inc., Aukland, New Zealand), using not more than five sequences per epitope variant.

## 3. RESULTS

### 3.1. Fine mapping of the VACV A27 epitopes by SPOTs membrane

The targets of six anti-A27 mAbs were mapped by SPOT synthesis (Table S1). The A27 protein of VACV Copenhagen was synthesized on a SPOTs membrane in form of 101 decapeptides with 9 aa overlap to cover the whole sequence of 110 aa. Immunodetection

was carried out with the six OPXV-specific mAbs (Fig. 1). A positive reaction was indicated by blue coloration of those spots binding the corresponding antibody. The complex of the four closely related antigenic sites #1A-D was identified and located within the range of aa 26–39. Epitope #1A (mAb: 5B4/2F2) was directed against the sequence region of eight aa 32-REAIVKAD-39 (Fig. S2). In case of the mAb 5B4/2F2, seven spots were recognized (No. 10-16), from which spots 11-13 showed the strongest reactivity to the mAb, which indicated an optimal antibody binding condition and only these peptides were used for defining the epitope. By the same procedure, epitope #1B (mAb 2C11/1B4) was assigned to the six aa 28-PEAKRE-33, epitope #1C (mAb 3F5/2D5) to the six aa 26-KKPEAK-31, and epitope #1D (mAb 1D5/2D11) to seven aa 28-PEAKREA-34 (Fig. 1 and Table S2). Epitope #4 (mAb 2G8/1E4) was located at aa positions 9-DDDLAI-14, whereas epitope #5 (mAb 5B1/1A11) was represented by the four aa 68-IEKC-71.

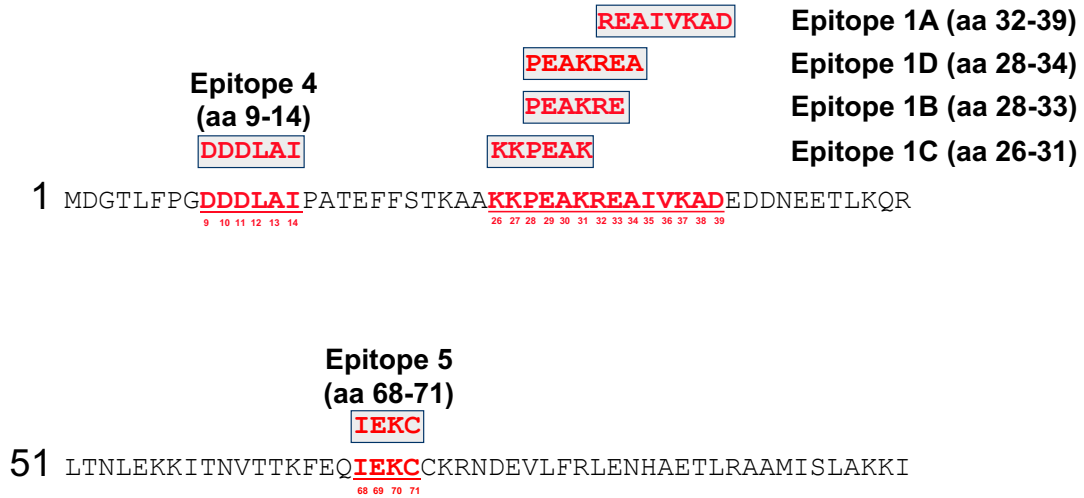



**FIG 1** Mapping of the antigenic sites of the six A27-specific mAbs on a SPOTs membrane.

### 3.2. Fine mapping of the VACV A27 epitopes by microarray analysis

Similar mapping results were obtained when using the OPXV microarray chip imprinted with 521 pentdecapeptides with 12 aa overlap. Epitope #1A (mAb 5B4/2F2) was only one aa longer compared to the SPOTs membrane and was, therefore, directed to the sequence region aa 31-KREAIVKAD-39. Epitopes #1B (mAb 2C11/1B4), #1C (mAb 3F5/2D5) and #1D (mAb 1D5/2D11) were all assigned to the aa region 28-PEAKRE-33. For epitope #1B, the microarray chip and the SPOTs membrane yielded identical results. The epitope #1D was mapped to the same region, but only one aa shorter on the microarray chip. Epitope #4 (mAb 2G8/1E4) was allocated to aa 7-PGDDDLAIPATE-18 and, therefore, by 6 aa longer compared to results from the SPOTs membrane. MAb 5B1/1A11 (epitope #5), however, did not react with any of the peptides on the chip, although the target sequences detected on the SPOTs membranes were present in the microarray spots no. 20-23 and 493-496 (Tables S1 and S2 and Fig. S3). In the following investigations, we refer, therefore, to the epitope locations provided by the SPOTs membrane, because they were regarded to be more accurate due to the shorter aa offset of one aa compared to three aa in the microarrays.

### 3.3. Identification of neutralization-mediating epitopes with/without complement

Epitopes able to induce neutralizing antibodies were detected by PRT. Protein G purified mAbs against the six antigenic sites mapped on the A27 protein were incubated with VACV Elstree either in the presence or absence of 1% human complement. Complement was used to increase the footprints of the mAbs on the viral surface. The mAbs 5B4/2F2 (epitope #1A), 2C11/1B4 (epitope #1B), and 2G8/1E4 (epitope #4) neutralized VACV Elstree (measured as 50% plaque reduction) in the absence of complement at concentrations of 12.5, 25 and 200 µg/ml, respectively (Table 1 and Fig. 2). An eight to

sixteen-fold increase in the neutralization strength of these mAbs was observed in the presence of complement (5B4/2F2: 1.6 µg/ml; 2C11/1B4: 3.1µg/ml; 2G8/1E4: 12.5 µg/ml). The mAbs 3F5/2D5 (epitope #1C) and 1D5/1E10 (epitope #1D) neutralized VACV Elstree only in the presence of 1% complement at concentrations of 200 µg/ml and 100 µg/ml, respectively, while no neutralization was observed with the mAb 5B1/2G6 (epitope #5).

**Table 1** Neutralization efficiency of six different purified anti-A27 monoclonal antibodies against several epitopes with and without complement binding.

| Epitope | Position (aa) | MAb | Virus strain used for mAb production | Isotype | Neutralization without complement (µg/ml) | Neutralization with 1% complement (µg/ml) |
|---|---|---|---|---|---|---|
| 1A | 32-39 | 5B4/2F2 | VACV MVA | IgG2a | 12.5 | 1.6 |
| 1B | 28-33 | 2C11/1B4 | VACV MVA | IgG2b | 25.0 | 3.1 |
| 1C | 26-31 | 3F5/2D5 | CPXV KR2 Brighton | IgG1 | - | 200.0 |
| 1D | 28-34 | 1D5/2D11 | CPXV KR2 Brighton | IgG1 | - | 100.0 |
| 4 | 9-14 | 2G8/1E4 | ECTV Munich 1 | IgG3 | 200.0 | 12.5 |
| 5 | 68-71 | 5B1/2G6 | ECTV Munich 1 | IgG2a | - | - |

-: No neutralization observed.

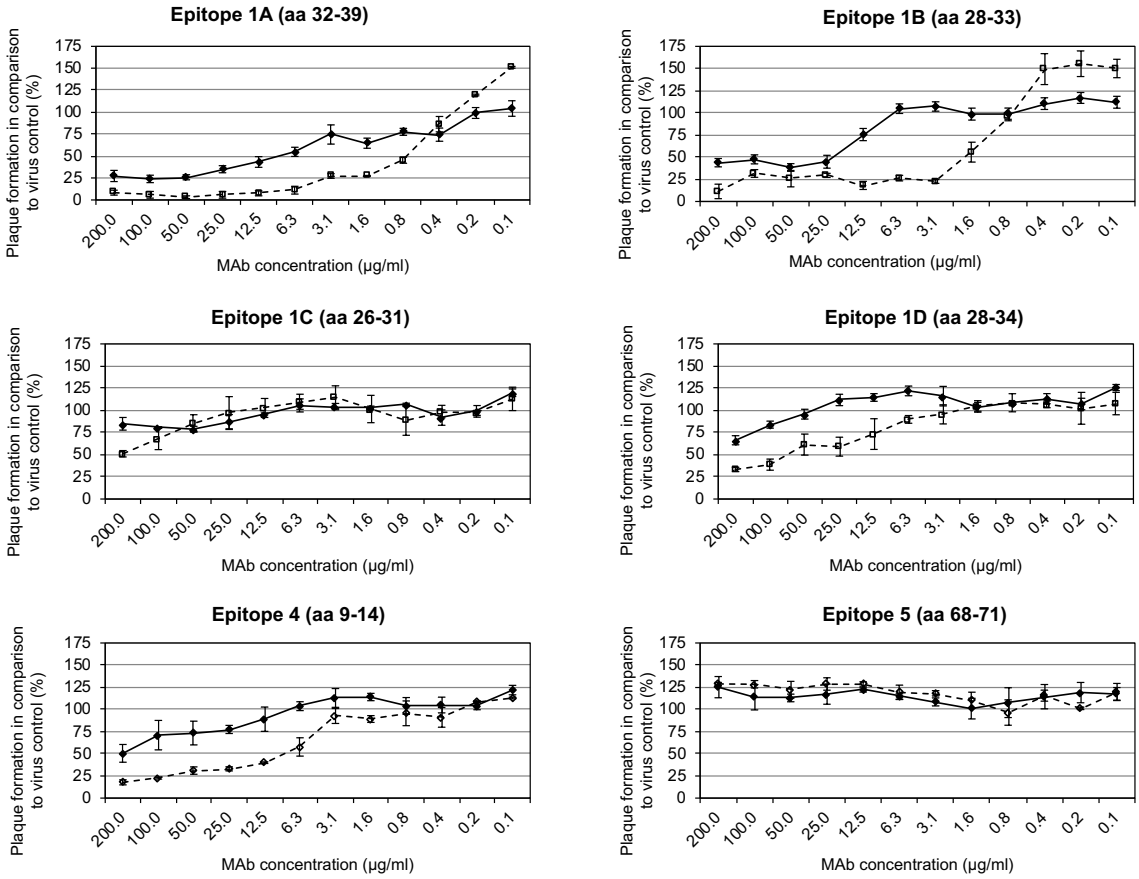



**FIG 2** Neutralization-mediating epitopes were detected by plaque reduction test (PRT). Six mapped anti-A27 mAbs were incubated with VACV Elstree either in the presence or absence of 1% human complement. The plaque formation in comparison to virus control in percent is shown as a function of the respective concentration in µg/ml of the mAbs. The solid line (♦) shows the antibody alone, the dashed line (■) the antibody together with 1% complement. The mAbs 5B4/2F2, 2C11/1B4, and 2G8/1E4 neutralized VACV Elstree in the absence of complement. Neutralization could be improved in the presence of complement. The mAbs 3F5/2D5 and 1D5/1E10 neutralized the VACV Elstree only in the presence of 1% complement, while no neutralization was observed with the mAb 5B1/2G6.

### 3.4. Inhibition of cell fusion

A27 was initially designated as the fusion protein (Gong et al., 1990; Moss, 2011; Rodriguez et al., 1987). However, more recent evidence indicates that there is not only one fusion protein in the envelope of IMV, but rather a fusion complex consisting of at least 11 proteins (Diesterbeck et al., 2018; Moss, 2012, 2016). Evidence now suggests that the A27 protein is not integrated into the fusion complex (Moss, 2016; Senkevich et al., 2005). Other investigations reported a second fusion complex consisting of A17 and A27 (Kochan et al., 2008), where the fusion event of VACV WR at pH 4.8 was inhibited

by anti-A27 mAbs. Therefore, we retested this effect using three epitope-mapped anti-A27 mAbs from our collection to cover the entire target region. Fusion of infected BS-C-1 cells was indicated by the formation of large areas of fused cells, rather than separate individual cells (Fig. 3A). Fusion was inhibited by the mAb 5B4/2F2 directed to epitope #1A (aa 32-39) (Fig 3B). The mAb 3F5/2D5 against epitope #1C (aa 26-31) was binding upstream of the mAb 5B4/2F2 and was not able to block cell fusion (Fig. 3C). The same was observed for mAb 5B1/2G6 binding to the C-terminal epitope #5 (aa 68-71) (Fig. 3D).

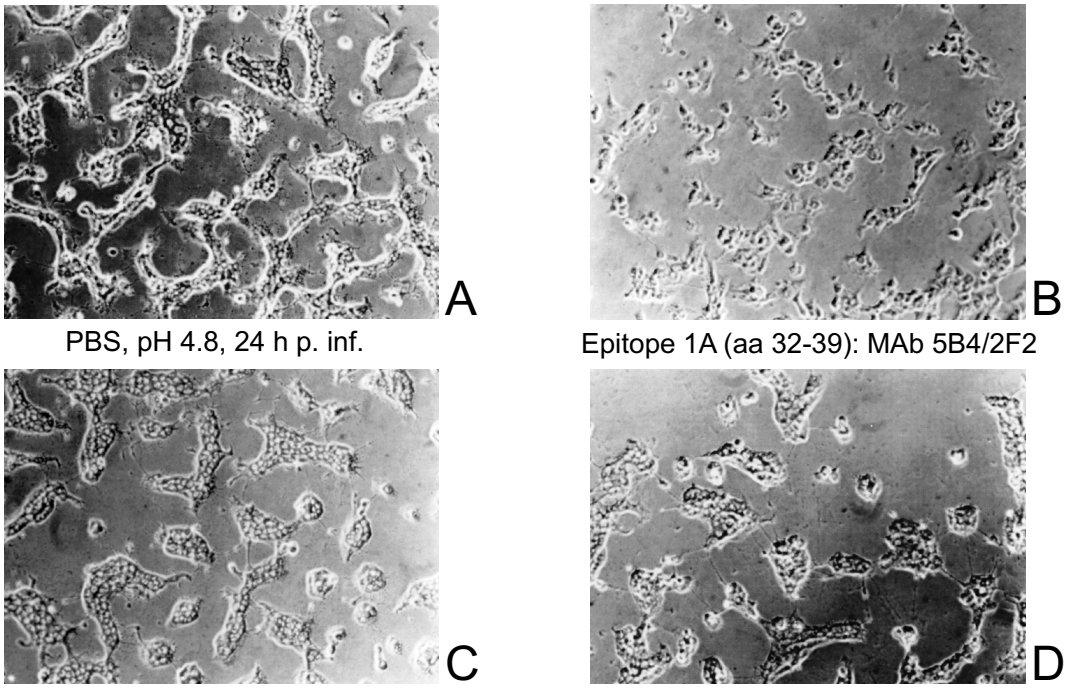



**FIG 3** Acid-induced fusion inhibition experiments with VACV WR and three of the six anti-A27 mAbs. A: Fusion of infected BS-C-1 cells was indicated by the formation of larger, structureless, and fused cell areas. B: Fusion was inhibited by the mAb 5B4/2F2 directed to epitope #1A (aa 32-39). C: The mAb 3F5/2D5 against epitope #1C (aa 26-31) was binding upstream of the mAb 5B4/2F2 and not able to block cell fusion. D: The mAb 5B1/2G6 binding to the C-terminal epitope #5 (aa 68-71) was also not able to inhibit fusion.

### 3.5. Binding affinities of the mAbs to various variants of OPXVs

Binding affinities of the purified mAbs to the six A27 epitopes detected in VACV Elstree

were determined by indirect ELISAs on microplates coated with the purified reference strains VACV-MVA, VACV, CPXV KR2 Brighton, CMLV CP1, ECTV Munich 1, and MPXV Copenhagen. The binding curves were determined in triplicates for each virus strain. In case of the VACV strains, with the exception of VACV MVA, all data were calculated as mean values. VACV MVA was presented alone in order to compare affinity data directly to other VACV strains (Fig. 4). All mAbs directed to epitope complex #1 showed strong binding activity to VACV, CPXV and CMLV, but did not react with or bound only weakly to ECTV and MPXV. In all VACV strains, the mAb 5B4/2F2 bound to its epitope #1A equally well. There was no difference in the amino acid sequence of the respective epitope. An 11.5-23-fold decrease in binding activity was observed with CPXV KR2 Brighton and CMLV CP1. Responsible for this finding were obviously the aa exchanges D39E in CPXV and V36I in CMLV. In ECTV Munich 1 and MPXV Copenhagen, the epitope #1A could not be detected, apparently due to aa exchanges R32H and I35T in ECTV and D39Y in MPXV. Epitope #1B was detected by the mAb 2C11/1B4 in VACVs, CPXV and CMLV with a similar affinity, whereas aa exchanges A30D and R32H in ECTV and A30T in MPXV caused the loss of the mAb reaction. Epitope #1C was also detected equally well in VACVs, CPXV and CMLV by the corresponding mAb 3F5/2D5. In ECTV, the kinetics of the mAb were reduced 25 – 53-fold according to the aa exchange A30D. In MPXV, the epitope was only very weakly detectable. The mAb 1D5/2D11 against epitope #1D, which is only one aa longer than epitope #1B (A at position 34), reacted equally well with VACVs, CPXV and CMLV. Despite the aa exchanges A30D and R32H in ECTV, which were also present, the mAb detected the epitope with 2.6 to 7.6-fold weaker affinity compared to VACVs, CPXV and CMLV. Even in MPXV, the epitope #1D was detected by the mAb, albeit with a 4.6-(ECTV) to 34.8-fold (VACVs) weaker intensity. In contrast to the heterogeneous species-specific binding behavior of mAbs directed to the epitope complex #1A-D, the mAbs

targeting epitopes #4 and #5 showed the same strong binding activities to all OPXVs tested. $V_{max}$- and $K_m$-values were in the same range.

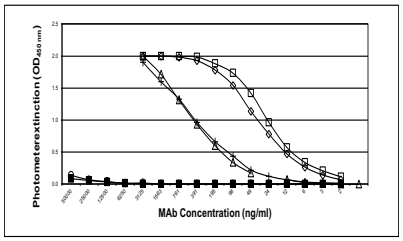
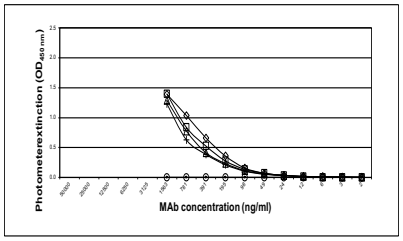
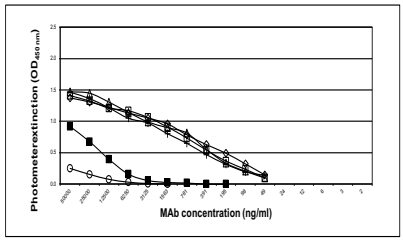
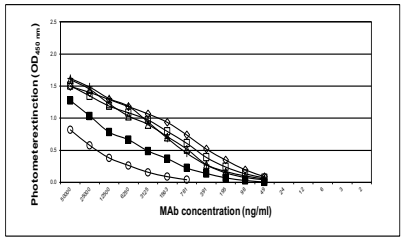
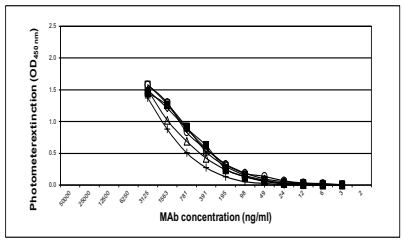
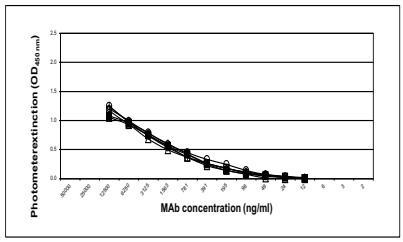
| Epitope | OPXV | $V_{max}$ | $K_m$ (ng/ml) | Amino acid | Binding affinities |
|---|---|---|---|---|---|
| 1A (5B4/2F2) (aa 32-39) | ◊ VACV* | 2.08 | 39 | 32-REAIVKAD-39 |  |
| | □ MVA | 2.08 | 26 | 32-.....-39 | |
| | + CPXV | 2.11 | 444 | 32-.....E-39 | |
| | Δ CMLV | 2.38 | 606 | 32-....I...-39 | |
| | ■ ECTV | - | - | 32-H..T....-39 | |
| | ○ MPXV | - | - | 32-.....Y-39 | |
| 1B (2C11/1B4) (aa 28-33) | ◊ VACV* | 2.33 | 1,031 | 28-PEAKRE-33 |  |
| | □ MVA | 3.41 | 2,285 | 28-.....-33 | |
| | + CPXV | 6.08 | 6,236 | 28-.....-33 | |
| | Δ CMLV | 3.97 | 3,238 | 28-.....-33 | |
| | ■ ECTV | - | - | 28-..D.H.-33 | |
| | ○ MPXV | - | - | 28-..T...-33 | |
| 1C (3F5/2D5) (aa 26-31) | ◊ VACV* | 1.27 | 399 | 26-KKPEAK-31 |  |
| | □ MVA | 1.32 | 608 | 26-.....-31 | |
| | + CPXV | 1.35 | 899 | 26-.....-31 | |
| | Δ CMLV | 1.39 | 694 | 26-.....-31 | |
| | ■ ECTV | 1.88 | 49,902 | 26-....D.-31 | |
| | ○ MPXV | - | - | 26-.N..T.-31 | |
| 1D (1D5/2D11) (aa 28-34) | ◊ VACV* | 1.40 | 733 | 28-PEAKREA-34 |  |
| | □ MVA | 1.39 | 1,149 | 28-.....-34 | |
| | + CPXV | 1.62 | 2,168 | 28-.....-34 | |
| | Δ CMLV | 1.53 | 2,028 | 28-.....-34 | |
| | ■ ECTV | 1.31 | 5,567 | 28-..D.H..-34 | |
| | ○ MPXV | 1.21 | 25,543 | 28-..T....-34 | |
| 4 (2G8/1E4) (aa 9-14) | ◊ VACV* | 1.93 | 923 | 9-DDDLAI-14 |  |
| | □ MVA | 2.18 | 1,141 | 9-.....-14 | |
| | + CPXV | 3.25 | 4,259 | 9-.....-14 | |
| | Δ CMLV | 2.39 | 1,932 | 9-.....-14 | |
| | ■ ECTV | 1.93 | 918 | 9-.....-14 | |
| | ○ MPXV | 2.16 | 1,118 | 9-.....-14 | |
| 5 (5B1/2G6) (aa 68-71) | ◊ VACV* | 1.31 | 1,769 | 68-IEKC-71 |  |
| | □ MVA | 1.29 | 1,963 | 68-....-71 | |
| | + CPXV | 1.42 | 2,442 | 68-....-71 | |
| | Δ CMLV | 1.21 | 2,038 | 68-....-71 | |
| | ■ ECTV | 1.20 | 1,647 | 68-....-71 | |
| | ○ MPXV | 1.30 | 1,583 | 68-....-71 | |

**FIG 4** Binding affinities of the six anti-A27 mAbs determined by indirect ELISAs towards the reference strains VACV MVA, a mean of 11 different VACV strains, CPXV KR2 Brighton, CMLV CP1, ECTV Munich 1, and MPXV Copenhagen. In case of the VACV strains, with the exception of VACV MVA, all data were calculated as mean values from the individual binding curves. The optical density ($OD_{450\,nm}$) is shown as a

function of the respective concentration (ng/ml) of the mAb. Moreover, the aa sequence matches and differences of the epitopes, as well as the $V_{max}$ and $K_m$ values are shown. All mAbs directed to epitope complex #1A-D showed strong binding activity to VACV, CPXV and CMLV, but did not react or bound weakly to ECTV and MPXV. In contrast the mAbs targeting epitopes #4 and #5 showed the same strong binding activities to all OPXVs tested. All the six antigenic sites were recognized equally well in VACV MVA and in all the other VACVs. ◊ = Mean of 11 different VACV strains; □ = VACV MVA; + = CPXV KR2 Brighton; ∆ = CMLV CP1; ■ = ECTV Munich 1; ○ = MPXV Copenhagen.

### 3.6. Species-specific epitope conservation and variation among the OPXV members

A total of 391 amino acid sequences of the OPXV A27 protein homologs from the GenBank were analyzed with respect to species-specific conservation or variation of the six sequential antigenic sites mapped.

Epitope #4, located at the N-terminus of the A27 protein between aa residues 9-14 (9-DDDLAI-14), is highly conserved within the genus OPXV (Tables S3, S4, and S5). This motif was found in 372 of the 391 analyzed sequences.

The motif 68-IEKC-71 of epitope #5 is nearly genus-specific in OPXVs (Tables S4, S5, and S6). It was present in almost all OPXV strains (389/391). In case of 3/3 SkPXVs, the motif 68-IEKC-71 is postponed backward (94-IEKC-97) due to an insertion of 10 aa in the epitope complex #1A-D (as stated below) and 16 aa between epitope complex #1A-D and #5. The same displaced epitope region (94-IEKC-97) was observed in the case of VACV WR 65-16 (P26312.1) as well, because the A27 of this virus strain starts at aa 73L.

The highest number of variations was found within the four antigenic sites of the epitope complex #1A-D located at an A27 surface domain between aa 26–39 (KKPEAKREAIVKAD; epitope #1A: aa 32-39, #1B: aa 28-33, #1C: aa 26-31, #1D: 28-34). This complex is highly conserved in sequences of VARV major (66/67), VARV minor (2/2), VACVs (59/61), BPXV (26/26), HSPV (2/2), RPXV (2/2) and TaPXV (2/3) (Tables S7, S4, and S5). All CMLVs (18/18) showed a unique aa exchange V36I leading to the motif 26-KKPEAKREAIIKAD-39. The exchanges A30D, R32H, and I35T (26-

KKPEDKHEATVKAD-39) were characteristic for ECTV (14/14). The three aa exchanges K27N, A30T and D39Y were specific for all MPXVs (57/57) and resulted in the motif 26-KNPETKREAIVKAY-39. The New World OPXVs, including RCNV (1/1), SkPXV (3/3) and VPXV (1/1) showed the most different, however, species-specifically conserved aa sequence. In CPXVs, a much more polyphyletic arrangement was found, resulting in seven different CPXV motifs (Fig. 5). These results were confirmed by the peptide microarrays including various variants (Fig. S3 and Table S1).

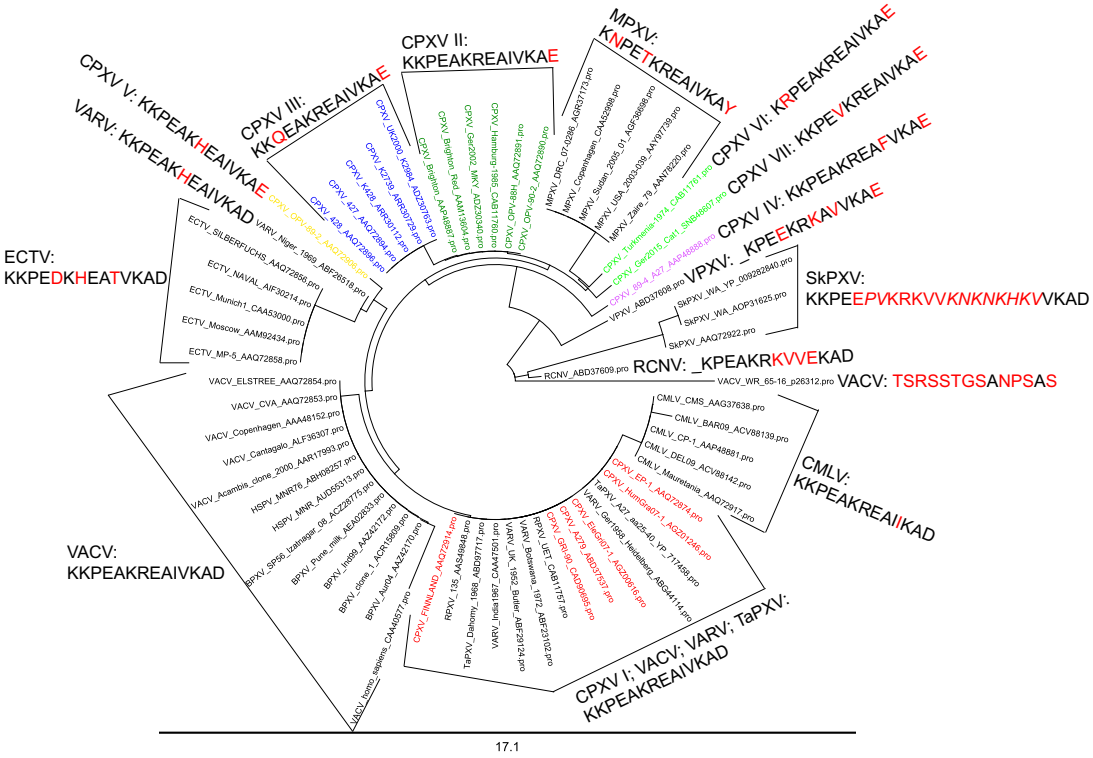



**FIG 5** Phylogenetic analysis is based on the different OPXV amino acid sequences of the epitope complex #1A-D. The sequences were divided into two distinct groups: the monophyletic sequences, including VACV, VARV, ECTV, MPXV, CMLV, HSPV, RPXV, TaPXV, SkPXV, RCNV and VPXV are shown in black, as well as the polyphyletic CPXVs, which are color coded. The sequence differences compared to VACV/VARV are highlighted in red. Because of their polyphyletic behavior, the CPXVs could be subdivided into seven different variants. Red CPXV I; green CPXV II; blue CPXV III; violet CPXV IV; yellow CPXV V; bright green CPXV VI and CPXV VII.

### 3.7. Silent mutations in the epitope sequences among the OPXV members

When looking at the nucleotide sequences within the regions coding for the epitopes #1A-D, #4, and #5, we also searched for silent mutations in all three epitope regions. VACV

(61/61) and its variants including HSPV (2/2), BPXV (26/26) and RPXV (2/2) showed no silent mutations. However, within the monophyletic groups such as CMLV (n: 17/18; ntx: gac28-30gat), ECTV (n: 12/14; ntx: gac28-30gat and n: 14/14; ntx: ata202-204atc), SkPXV (n: 3/3; ntx: gac28-30gat, gat31-33gac, gca37-39gcg, aag79-81aaa, cgc94-96cgt100-102, gcc112-114gca142-144, gaa283-285gag, aag286-288aaa and tgt289-291tgc) and VARV major and minor (n: 68/69; ntx: gac28-30gat) a few silent mutations were confirmed, and could be used for classification, because they were species-specific. In case of the polyphyletic CPXVs we found eight silent mutations in the epitope coding regions of the A27L gene (n: 5/134 ntx: gac28-30gat; n: 19/134 ntx: ctt34-36ctc; n: 42/134 ntx: aaa76-78aag; n: 1/134 ntx: aag79-81aaa; n: 4/134 ntx: cgc94-96cgt; n: 1/134 ntx: gca100-102gcc; n: 85/134 ntx: gcc112-114gct; n: 32/134 ntx: ata202-204atc). Due to their irregular distribution among the different CPXV strains, they were not suitable for a taxonomic classification.

## 4. DISCUSSION

The A27 protein is immunogenic and highly conserved within the members of OPXV (Chang et al., 2013; Kochan et al., 2008; Rodriguez et al., 1985). In this study, six antigenic sites on the A27 protein (epitope #4: aa region 9-14, epitope complex #1A-D: between aa 26 and 39 and epitope #5: aa region 68-71) were mapped. With respect to epitopes #4 and #1A-D, the mapping with the SPOT synthesis and microarray chip showed similar results with only few amino acids divergence. By contrast, epitope #5 could not be detected when the OPXV microarray chip was employed. Although the granularity of membranes is higher with an offset of one, compared to an offset of three in the microarrays, also the resolution of the microarray slide is sufficient to detect epitope #5 with a length of four spots. One reason may be degradation of the respective antibodies

over time, as a time lag >5 years between the former spot measurement and the more recent microarrays existed. Another reason may be the use of different side chain protection groups. For production of membranes, Cys(acetyl-aminomethyl) was used, whereas for peptides in the microarray assay Cys(triphenylmethyl) was used that was deprotected during slide preparation via trifluoroacetic acid (TFA). As a cysteine residue is involved in binding, a formation of disulfide bonds or incomplete Cys deprotection may have altered antibody binding properties, thereby leading to different signals in the microarrays compared to the membranes (Abd El Wahed, 2011). The OPXV microarray chip, however, was used to screen for additional species-specific epitope variations by aa exchanges according to the GenBank database entries.

Epitope #4 was conserved among all OPXVs and nearly genus-specific, because the main motif 9-DDDLAI-14 was found in 372/391 database entries. In 13 OPXV strains, the N-terminal sequences could not be assessed due to truncations in the sequences uploaded from GenBank. In 3/26 BPXVs, the motif was 9-DDDLAT-14, whereas all three SkPXVs contain the motif 9-DDDMAI-14. The changed aa sequence (L12M) was represented by spot 479 of the OPXV microarray chip and mAb 2G8/1E4 reacted with this spot (Table S7). In 2D predictions of the secondary structure of the A27 protein, a β-turn was evident in this area with a high antigenicity. This was expected as in previously published data epitopes often were identified in the region of β-turns (Chou and Fasman, 1979; Fanning et al., 1986; Garnier et al., 1978; Novotny et al., 1986). Predictions on hydrophilicity (Kyte and Doolittle, 1982) and surface probability (Emini et al., 1985) did not show any special features for this region of the A27 protein. Nevertheless, the mAb 2G8/1E4 against epitope #4 showed equally well binding affinities to all OPXV reference strains tested. Its neutralization capacity could be enhanced by the addition of complement. However, in previous studies the mAb 2G8/1E4 showed no neutralizing abilities against the tested ECTV M1 (Czerny et al., 1994). The discrepancy is caused by another test setup

including another OPXV strain (VACV Elstree instead of ECTV M1) and different cells (Vero cells instead of MA-cells) as well as by a 20-fold higher initial concentration of the antibody. The same was true for mAbs 3F5/2D5 and 1D5/2D11 (epitope complex #1 as mentioned further below).

Epitope #5 was the most highly conserved one in A27 as the motif 68-IEKC-71 was present in 389/391 aa sequences. In all three SkPXVs and 1/61 VACV sequences the epitope was shifted downstream to 93-IEKC-96. In 1/134 CPXV strains, the motif was 68-IEKY-71 while in another CPXV the epitope was missing because the C-terminus was truncated. The mAb 5B1/2G6 against epitope #5 was not neutralizing. However, the antigenic site is located in a functionally very important area within the C-terminus of the A27 protein. In this hydrophilic region, the two cysteines at positions 71 and 72 are responsible for formation of disulfide bonds and, therefore, play an important role for a functionally active trimeric A27 structure (Rodriguez and Esteban, 1987; Rodriguez et al., 1987). In 2D predictions of the secondary structure, a β-sheet (aa 58-83) followed by two β-turns (aa 70-75) was evident in this constant area. Two β-turns led generally to a high antigenicity (Chou and Fasman, 1979). Predictions on hydrophilicity (Kyte and Doolittle, 1982) and surface probability (Emini et al., 1985) did not reveal any special features. The mAb 5B1/2G6 showed similar binding affinities to all OPXV reference strains tested. This was expected because of the high sequence conservation of the targeted epitope among OPXV.

The most important antigenic region of the A27 protein was confined by aa 26-39. This has already been known from a previous investigation that identified functional domains in the A27 envelope protein (Vazquez and Esteban, 1999). In our present study, a complex of four closely related epitopes (#1A-D) could be allocated to this region. The narrow location of these epitopes has already been predicted previously from data obtained with

two overlapping oligopeptides (Meyer et al., 1994) and from quantitative competitive ELISAs performed with purified mAbs and viruses (Czerny et al., 1994). Two of the four mAbs binding to these epitopes were neutralizing in vitro. In this study, the four mAbs could enhance virus inhibition after adding complement. Other authors identified this region also as a strong target for binding of mAbs (Kaever et al., 2016). An epitope with a larger extension (aa 21-40) comprised the area of the epitope complex #1A-D completely (aa 26-39). However, those mAbs directed to this epitope region only neutralized in the presence of complement. According to 2-D structure predictions and published data (Massung et al., 1994; Meyer et al., 1994; Rodriguez and Esteban, 1987), the A27 region with the four epitopes #1A-D was classified as hydrophilic. Between aa residues 25 and 45 a hypervariable structure region was found. In case of VACV and VARV, it started with an α-helix up to aa 40, followed by two β-turns. In CPXV and CMLV, however, the α-helix changed at aa 37 into three and four β-turns, respectively. MPXV showed two β-turns at aa residues 25-34 followed by an α-helix up to aa 39 and three β-turns. In ECTV, the β-sheet structure was found up to aa 30, followed by an α-helix up to aa 37 and three β-turns. These highly variable structural conditions led to a significant species-specific difference in the overall structure of the investigated A27 proteins. Thus, the proteins of the species VACV, VARV and CMLV had a more linear form, while the proteins of the species MPXV, CPXV and ECTV were folded to a larger extent. Considering the epitope complex #1A-D, the aa main motif was 26-KKPEAKREAIVKAD-39. Based on GenBank database entries for 391 A27 protein sequences, this motif in the complete form (aa 26-39) was found in 210/391 OPXVs (68/69 VARV, 59/61 VACV, 26/26 BPXV, 2/2 HSPV, 2/2 RPXV, 51/134 CPXV, 2/3 TaPXV). The database entries for the 391 A27 protein sequences also indicated that this region could be defined as a very variable area with a lot of aa exchanges and structural differences. Affinity experiments showed, that the binding of the four mAbs to their

respective targets was different and obviously dependent on aa exchanges. The epitopes #1A and #1B were completely absent in MPXV and ECTV. Especially in case of MPXV, three aa exchanges led to the motif variation 26-KNPETKREAIVKAY-39, independent of the geographic distribution of isolates. In ECTVs, three different aa exchanges in comparison to VACV led to the motif to 26-KKPEDKHEATVKAD-39. In both OPXV genera, these aa exchanges were absolutely species-specific (57/57 MPXV; 14/14 ECTV). To investigate the direct influence of the exchanged amino acids on the binding of the corresponding mAbs, the epitope #1A was re-synthesized on a SPOTs membrane in the unchanged (VACV) and changed (MPXV and ECTV) design. The aa exchanges led unequivocally to the loss of mAb binding to its epitope #1A (Figure S4) which was confirmed by lack of binding to spots 488 and 489 on the OPXV microarray chip (Table S7). The variations between aa 26 and 39 also led to a significant structural change of the A27 protein homologs of MPXV and ECTV. The change of the aspartic acid at position 39 of MPXV to tyrosine, containing a benzene ring, was mainly responsible for the loss of the mAb reactivity. The binding site of the main immunogenic epitope #1A was defined as an octapeptide of 31-REAIVKAD-39, when the three decapeptides on the SPOTs membrane with the highest spot intensity (No. 11-13) were taken for epitope determination (Fig. S2). However, in all seven decapeptides (No. 10-16), even those with weaker reactions, used for the evaluation, it became clear that the tetrapeptide 35-IVKA-38 was the most important factor for binding of the mAb 5B4/2F2 (epitope 1A). It was apparent, that the VACV tetrapeptide 35-IVKA-38 was present in the CMLV variation 35-IIKA-38. If the CMLV specificity is referred to the whole region of epitope complex #1A-D, database analysis will reveal that the aa exchange V36I is unique for all 18/18 CMLVs, independent of their geographical origin (Africa, Asia), thereby leading to the two motifs 26-KKPEAKREAIIKAD-39 (17/18) and 26-KRPEAKREAIIKAD-39 (1/18). The most prominent but species-specifically conserved aa sequence difference in the

epitope complex #1A-D was found in New World OPXVs with the motifs 26-_KPEAKRKVVEKAD-39 in RCNV (1/1), 26-_KPEEKRKAVVKAE-39 in VPXV (1/1), and 26-KKPEEPVKRKVVKNKNKHKVVKAD-49 in SkPXV (3/3). In spite of the 4 aa exchanges in RCNV, the mAbs against epitopes #1B-D gave a weak signal on spot 490 of the OPXV microarray chip (Table S7), whereas the epitope #1A was not detected. In VPXV and SkPXV (spots 491 and 492), the epitope complex #1A-D could not be detected by any of the mAbs.

Because of the fact that the sequence differences in the A27 region, representing the epitope complex #1A-D, were species-specifically conserved, the Old World OPXVs, such as VARV, VACV, HSPV, RPXV, BPXV, ECTV, MPXV, CMLV, and TaPXV, as well as the New World OPXVs, like SkPXV, RCNV and VPXV, revealed a monophyletic character. The sequence variations in this area, however, were not species-specifically conserved in CPXVs, which is why this group was regarded as polyphyletic. This taxonomic arrangement was concordant with previous investigations, where CPXVs were classified into different clades based on whole genome analysis (Carroll et al., 2011; Dabrowski et al., 2013; Franke et al., 2017). According to the most recent findings, CPXVs were divided into four clades, CPXV-like 1, CPXV-like 2, VACV-like and VARV-like (Franke et al., 2017). In our study, we could identify seven CPXV variants when referring only to the amino acid sequences of the epitope complex #1A-D (Fig. 5).

The A27 protein was formerly categorized as a fusion protein (Gong et al., 1990; Rodriguez and Esteban, 1987; Rodriguez et al., 1985) and believed to mediate the direct fusion of virus and cytoplasm membranes (“fusion from without”) (Rodriguez and Esteban, 1987; Rodriguez et al., 1987). Hitherto, A27 is not settled to be a part of a fusion complex consisting of at least 11 different proteins (A16, A21, A28, F9, G3, G9, H2, J5,

L1, L5 and O3), being conserved in all OPXVs (Diesterbeck et al., 2018; Moss, 2012, 2016). Still, there is also evidence in the literature that the A27 and A17 proteins form a second fusion complex (Kochan et al., 2008), which was assigned to fusion proteins type I. Typical for type I viral fusion proteins is the presence of a coiled-coil structure (Vazquez et al., 1998), which is, beside the A27, also seen in influenza virus HA2 (Chen et al., 1995), Ebola GP2 (Dessen et al., 2000; Weissenhorn et al., 1998) and HIV gp41 (Skehel and Wiley, 1998). The authors suggested that the A17-A27-complex is transported to the cell membrane during viral replication and mediates fusion of the infected cells ("fusion from within"), meaning that A17 is the membrane-anchoring domain with the fusion peptide (aa 18-34) and A27 is responsible for the oligomerization as well as the membrane-attachment (Kochan et al., 2008). A27 binds to the GAG heparan sulfate of neighboring cells. This binding is mediated through the aa residues 26-KKPE-29 (Chang et al., 2013; Chung et al., 1998; Hsiao et al., 1998; Shih et al., 2009), resulting in an accumulation of cells in the immediate vicinity (Kochan et al., 2008). In several studies, mAbs against the A27 protein were able to block the "fusion from within" in a model described previously (Gong et al., 1990; Rodriguez and Esteban, 1987; Rodriguez et al., 1985). Therefore, we used this model to test inhibition of the fusion by three anti-A27-mAbs from our collection, whose antigenic sites were exactly mapped. Through low-pH treatment (Doms et al., 1990; Gong et al., 1990), we were able to induce fusion of VACV infected cells. This "fusion from within" was indicated by the formation of large and structureless fused cell areas known as syncytia (Gong et al., 1990). The mAb 3F5/2D5 directed to epitope #1C (aa 26-KKPEAK-31) was not able to block the fusion, although the GAG binding site being inside its epitope. Acid-induced syncytia were formed. By adding the mAb 5B4/2F2 directed to epitope #1A (aa 32-REAIVKAD-39) and binding just more to the C-terminus of the mAb 3F5/2D5, fusion could be inhibited. The reason for the inhibition is not clear at the moment. However, at least a steric hindrance of the

mAb could be ascertained. Moreover, antibodies may directly interfere with interactions by occupying binding sites or sterically hindering binding sites in close proximity. In addition, antibody binding affects protein conformation, and different antibodies have different effects on protein conformation that may alter distant interacting sites. The mAb 5B1/2G6 binding to the C-terminal epitope #5 (aa 68-71) failed to block the fusion by showing polykaryon formation, too. The epitope of this mAb is directly related to the binding site (aa 71-72) of the A26 fusion suppressor protein to the A27 protein, but there was no direct influence on the fusion event.

In summary, we mapped six antigenic sites on the A27 protein of VACV. This enabled us to interpret species-specific epitope variations and conservations of various OPXVs to get an impression of their phylogenic relationships. To elucidate structure function relationships in more detail, co-crystallization might be helpful for future investigations. Moreover, the data on antigenic sites for cross-reacting or monospecific neutralizing antibodies are of high relevance for target directed screening of human immunoglobulin libraries to generate specifically engineered human recombinant antibodies, which might help for controlling any future outbreak of zoonotic orthopoxviruses.

**ACKNOWLEDGEMENTS**

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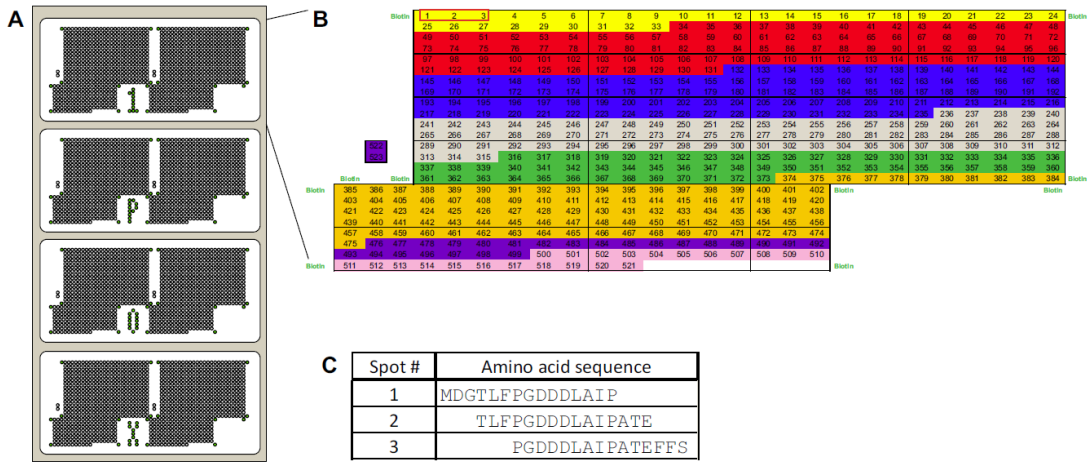
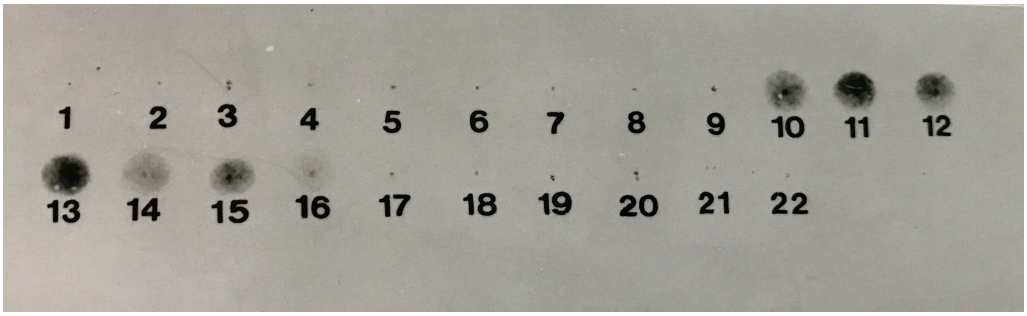
## SUPPLEMENTAL MATERIAL

### Supplemental figures




| Spot # | Amino acid sequence |
|---|---|
| 1 | MDGTLFPGDDDLAIP |
| 2 | TLFPGDDDLAIPATE |
| 3 | PGDDDLAIPATEFFS |

**FIG S1** Layout of the poxvirus microarray chip. A: The chip contains eight arrays arranged in the same manner. B: Arrangement of spots on each array: yellow is peptides representing A27 antigen of Vaccinia virus Western Reserve; red, D8; blue, H3; grey, L1; green, A33; orange, B5. The lower part of the array contains peptide sequence variations of A27 (lilac) and D8 (pink) antigens of the other orthopoxviruses. Sites of the sample deposition depicted by the plus symbol. The number serves as the batch identification and letters represent the chip name. C: Amino acid sequence of the first three peptides covering A27, corresponding to spots #1-#3 in B.




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptide Nr. 10: | E | A | K | R | E | A | I | V | K | A | | |
| Peptide Nr. 11: | | A | K | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **D** | |
| Peptide Nr. 12: | | | K | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **D** | E |
| Peptide Nr. 13: | | | | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **D** | E D |
| Peptide Nr. 14: | | | | | E | A | I | V | K | A | D | E D D |
| Peptide Nr. 15: | | | | | | A | I | V | K | A | D | E D D N |
| Peptide Nr. 16: | | | | | | | I | V | K | A | D | E D D N E |

**FIG S2** The 14 kDa A27 protein of VACV Copenhagen was synthesized on a SPOTs membrane as 101 decapeptides with 9 aa overlap to cover the whole sequence of 110 aa. By immunodetection with the mAb 5B4/2F2 seven spots were identified (No. 10-

16) to carry the target sequences spots 11-13 showed the strongest signals. The eight amino acids the three peptides had in common were represented to the sequence region 32-REAIVKAD-39. The minimal sequence essential for binding is 35-IVKA-38 and marked in red.

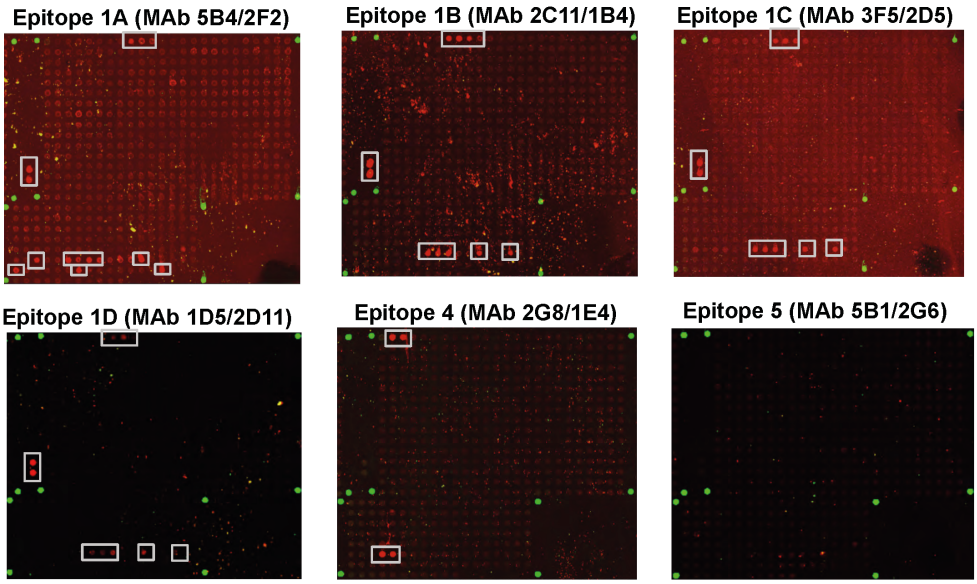



**FIG S3.** The OPXV microarray is based on 521 15-mer peptides overlapping by 12 aa. These peptides were spotted by SPOT technique on a chip. After immunodetection, the responding spots with the highest common intensity were chosen and outlined. Epitope #1A (mAb 5B4/2F2) was directed to a sequence region aa 31-KREAIVKAD-39. Epitopes #1B (mAb 2C11/1B4), #1C (mAb 3F5/2D5) and #1D (mAb 1D5/2D11) were all assigned to the aa region 28-PEAKRE-33. Epitope #4 (mAb 2G8/1E4) was allocated to aa 7-PGDDDLAIPATE-18. MAb 5B1/1A11 (epitope #5), however, did not react with any of the peptides on the chip.

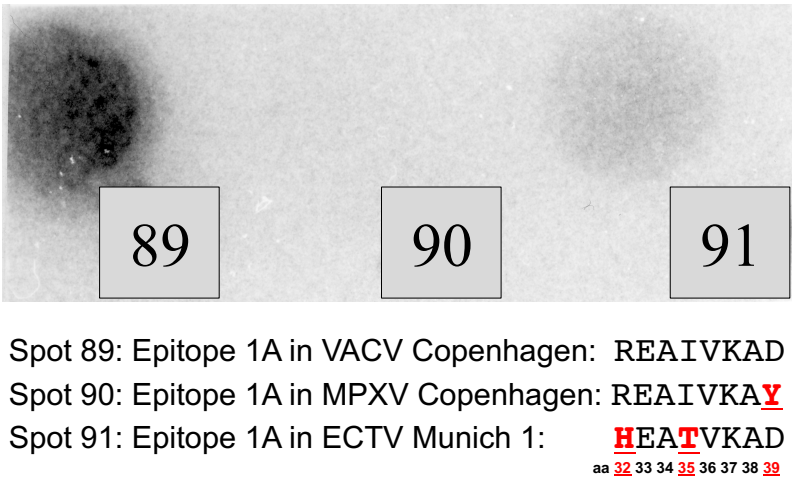



**FIG S4.** Immunological detection of ECTV- and MPXV-specific aa exchanges of the epitope #1A sequence on SPOTs membrane by the mAb 5B4/2F2. Spot 89: VACV epitope #1A (32-REAIVKAD-39). Spot 90: MPXV epitope #1A (32-REAIVKAY-39). Spot 91: ECTV epitope #1A (32-HEATVKAD-39). The aa exchanges led unequivocally to the loss of the binding of the mAb 5B4/2F2. The weak response on spot 91 was also observed with the secondary antibody used for detection.

## Supplemental tables

**Table S1** Reactivity of six A27-specific mAbs on the OPXV microarray chip. The amino acids marked in red are species-specific sequence variations to the VACV WR or epitope sequence exchanges.

| Spot ID | Spot Designation | Sequence Variations | Epitope 1A | Epitope 1B | Epitope 1C | Epitope 1D | Epitope 4 | Epitope 5 |
|---|---|---|---|---|---|---|---|---|
| | | | Epitope sequence according to SPOTs Membrane | | | | | |
| | | | REAIVKAD | PEAKRE | KKPEAK | PEAKREA | DDDLAI | IEKC |
| | | | Epitope sequence according to OPXV Microarray Chip | | | | | |
| | | | KREAIVKAD | PEAKRE | PEAKRE | PEAKRE | PGDDDLAIPATE | n.d. |
| 2 | VACV WR (AAO89429.1) | TLFPGDDDLAIPATE | | | | | | |
| 3 | | PGDDDLAIPATEFFS | | | | | | |
| 478 | VACV WR (AAO89429.1) | LFPGDDDLAIPATEF | | | | | | |
| 479 | SkPXV (AAQ72922.1)* | LFPGDDDMAIPATEF | | | | | | |
| 7 | VACV WR (AAO89429.1) | FFSTKAAKKPEAKRE | | | | | | |
| 8 | | TKAAKKPEAKREAIV | | | | | | |
| 9 | | AKKPEAKREAIVKAD | | | | | | |
| 10 | | PEAKREAIVKADEDD | | | | | | |
| 11 | | KREAIVKADEDDNEE | | | | | | |
| 481 | VARV (ABF26518.1) | AKKPEAKHEAIVKAD | | | | | | |
| 482 | VACV (AAA48152.1)* | DKKPEAKREAIVKAD | | | | | | |
| 522 | CMLV (CAA52999.1)* | AKKPEAKREAIIKAD | | | | | | |
| 483 | CPXV (AAP48887.1)* | AKKPEAKREAIVKAE | | | | | | |
| 484 | CPXV (AAP48888.1) | AKKPEAKREAFVKAE | | | | | | |
| 485 | CPXV (AAQ72894.1)* | AKKQEAKREAIVKAE | | | | | | |
| 486 | CPXV (AAQ72906.1) | AKKPEAKHEAIVKAE | | | | | | |
| 488 | ECTV (CAA53000.1)* | AKKPEDKHEATVKAD | | | | | | |
| 489 | MPXV (CAA52998.1)* | AKNPETKREAIVKAY | | | | | | |
| 490 | RCNV (ABD37609.1) | AKKPEAKRKVVEKAD | | | | | | |
| 491 | VPXV (ABD37608.1) | AKKPEEKRKAVVKAE | | | | | | |
| 492 | SkPXV (AAQ72922.1)* | AKKPEEPVKRKVVKN | | | | | | |
| 497 | VARV (CAA47501.1)* | TKFEQIEKCCKRNDE | | | | | | |

*Accession number is representative for several sequences of this type. All GenBank accession numbers can be found in Table S2. Different shades of grey represent strength of the fluorescence intensity. n.d.: not detected

Weak reaction
Strong reaction

**Table S2** Detection of the six A27 antigenic sites by the corresponding anti-OPXV-mAbs *via* SPOTs-membrane and OPXV peptide microarray chip.

| **Epitope** | **Epitope sequences determined on SPOTs-membrane:** | | **Epitope sequence determined on OPXV peptide micro-array:** | |
|---|---|---|---|---|
| | **AA position** | **AA composition** | **AA position** | **AA composition** |
| 1A | 32-39 | **REAIVKAD** | 31-39 | K**REAIVKAD** |
| 1B | 28-33 | **PEAKRE** | 28-33 | **PEAKRE** |
| 1C | 26-31 | KK**PEAK** | 28-33 | **PEAK**RE |
| 1D | 28-34 | **PEAKRE**A | 28-33 | **PEAKRE** |
| 4 | 9-14 | **DDDLAI** | 7-18 | PG**DDDLAI**PATE |
| 5 | 68-71 | IEKC | - | Not detected |

The matches within the epitope sequence are highlighted and underlined.

**Table S3** Mapping of epitope #4 based on 391 complete and partial amino acid sequences from the NCBI GenBank database.

| **Linear A27 epitope aa 9-14** | **OPXV genera** | **Number of DB entries** |
|---|---|---|
| DDDLAI | VARV major | 66/67 |
| | VARV minor | 2/2 |
| | VACV | 60/61 |
| | BPXV | 23/26 |
| | HSPV | 2/2 |
| | RPXV | 2/2 |
| | CMLV | 17/18 |
| | CPXV | 128/134 |
| | ECTV | 13/14 |
| | MPXV | 57/57 |
| | TaPXV | 2/3 |
| DDDLA**T** | BPXV | 3/26 |
| DDD**M**AI | SkPXV | 3/3 |
| **N-Terminus truncated** | VARV major | 1/67 |
| | CMLV | 1/18 |
| | CPXV | 6/133 |
| | ECTV | 1/14 |
| | TaPXV | 1/3 |
| | RCNV | 1/1 |
| | VPXV | 1/1 |
| **RARSPR** | VACV | 1/61 |

Differences within the epitope sequence are highlighted.

**Table S4** 391 amino acid sequences of the OPXV A27 protein homologs available so far in the GenBank, being analyzed with respect to species-specific conservation or variation of the six sequential antigenic sites mapped.

| OPXV Species | OPXV Strain/ *Isolate* | Number | ID Protein | Protein Sequence | Length | Source | Published |
|---|---|---|---|---|---|---|---|
| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| BPXV | *Aur04* | 1 | AAZ42170.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ117952.1 | 9/22/2006 |
| BPXV | *BP4* | 2 | AAZ42169.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ117951.1 | 9/22/2006 |
| BPXV | *Ind99* | 3 | AAZ42172.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ117954.1 | 9/22/2006 |
| BPXV | *Vij96* | 4 | AAZ42171.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ117953.1 | 9/22/2006 |
| BPXV | *Buffalopox virus clone 1* | 5 | ACR15809.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | FJ748495.1 | 5/23/2009 |
| BPXV | *Buffalopox virus clone 2* | 6 | ACR15810.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | FJ748496.1 | 5/23/2009 |
| BPXV | *Pune/09* | 7 | ACZ06589.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLESHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ443265.1 | 11/30/2009 |
| BPXV | *Pune/H/09* | 8 | ACZ06590.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ443266.1 | 11/30/2009 |
| BPXV | *SP69 Gujarat/08* | 9 | ACZ28777.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHVETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ464367.1 | 11/30/2009 |
| BPXV | *SP70 Hyderabad/08* | 10 | ACZ28778.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ464368.1 | 11/30/2009 |
| BPXV | *SP66 Pune/08* | 11 | ACZ28776.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ464366.1 | 11/30/2009 |
| BPXV | *Bareilly/99* | 12 | AEA02828.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008906.1 | 3/31/2011 |
| BPXV | *Bareilly/00* | 13 | AEA02829.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008907.1 | 3/31/2011 |
| BPXV | *Bangalore/03* | 14 | AEA02836.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AEA02836.1 | 3/31/2011 |
| BPXV | *Bangalore/09* | 15 | AEA02832.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008910.1 | 3/31/2011 |
| BPXV | *Vij/97* | 16 | AEA02830.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008908.1 | 3/31/2011 |
| BPXV | *Krisna/07* | 17 | AEA02827.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008905.1 | 3/31/2011 |
| BPXV | *Nel/06* | 18 | AEA02826.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008904.1 | 3/31/2011 |
| BPXV | *Pune 1/04* | 19 | AEA02824.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008902.1 | 3/31/2011 |
| BPXV | *Pune 2/04* | 20 | AEA02825.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008903.1 | 3/31/2011 |
| BPXV | *Pune/06* | 21 | AEA02831.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHVETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008909.1 | 3/31/2011 |
| BPXV | *Pune/07* | 22 | AEA02835.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008913.1 | 3/31/2011 |
| BPXV | *Karachi 2005* | 23 | AVO21155.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | MG599038.1 | 3/21/2018 |
| BPXV | *SP56 BPXV Ezatnagar/08* | 24 | ACZ28775.1 | MDGTLFPG**DDDLAT**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ464365.1 | 11/30/2009 |
| BPXV | *Pune/milk* | 25 | AEA02833.1 | MDGTLFPG**DDDLAT**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008911.1 | 3/31/2011 |
| BPXV | *Hyd 17/04* | 26 | AEA02834.1 | MDGTLFPG**DDDLAT**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM008912.1 | 3/31/2011 |
| | | | | | | | |
| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| CMLV | CMS | 1 | AAG37638.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY009089.1 | 7/30/2002 |
| CMLV | Cp-1 | 2 | CAA52999.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | X75156.1 | 7/31/2002 |
| CMLV | *M-96* | 3 | AAL73853.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AF438165.1 | 6/16/2003 |
| CMLV | Cp-1 | 4 | AAP48881.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY223496.1 | 5/12/2004 |
| CMLV | NIGER | 5 | AAQ72915.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY299081.1 | 6/30/2004 |
| CMLV | SAUDI | 6 | AAQ72916.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY299082.1 | 6/30/2004 |
| CMLV | MAURETANIA | 7 | AAQ72917.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY299083.1 | 6/30/2004 |
| CMLV | CP-14 | 8 | AAQ72918.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY299084.1 | 6/30/2004 |
| CMLV | CP-5 | 9 | AAQ72919.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY299085.1 | 6/30/2004 |
| CMLV | CP-202/95H | 10 | AAQ72920.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY299086.1 | 6/30/2004 |
| CMLV | CP-1260 | 11 | AAQ72921.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | AY299087.1 | 6/30/2004 |
| CMLV | *DEL09* | 12 | ACV88142.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ465930.1 | 8/16/2011 |
| CMLV | *BAR09* | 13 | ACV88140.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMVSLAKKIDVQTGRRPYE | 110 | GQ465928.1 | 8/16/2011 |
| CMLV | *JSL09* | 14 | ACV88141.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ465929.1 | 8/16/2011 |
| CMLV | *Al-Ahsaa* | 15 | AEX54892.1 | **DLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN805951.1 | 1/15/2012 |
| CMLV | *M-96* | 16 | NP_570536.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | NC_003391.1 | 11/22/2012 |
| CMLV | 0408151v | 17 | AKU40516.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYDNLTLLFN | 117 | KP768318.1 | 8/9/2015 |
| CMLV | *BAR09* | 18 | ACV88139.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**K**K**PEAKREAI**I**KAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | GQ465927.1 | 8/16/2011 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| CPXV | GRI-90 | 1 | CAB11758.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99060.1 | 3/19/2003 |
| CPXV | 98/5 | 2 | AAP48885.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223500.1 | 5/12/2004 |
| CPXV | EP-5 | 3 | AAP48882.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223497.1 | 5/12/2004 |
| CPXV | EXP-GR | 4 | AAP48884.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223499.1 | 5/12/2004 |
| CPXV | Revin | 5 | AAP48883.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223498.1 | 5/12/2004 |
| CPXV | EP-1 | 6 | AAQ72874.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299040.1 | 6/30/2004 |
| CPXV | EP-3 | 7 | AAQ72877.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299043.1 | 6/30/2004 |
| CPXV | EP-5 | 8 | AAQ72876.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299042.1 | 6/30/2004 |

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| CPXV | EP-7 | 9 | AAQ72878.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299044.1 | 6/30/2004 |
| CPXV | EP-8 | 10 | AAQ72879.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299045.1 | 6/30/2004 |
| CPXV | 28-00 | 11 | AAQ72880.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299046.1 | 6/30/2004 |
| CPXV | FINNLAND | 12 | AAQ72914.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299080.1 | 6/30/2004 |
| CPXV | OPV-89-1 | 13 | AAQ72887.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299053.1 | 6/30/2004 |
| CPXV | OPV-98-2 | 14 | AAQ72881.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299047.1 | 6/30/2004 |
| CPXV | OPV-99-1 | 15 | AAQ72882.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299048.1 | 6/30/2004 |
| CPXV | RAT-MOSCOW | 16 | AAQ72875.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299041.1 | 6/30/2004 |
| CPXV | GRI-90 | 17 | CAD90695.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X94355.2 | 4/18/2005 |
| CPXV | Austria 1999 | 18 | ADZ24156.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ407377.1 | 8/9/2011 |
| CPXV | Finland_2000_MAN | 19 | ADZ29269.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420893.1 | 8/9/2011 |
| CPXV | Germany_1998_2 | 20 | ADZ30126.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420897.1 | 8/9/2011 |
| CPXV | HumGri07/1 | 21 | AGZ01459.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813511.1 | 11/3/2013 |
| CPXV | EleGri07/1 | 22 | AGZ00616.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813507.1 | 11/3/2013 |
| CPXV | HumAac09/1 | 23 | AGZ00825.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEK_ITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 109 | KC813508.1 | 11/3/2013 |
| CPXV | HumGra07/1 | 24 | AGZ01246.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813510.1 | 11/3/2013 |
| CPXV | HumKre08/1 | 25 | AGZ01668.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813512.1 | 11/3/2013 |
| CPXV | HumLit08/1 | 26 | AGY97627.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813493.1 | 11/3/2013 |
| CPXV | RatAac09/1 | 27 | AGY99343.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813501.1 | 11/3/2013 |
| CPXV | RatGer09/1 | 28 | AGY99763.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813503.1 | 11/3/2013 |
| CPXV | RatKre08/2 | 29 | AGZ00194.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813505.1 | 11/3/2013 |
| CPXV | *CEPAD332* | 30 | AHB89673.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC592404.1 | 12/28/2013 |
| CPXV | *CEPAD333* | 31 | AHB89677.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC592406.1 | 12/28/2013 |
| CPXV | *CEPAD336* | 32 | AHB89674.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC592405.1 | 12/28/2013 |
| CPXV | RatPox09 | 33 | CRL86650.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LN864565.1 | 9/3/2015 |
| CPXV | *Ger/2017/Alpaca2* | 34 | SPQ84369.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896732.2 | 6/15/2018 |
| CPXV | *Ger/2015/Human2* | 35 | SPN69117.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT993232.1 | 6/15/2018 |
| CPXV | *Ger/2015/Prairie-dog* | 36 | SPN68837.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT993231.1 | 6/15/2018 |
| CPXV | *Ger/2017/Alpaca1* | 37 | SPN68556.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT993230.1 | 6/15/2018 |
| CPXV | *Ger/2017/common vole FMEtmka* | 38 | SPN68277.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT993228.1 | 6/15/2018 |
| CPXV | *Ger/2007/Vole* | 39 | SNB49364.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896722.1 | 6/15/2018 |
| CPXV | *89Lcow* | 40 | ABD36429.1 | **KPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEV | 52 | DQ385058.1 | 7/26/2016 |
| CPXV | *A280* | 41 | ABD37539.1 | **KPEAKREAIVKAD**GDENEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEV | 52 | DQ374558.1 | 7/26/2016 |
| CPXV | Kostroma_2015 | 42 | AQQ13014.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY369926.1 | 2/20/2017 |
| CPXV | Germany_1971_EP1 | 43 | ARB50380.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY463519.1 | 4/2/2017 |
| CPXV | *Ger/2017/Alpaca2* | 44 | SNB49500.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896732.1 | 6/23/2017 |
| CPXV | *Ger/2012/Alpaca* | 45 | SNB50568.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896726.1 | 6/15/2018 |
| CPXV | *Ger/2015/Human1* | 46 | SNB53463.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896720.1 | 6/15/2018 |
| CPXV | *Ger/2015/Cat2* | 47 | SNB69057.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896727.1 | 6/15/2018 |
| CPXV | *Ger/2015/Cat3* | 48 | SNB51754.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896733.1 | 6/15/2018 |
| CPXV | *Ger/2015/Cat4* | 49 | SNB50797.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896731.1 | 6/15/2018 |
| CPXV | *Ger/2014/Cat2* | 50 | SNB56801.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896725.1 | 6/15/2018 |
| CPXV | *Ger/2010/Cat* | 51 | SNB50433.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896729.1 | 6/15/2018 |
| CPXV | *Ger/2010/Racoon* | 52 | SNB48328.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896730.1 | 6/15/2018 |
| CPXV | *Ger 2010 MKY* | 53 | SNB54163.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDENEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLESHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896721.1 | 6/15/2018 |
| CPXV | Brighton | 54 | CAA53001.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X75158.1 | 7/31/2002 |
| CPXV | Hamburg-1985 | 55 | CAB11760.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLREAMISLAKEIDVQTGRRPYE | 110 | Z99062.1 | 3/19/2003 |
| CPXV | Brighton | 56 | AAP48887.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223502.1 | 5/12/2004 |
| CPXV | EP-2 | 57 | AAP48889.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223504.1 | 5/12/2004 |
| CPXV | EP-4 | 58 | AAP48886.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223501.1 | 5/12/2004 |
| CPXV | OPV-89-3 | 59 | AAQ72892.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299058.1 | 6/30/2004 |
| CPXV | OPV-89-5 | 60 | AAQ72886.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299052.1 | 6/30/2004 |
| CPXV | OPV-90-1 | 61 | AAQ72883.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299049.1 | 6/30/2004 |
| CPXV | OPV-90-2 | 62 | AAQ72890.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299056.1 | 6/30/2004 |
| CPXV | OPV-90-3 | 63 | AAQ72904.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299070.1 | 6/30/2004 |
| CPXV | OPV-90-4 | 64 | AAQ72905.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299071.1 | 6/30/2004 |
| CPXV | OPV-90-5 | 65 | AAQ72903.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299069.1 | 6/30/2004 |
| CPXV | OPV-91-1 | 66 | AAQ72885.1 | MDGTLFPGD**DDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299051.1 | 6/30/2004 |
| CPXV | OPV-91-3 | 67 | AAQ72884.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299050.1 | 6/30/2004 |
| CPXV | OPV-91-4 | 68 | AAQ72898.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299064.1 | 6/30/2004 |
| CPXV | OPV-98-1 | 69 | AAQ72893.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKA**EGDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299059.1 | 6/30/2004 |

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| CPXV | OPV-98-3 | 70 | AAQ72909.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299075.1 | 6/30/2004 |
| CPXV | OPV-98-4 | 71 | AAQ72910.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299076.1 | 6/30/2004 |
| CPXV | SWEDEN1 | 72 | AAQ72899.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299065.1 | 6/30/2004 |
| CPXV | SWEDEN2 | 73 | AAQ72901.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299067.1 | 6/30/2004 |
| CPXV | NANCY | 74 | AAQ72908.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299074.1 | 6/30/2004 |
| CPXV | NW-MAN | 75 | AAQ72889.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299055.1 | 6/30/2004 |
| CPXV | OPV-88H | 76 | AAQ72891.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299057.1 | 6/30/2004 |
| CPXV | EP-RIEMS | 77 | AAQ72911.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299077.1 | 6/30/2004 |
| CPXV | EP-6 | 78 | AAQ72907.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299073.1 | 6/30/2004 |
| CPXV | BIBER | 79 | AAQ72912.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299078.1 | 6/30/2004 |
| CPXV | CATPOX3 | 80 | AAQ72902.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299068.1 | 6/30/2004 |
| CPXV | CATPOX5 | 81 | AAQ72897.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299063.1 | 6/30/2004 |
| CPXV | NW-CAT | 82 | AAQ72888.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299054.1 | 6/30/2004 |
| CPXV | 780 | 83 | AAQ72913.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299079.1 | 6/30/2004 |
| CPXV | Brighton Red | 84 | AAM13604.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AF482758.2 | 8/1/2006 |
| CPXV | Brighton Red | 85 | AF482758.2 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AF482758.2 | 8/1/2006 |
| CPXV | Germany 91-3 | 86 | ABD97498.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437593.1 | 8/19/2006 |
| CPXV | Calpox | 87 | ADW95399.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ891540.1 | 2/12/2011 |
| CPXV | France_2001_Nancy | 88 | ADZ29482.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420894.1 | 8/9/2011 |
| CPXV | Germany_1980_EP4 | 89 | ADZ29697.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420895.1 | 8/9/2011 |
| CPXV | Germany_1990_2 | 90 | ADZ29910.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420896.1 | 8/9/2011 |
| CPXV | Germany_2002_MKY | 91 | ADZ30340.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420898.1 | 8/9/2011 |
| CPXV | Norway_1994_MAN | 92 | ADZ30551.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420899.1 | 8/9/2011 |
| CPXV | RatHei09/1 | 93 | AGY99977.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813504.1 | 11/3/2013 |
| CPXV | HumLan08/1 | 94 | AGY97413.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813492.1 | 11/3/2013 |
| CPXV | HumLue09/1 | 95 | AGY97847.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813494.1 | 11/3/2013 |
| CPXV | HumMag07/1 | 96 | AGY98067.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813495.1 | 11/3/2013 |
| CPXV | HumPad07/1 | 97 | AGY98272.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813496.1 | 11/3/2013 |
| CPXV | JagKre08/2 | 98 | AGY98704.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813498.1 | 11/3/2013 |
| CPXV | MarLei07/1 | 99 | AGY98923.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813499.1 | 11/3/2013 |
| CPXV | MonKre08/4 | 100 | AGY99134.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813500.1 | 11/3/2013 |
| CPXV | HumBer07/1 | 101 | AGZ01034.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813509.1 | 11/3/2013 |
| CPXV | BeaBer04/1 | 102 | AGY97197.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813491.1 | 11/3/2013 |
| CPXV | CatBer07/1 | 103 | AGY99547.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813502.1 | 11/3/2013 |
| CPXV | CatPot07/1 | 104 | AGZ00407.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813506.1 | 11/3/2013 |
| CPXV | JagKre08/1 | 105 | AGY98488.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC813497.1 | 11/3/2013 |
| CPXV | *CEPAD335* | 106 | AHB89678.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMIPLAKKIDVQTGRRPYQ | 110 | KC592407.1 | 12/28/2013 |
| CPXV | FM2292 | 107 | CRL86944.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LN864566.1 | 9/3/2015 |
| CPXV | Brighton Red | 108 | NP_619946.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | NC_003663.2 | 1/28/2016 |
| CPXV | *A220* | 109 | ABD37523.1 | **KPEAKREAIVKAE**GDDNEETFKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEV | 52 | DQ374542.1 | 7/26/2016 |
| CPXV | *A247* | 110 | ABD37531.1 | **KPEAKREAIVKAE**GDENEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEV | 52 | DQ374550.1 | 7/26/2016 |
| CPXV | CPXV Amadeus 2015 | 111 | CUI02420.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LN879483.1 | 7/29/2016 |
| CPXV | *CPXV_1639* | 112 | ARR30529.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY549148.1 | 5/21/2017 |
| CPXV | *CPXV_Catpox5wv1* | 113 | ARR29908.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY549144.1 | 5/21/2017 |
| CPXV | *Norwayfeline* | 114 | ARR31101.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY549151.1 | 5/21/2017 |
| CPXV | *Ger/2014/Cat1* | 115 | SNB57931.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896723.1 | 6/15/2018 |
| CPXV | *Ger/2010/Alpaca* | 116 | SNB50383.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896718.1 | 6/15/2018 |
| CPXV | *Ger/2010/Rat* | 117 | SNB52980.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLINLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896728.1 | 6/23/2017 |
| CPXV | *Ger/2013/Alpaca* | 118 | SNB48988.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896719.1 | 6/15/2018 |
| CPXV | *Ger/2014/Human* | 119 | SPN67997.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT993226.1 | 6/15/2018 |
| CPXV | 427 | 120 | AAQ72894.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299060.1 | 6/30/2004 |
| CPXV | 428 | 121 | AAQ72896.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299062.1 | 6/30/2004 |
| CPXV | 2739 | 122 | AAQ72895.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299061.1 | 6/30/2004 |
| CPXV | UK2000_K2984 | 123 | ADZ30763.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ420900.1 | 8/9/2011 |
| CPXV | *A009* | 124 | ABD37458.1 | **KQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEV | 110 | DQ374477.1 | 7/26/2016 |
| CPXV | *CPXV_K428* | 125 | ARR30112.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY549145.1 | 5/21/2017 |
| CPXV | *CPXV_K2739* | 126 | ARR30729.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY549149.1 | 5/21/2017 |
| CPXV | *CPXV_K4207* | 127 | ARR30930.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY549150.1 | 5/21/2017 |
| CPXV | *CPXV_Catpox3L97* | 128 | ARR29711.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKQEAKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY549143.1 | 5/21/2017 |
| CPXV | Turkmenia-1974 | 129 | CAB11761.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KRPEAKREAIVKAE**GDDNEETLKQRLTNLEKKITIITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99063.1 | 3/19/2003 |
| CPXV | *Ger/2015/Cat1* | 130 | SNB48607.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEVKREAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | LT896724.1 | 6/15/2018 |

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| CPXV | OPV-89-2 | 131 | AAQ72906.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKHEAIVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299072.1 | 6/30/2004 |
| CPXV | 89/4 | 132 | AAP48888.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAFVKAE**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223503.1 | 5/12/2004 |
| CPXV | SWEDEN111 | 133 | AAQ72900.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KSQRLNAKQLLKLR**EMTMKKLSNND | 50 | AY299066.1 | 6/30/2004 |
| CPXV | *A279* | 134 | ABD37537.1 | **KPEAKREAIVKAD**GDENEETLKQRLTNLEKKITNVTTKFEQ**IEKY**CKRNDEV | 52 | DQ374556.1 | 7/26/2016 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| ECTV | Munich-1 | 1 | CAA53000.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X75157.1 | 7/31/2002 |
| ECTV | Moscow | 2 | AAM92434.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AF012825.2 | 8/6/2002 |
| ECTV | K-1 (Ect 3) | 3 | CAB11753.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99055.1 | 3/19/2003 |
| ECTV | MP-2 | 4 | AAQ72857.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299023.1 | 6/30/2004 |
| ECTV | C99-505 | 5 | AAQ72860.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299026.1 | 6/30/2004 |
| ECTV | MP-5 | 6 | AAQ72858.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299024.1 | 6/30/2004 |
| ECTV | MP-33221 | 7 | AAQ72859.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299025.1 | 6/30/2004 |
| ECTV | SILBERFUCHS | 8 | AAQ72856.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299022.1 | 6/30/2004 |
| ECTV | MPV-CC | 9 | AAX73202.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY973172.1 | 4/11/2005 |
| ECTV | Moscow | 10 | NP_671648.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | NC_004105.1 | 11/22/2012 |
| ECTV | ERPV | 11 | AFH54695.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JQ410350.1 | 1/24/2014 |
| ECTV | *NAVAL* | 12 | AIF30214.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KJ563295.1 | 5/11/2015 |
| ECTV | *Hamptead* | 13 | AUO16292.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY554976.1 | 1/16/2018 |
| ECTV | Ectromelia virus | 14 | ABA61050.1 | **KPEDKHEATVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEV | 52 | DQ178390.1 | 7/26/2016 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| HSPV | *MNR-76* | 1 | ABH08257.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ792504.1 | 8/30/2006 |
| HSPV | MNR | 2 | AUD55313.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY349117.1 | 7/19/2018 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| MPXV | Copenhagen | 1 | CAA52998.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X75155.1 | 7/31/2002 |
| MPXV | Zaire 79 | 2 | AAN78220.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKXNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY160186.1 | 3/18/2003 |
| MPXV | Sierra Leone 70-0666 | 3 | CAB11763.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99065.1 | 3/19/2003 |
| MPXV | *DRC 07-0093* | 4 | AGR36218.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878416.1 | 1/14/2004 |
| MPXV | CDC#v70-I-187 | 5 | AAP48866.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | AY223481.1 | 5/12/2004 |
| MPXV | CDC#v78-I-3945 | 6 | AAP48865.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | AY223480.1 | 5/12/2004 |
| MPXV | CDC#v79-I-005 | 7 | AAP48864.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223479.1 | 5/12/2004 |
| MPXV | CDC#v97-I-004 | 8 | AAP48863.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223478.1 | 5/12/2004 |
| MPXV | Congo 8 | 9 | AAP48862.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223477.1 | 5/12/2004 |
| MPXV | AP-4 | 10 | AAQ72865.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299031.1 | 6/30/2004 |
| MPXV | AP-2 | 11 | AAQ72866.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299032.1 | 6/30/2004 |
| MPXV | AP-6 | 12 | AAQ72867.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | AY299033.1 | 6/30/2004 |
| MPXV | AP-5 | 13 | AAQ72868.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | AY299034.1 | 6/30/2004 |
| MPXV | MPXV-WRAIR7-61; Walter Reed 267 | 14 | AAU01342.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | AY603973.1 | 9/2/2005 |
| MPXV | *Sierra Leone MPXV-SL-132* | 15 | AAW67890.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | AY741551.1 | 9/7/2005 |
| MPXV | COP-58 | 16 | AAX09233.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | AY753185.1 | 9/7/2005 |
| MPXV | Congo_2003_358 | 17 | AAY97141.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ011154.1 | 9/28/2005 |
| MPXV | Liberia_1970_184 | 18 | AAY97540.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | DQ011156.1 | 9/28/2005 |
| MPXV | USA_2003_039 | 19 | AAY97739.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | DQ011157.1 | 9/28/2005 |
| MPXV | USA_2003_044 | 20 | AAY96941.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ011153.1 | 9/28/2005 |
| MPXV | Zaire_1979-005 | 21 | AAY97342.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ011155.1 | 9/28/2005 |
| MPXV | Zaire-96-I-16 | 22 | AAL40597.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AF380138.1 | 3/4/2009 |
| MPXV | Zaire 1979-005 | 23 | ADK39167.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HM172544.1 | 7/25/2010 |
| MPXV | D14L knockout | 24 | ADX22985.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKXNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ857563.1 | 9/2/2011 |
| MPXV | V79-I-005 | 25 | ADX22789.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKXNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | HQ857562.1 | 9/2/2011 |
| MPXV | Zaire-96-I-16 | 26 | NP_536566.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | NC_003310.1 | 8/28/2012 |
| MPXV | Sudan 2005_01 | 27 | AGF36698.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC257459.1 | 2/20/2013 |
| MPXV | DRC Yandongi 1985 | 28 | AGF36905.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC257460.1 | 2/20/2013 |
| MPXV | *DRC 06-0950* | 29 | AGR34500.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878407.1 | 1/14/2014 |
| MPXV | *DRC 06-0970* | 30 | AGR34690.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878408.1 | 1/14/2014 |
| MPXV | *DRC 06-1070* | 31 | AGR35072.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878410.1 | 1/14/2014 |
| MPXV | *DRC 07-0120* | 32 | AGR36600.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878418.1 | 1/14/2014 |
| MPXV | *DRC 06-0999* | 33 | AGR34881.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878409.1 | 1/14/2014 |
| MPXV | *DRC 06-1075* | 34 | AGR35263.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878411.1 | 1/14/2014 |
| MPXV | *DRC 06-1076* | 35 | AGR35454.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878412.1 | 1/14/2014 |

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| MPXV | *DRC 07-0045* | 36 | AGR35645.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878413.1 | 1/14/2014 |
| MPXV | *DRC 07-0046* | 37 | AGR35836.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878414.1 | 1/14/2014 |
| MPXV | *DRC 07-0092* | 38 | AGR36027.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878415.1 | 1/14/2014 |
| MPXV | *DRC 07-0104* | 39 | AGR36409.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878417.1 | 1/14/2014 |
| MPXV | *DRC 07-0275* | 40 | AGR36791.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878419.1 | 1/14/2014 |
| MPXV | *DRC 07-0283* | 41 | AGR36982.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878420.1 | 1/14/2014 |
| MPXV | *DRC 07-0286* | 42 | AGR37173.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878421.1 | 1/14/2014 |
| MPXV | *DRC 07-0287* | 43 | AGR37364.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878422.1 | 1/14/2014 |
| MPXV | *DRC 07-0337* | 44 | AGR37555.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878423.1 | 1/14/2014 |
| MPXV | *DRC 07-0338* | 45 | AGR37745.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878424.1 | 1/14/2014 |
| MPXV | *DRC 07-0354* | 46 | AGR37935.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878425.1 | 1/14/2014 |
| MPXV | *DRC 07-0450* | 47 | AGR38125.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878426.1 | 1/14/2014 |
| MPXV | *DRC 07-0480* | 48 | AGR38316.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878427.1 | 1/14/2014 |
| MPXV | *DRC 07-0514* | 49 | AGR38507.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878428.1 | 1/14/2014 |
| MPXV | *DRC 07-0662* | 50 | AGR38698.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX878429.1 | 1/14/2014 |
| MPXV | Ivory Coast 2012 | 51 | n.a. | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKHNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KJ136820.1 | 5/29/2014 |
| MPXV | UTC | 52 | AIE40576.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | KJ642614.1 | 5/11/2015 |
| MPXV | W-Nigeria | 53 | AIE40752.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | KJ642615.1 | 5/11/2015 |
| MPXV | PCH | 54 | AIE40930.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | KJ642616.1 | 5/11/2015 |
| MPXV | Nigeria-SE-1971 | 55 | AIE41105.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | KJ642617.1 | 5/11/2015 |
| MPXV | *Cote d'Ivoire_1971* | 56 | AKG51301.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | KP849470.1 | 5/13/2015 |
| MPXV | *MPXV_Nig_2017_297957* | 57 | AUW64219.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KNPETKREAIVKAY**GDDNEETLKQRLTNLEKKITNITTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | MG693723.1 | 2/6/2018 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| RPXV | Rabbitpox virus Uttrecht | 1 | CAB11757.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99059.1 | 3/19/2003 |
| RPXV | Rabbitpox virus | 2 | AAS49848.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY484669.1 | 10/21/2005 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| RCNV | Herman | 1 | AKJ93776.1 | MEGTLFPG**DDDIAI**PATEFFVNKAA**KKPEKPAKRKVVKAD**DAEEKADEEEDAEEDIKGRLTNLEKKITNVTTKFAQ**IEKC**CKRNDEVLFRLENHAETLRVAMLSLAKKIDIQTGRQRYE | 119 | KP143769.1 | 6/10/2015 |
| RCNV | Raccoonpoxvirus | 2 | ABD37609.1 | **KPEAKRKVVEKAD**EEEDEEDIKGRLTNLEKKITNVTTKFAQ**IEKC**CKRNDEV | 52 | DQ374628.1 | 7/26/2016 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| SkPXV | SKUNK | 1 | AAQ72922.1 | MDGTLFPG**DDDMAI**PATEFFVTRAA**KKPEEPVKRKVVKNKNKHKVVKAD**GEDDPDEDDEDDDDEEDDDAEETIKHRLTNLEKKITNVTTKFAQ**IEKC**CKRNDEVLFRLENHAETLRAAMLTLAKKIDVQTGRQRYE | 136 | AY299088.1 | 6/30/2004 |
| SkPXV | WA | 2 | YP_009282840.1 | MDGTLFPG**DDDMAI**PATEFFVTRAA**KKPEEPVKRKVVKNKNKHKVVKAD**GEDDPDEDDEDDDDEEDDDAEETIKHRLTNLEKKITNVTTKFAQ**IEKC**CKRNDEVLFRLENHAETLRAAMLTLAKKIDVQTGRQRYE | 136 | NC_031038.1 | 9/23/2016 |
| SkPXV | WA | 3 | AOP31625.1 | MDGTLFPG**DDDMAI**PATEFFVTRAA**KKPEEPVKRKVVKNKNKHKVVKAD**GEDDPDEDDEDDDDEEDDDAEETIKHRLTNLEKKITNVTTKFAQ**IEKC**CKRNDEVLFRLENHAETLRAAMLTLAKKIDVQTGRQRYE | 136 | KU749310.1 | 9/21/2016 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| TaPXV | Dahomey 1968 | 1 | YP_717458.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GYDNEETLKQRLTNLEKKITNVTIKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | NC_008291.1 | 8/19/2006 |
| TaPXV | Dahomey 1968 | 3 | ABD97717.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GYDNEETLKQRLTNLEKKITNVTIKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRHPYE | 110 | DQ437594.1 | 8/19/2006 |
| TaPXV | Taterapox virus | 3 | ABD37615.1 | **KPEAKREAIVKAD**GYDNEETLKQRLTNLEKKITNVTIKFEQ**IEKC**CKRNDEV | 52 | DQ374634.1 | 7/26/2016 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| VACV | Vaccinia virus | 1 | AAA48248.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | M18173.1 | 8/3/1993 |
| VACV | Tian Tan | 2 | AAF34032.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYG | 110 | AF095689.1 | 2/14/2000 |
| VACV | LIVP-1 | 3 | CAB11755.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRHMSNLTLLLIKSIFKK | 123 | Z99057.1 | 3/19/2003 |
| VACV | COP-2 | 4 | CAB11754.1 | MDGTLFPG**DDDLAI**PATEFFSTKAD**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99056.1 | 3/19/2003 |
| VACV | LIVP-2 | 5 | CAB11756.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**VDDNEETLKQRLTNLEKKITNVTTKFKQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99058.1 | 3/19/2003 |
| VACV | Wyeth | 6 | CAB11762.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Z99064.1 | 3/19/2003 |
| VACV | Ankara | 7 | AAB96472.1 | MDGTLFPG**DDDLAI**PATEFFSTTAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | U94848.1 | 4/14/2003 |
| VACV | Acambis 3000 Modified Virus Ankara (MVA) | 8 | AAT10536.1 | MDGTLFPG**DDDLAI**PATEFFSTTAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY603355.1 | 5/15/2004 |
| VACV | BP-1 | 9 | AAQ72850.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299016.1 | 6/30/2004 |
| VACV | IHD | 10 | AAQ72851.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299017.1 | 6/30/2004 |
| VACV | LEVADITI | 11 | AAQ72852.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299018.1 | 6/30/2004 |
| VACV | CVA | 12 | AAQ72853.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299019.1 | 6/30/2004 |
| VACV | ELSTREE | 13 | AAQ72854.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299020.1 | 6/30/2004 |
| VACV | EP-MARINA | 14 | AAQ72855.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY299021.1 | 6/30/2004 |
| VACV | Connaught | 15 | AAN78218.2 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY160184.2 | 1/25/2005 |
| VACV | LC16m8 | 16 | AAW23581.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY678275.1 | 9/8/2005 |
| VACV | LC16mO | 17 | AAW23863.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY678277.1 | 9/8/2005 |
| VACV | WR (Western Reserve) | 18 | AAO89429.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY243312.1 | 3/14/2006 |
| VACV | Copenhagen | 19 | AAA48152.1 | MDGTLFPG**DDDLAI**PATEFFSTKAD**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | M35027.1 | 3/15/2006 |

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| VACV | 3737 | 20 | ABD57680.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ377945.1 | 4/19/2006 |
| VACV | DUKE | 21 | ABD98620.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ439815.1 | 10/27/2006 |
| VACV | Lister clone VACV107 | 22 | ABD52635.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ121394.1 | 6/12/2007 |
| VACV | chorioallantois vaccinia virus Ankara (CVA) | 23 | CAM58321.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AM501482.1 | 1/30/2009 |
| VACV | GLV-1h68 | 24 | ABZ80105.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | EU410304.1 | 9/29/2009 |
| VACV | unknown Sequence 318 from patent US 7645456 | 25 | ADC23632.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | ADC23632.1 | 1/28/2010 |
| VACV | Acambis clone 3 | 26 | AAQ93247.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY313848.1 | 10/5/2010 |
| VACV | Acambis clone 2000 | 27 | AAR17993.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY313847.1 | 10/5/2010 |
| VACV | unknown Sequence 8 from patent US7807180 | 28 | ADS58159.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | ADS58159.1 | 12/13/2010 |
| VACV | Dryvax clone DPP12 | 29 | AEY73438.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654979.1 | 1/28/2012 |
| VACV | Dryvax clone DPP9 | 30 | AEY72723.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654976.1 | 1/28/2012 |
| VACV | Dryvax clone DPP10 | 31 | AEY72961.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654977.1 | 1/28/2012 |
| VACV | Dryvax clone DPP11 | 32 | AEY73196.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654978.1 | 1/28/2012 |
| VACV | Dryvax clone DPP13 | 33 | AEY73678.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654980.1 | 1/28/2012 |
| VACV | Dryvax clone DPP15 | 34 | AEY73908.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654981.1 | 1/28/2012 |
| VACV | Dryvax clone DPP16 | 35 | AEY74148.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654982.1 | 1/28/2012 |
| VACV | Dryvax clone DPP17 | 36 | AEY74389.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654983.1 | 1/28/2012 |
| VACV | Dryvax clone DPP19 | 37 | AEY74617.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654984.1 | 1/28/2012 |
| VACV | Dryvax clone DPP20 | 38 | AEY74857.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654985.1 | 1/28/2012 |
| VACV | Dryvax clone DPP21 | 39 | AEY75097.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JN654986.1 | 1/28/2012 |
| VACV | IHD-W | 40 | AGB75866.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC201194.1 | 1/9/2013 |
| VACV | TianTan clone TP3 | 41 | AGK06618.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC207810.1 | 4/27/2013 |
| VACV | TianTan clone TP5 | 42 | AGK06831.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KC207811.1 | 4/27/2013 |
| VACV | TianTan clone TT8 | 43 | AGJ91342.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX489135.1 | 4/24/2013 |
| VACV | TianTan clone TT9 | 44 | AGJ91614.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX489136.1 | 4/24/2013 |
| VACV | TianTan clone TT10 | 45 | AGJ91884.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX489137.1 | 4/24/2013 |
| VACV | TianTan clone TT11 | 46 | AGJ92158.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX489138.1 | 4/24/2013 |
| VACV | TianTan clone TT12 | 47 | AGJ92432.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | JX489139.1 | 4/24/2013 |
| VACV | Brazil Serro 2 | 48 | AHB35788.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KF179385.1 | 12/4/2014 |
| VACV | L-IVP | 49 | AIZ72898.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KP233807.1 | 12/17/2014 |
| VACV | Dryvax clone DPP25 | 50 | AIX98845.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KJ125438.1 | 4/21/2015 |
| VACV | Tashkent cloneTKT3 | 51 | AIX99269.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KM044309.1 | 4/21/2015 |
| VACV | Tashkent cloneTKT4 | 52 | AIX99474.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KM044310.1 | 4/21/2015 |
| VACV | *WAU86/88-1* | 53 | AHB23585.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KF866253.1 | 5/11/2015 |
| VACV | WR (Western Reserve) | 54 | YP_233032.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | NC_006998.1 | 8/6/2015 |
| VACV | IOC cloneB141 | 55 | ALF05145.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KT184690.1 | 9/26/2015 |
| VACV | IOC cloneB388 | 56 | ALF05396.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KT184691.1 | 9/26/2015 |
| VACV | Cantagalo | 57 | ALF36307.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KT013210.1 | 10/8/2015 |
| VACV | Lister clone: Lister Butantan | 58 | AND73983.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KX061501.1 | 5/10/2016 |
| VACV | Vaccinia virus WR | 59 | P11258.3 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | P11258.3 | 2/15/2017 |
| VACV | Vaccinia virus | 60 | CAA40577.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPDRKREQIVKAD**EDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X57318.1 | 7/26/2016 |
| VACV | *WR 65-16* | 61 | P26312.1 | MLEFFRPP**RARSPRE**LVQLLPEAWT**TSRSSTGSANPSA**SRKPARYPRIHAPELQSGEARWPLWSRIRPLEDPLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDEVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 136 | | 5/10/2017 |
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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| VARV major | India-1967 | 1 | CAA47501.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDNNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X67115.1 | 7/26/1994 |
| VARV major | Bangladesh-1975, ssp. major | 2 | AAA60882.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | L22579.1 | 1/12/1995 |
| VARV major | Harvey, ssp. major | 3 | CAA46494.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X65517.1 | 1/22/1999 |
| VARV major | India378 | 4 | AAP48868.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223483.1 | 5/12/2004 |
| VARV major | M-Sur-60 | 5 | AAP48870.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223485.1 | 5/12/2004 |
| VARV major | 6/58 | 6 | AAP48877.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDNNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223492.1 | 5/12/2004 |
| VARV major | Aslam | 7 | AAP48875.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223490.1 | 5/12/2004 |
| VARV major | Brazil128 | 8 | AAP48879.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223494.1 | 5/12/2004 |
| VARV major | Brazil131 | 9 | AAP48880.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223495.1 | 5/12/2004 |
| VARV major | Butler | 10 | AAP48878.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223493.1 | 5/12/2004 |
| VARV major | India164 | 11 | AAP48869.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223484.1 | 5/12/2004 |
| VARV major | Khateen | 12 | AAP48876.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223491.1 | 5/12/2004 |
| VARV major | M-A-60 | 13 | AAP48872.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223487.1 | 5/12/2004 |
| VARV major | M-Bl-60 | 14 | AAP48873.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223488.1 | 5/12/2004 |
| VARV major | M-N-60 | 15 | AAP48874.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223489.1 | 5/12/2004 |
| VARV major | M-Sok-60 | 16 | AAP48871.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223486.1 | 5/12/2004 |
| VARV major | TajBarin | 17 | AAP48867.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | AY223482.1 | 5/12/2004 |

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|---|---|---|---|---|---|---|---|
| VARV major | India-1967, ssp. major | 18 | CAA49075.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDNNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X69198.1 | 4/18/2005 |
| VARV major | United Kingdom 1947 Higgins (Staffordshire) | 19 | ABF28921.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441446.1 | 8/19/2006 |
| VARV major | Afghanistan 1970 Variolator 4 | 20 | ABG43304.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437580.1 | 8/19/2006 |
| VARV major | Bangladesh 1974 (nur islam) | 21 | ABF23711.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441420.1 | 8/19/2006 |
| VARV major | Bangladesh 1974 (Shahzaman) | 22 | ABF23908.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441421.1 | 8/19/2006 |
| VARV major | Bangladesh 1974 (Solaiman) | 23 | ABF24105.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441422.1 | 8/19/2006 |
| VARV major | Bangladesh 1975 v75-550 Banu | 24 | ABG43507.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437581.1 | 8/19/2006 |
| VARV major | Benin, Dahomey 1968 (v68-59) | 25 | ABF22898.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441416.1 | 8/19/2006 |
| VARV major | Botswana 1972 (v72-143) | 26 | ABF23102.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441417.1 | 8/19/2006 |
| VARV major | Botswana 1973 (v73-225) | 27 | ABF23304.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441418.1 | 8/19/2006 |
| VARV major | Brazil 1966 (v66-39 Sao Paulo) | 28 | ABF23508.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441419.1 | 8/19/2006 |
| VARV major | China Horn 1948; Sabin Lab July 1948 | 29 | ABG43708.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437582.1 | 8/19/2006 |
| VARV major | Congo 9 1970 (v74-227 Gispen) | 30 | ABF24302.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441423.1 | 8/19/2006 |
| VARV major | Congo 1970 v70-46 Kinshasa | 31 | ABG43912.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437583.1 | 8/19/2006 |
| VARV major | Ethiopia 1972 (Eth16 R14-1X-72 Addis) | 32 | ABF24503.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441424.1 | 8/19/2006 |
| VARV major | Ethiopia 1972 (Eth17 R14-1X-72 Addis) | 33 | ABF24705.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441425.1 | 8/19/2006 |
| VARV major | Germany 1958 Heidelberg | 34 | ABG44114.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437584.1 | 8/19/2006 |
| VARV major | Guinea 1969 (005) | 35 | ABF24906.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441426.1 | 8/19/2006 |
| VARV major | India 1953 (Kali-Muthu-M50 Madras) | 36 | ABF25108.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441427.1 | 8/19/2006 |
| VARV major | India 1953 (New Delhi) | 37 | ABF25308.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441428.1 | 8/19/2006 |
| VARV major | India 1964 7124 Vellore | 38 | ABG44317.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437585.1 | 8/19/2006 |
| VARV major | India 1964 7125 Vellore | 39 | ABG44521.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437586.1 | 8/19/2006 |
| VARV major | Iran 1972 2602 Tabriz | 40 | ABG44723.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437587.1 | 8/19/2006 |
| VARV major | Japan 1946 (Yamada MS-2(A) Tokyo) | 41 | ABF25510.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441429.1 | 8/19/2006 |
| VARV major | Japan 1951 (Harper, Masterseed) | 42 | ABF25712.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441430.1 | 8/19/2006 |
| VARV major | Japan 1951 (Stillwell, Masterseed) | 43 | ABF25913.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441431.1 | 8/19/2006 |
| VARV major | Korea 1947 (Lee, Masterseed) | 44 | ABF26115.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441432.1 | 8/19/2006 |
| VARV major | Kuwait 1967 (K1629) | 45 | ABF26317.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441433.1 | 8/19/2006 |
| VARV major | Nepal 1973 V73-175 | 46 | ABG44926.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437588.1 | 8/19/2006 |
| VARV major | Pakistan 1969 (Rafiq Lahore) | 47 | ABG45128.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437589.1 | 8/19/2006 |
| VARV major | Sierra Leone 1969 (V68-258) | 48 | ABF27124.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441437.1 | 8/19/2006 |
| VARV major | Somalia 1977 (V77-1252) | 49 | ABF27326.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRGCPYE | 110 | DQ441438.1 | 8/19/2006 |
| VARV major | SOM77_1605_139 | 50 | ABF27525.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRCPYE | 110 | DQ441439.1 | 8/19/2006 |
| VARV major | Somalia 1977; V77-2479 | 51 | ABG45331.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRCPYE | 110 | DQ437590.1 | 8/19/2006 |
| VARV major | South Africa 1965 (102 Natal, Ingwavuma) | 52 | ABF26721.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441435.1 | 8/19/2006 |
| VARV major | South Africa 1965 (103 T'vaal, Nelspruit) | 53 | ABF26921.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441436.1 | 8/19/2006 |
| VARV major | United Kingdom 1946 Harvey | 54 | ABF28521.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441444.1 | 8/19/2006 |
| VARV major | Sudan 1947 (Juba) | 55 | ABF27724.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441440.1 | 8/19/2006 |
| VARV major | Sudan 1947 (Rumbec) | 56 | ABF27924.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441441.1 | 8/19/2006 |
| VARV major | Sumatra 1970 V70-228 | 57 | ABF28123.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441442.1 | 8/19/2006 |
| VARV major | Syria 1972 V72-199 | 58 | ABG45735.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437592.1 | 8/19/2006 |
| VARV major | Tanzania 1965 kembula | 59 | ABF28322.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441443.1 | 8/19/2006 |
| VARV major | United Kingdom 1946 Hinden (Middlesex) | 60 | ABF28722.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441445.1 | 8/19/2006 |
| VARV major | United Kingdom 1952 Butler | 61 | ABF29124.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441447.1 | 8/19/2006 |
| VARV major | Yugoslavia 1972 V72-164 | 62 | ABF29328.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441448.1 | 8/19/2006 |
| VARV major | Sumatra 1970 V70-222 | 63 | ABG45532.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ437591.1 | 8/19/2006 |
| VARV major | India-1967, ssp. major | 64 | NP_042178.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDNNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | NC_001611.1 | 11/23/2010 |
| VARV major | *PoxSib* | 65 | AFX98076.1 | TNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETL | 31 | JX080526.1 | 12/3/2012 |
| VARV major | VD21, 17th century | 66 | APR62872.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | KY358055.1 | 1/3/2017 |
| VARV major | Niger 1969 (001, importation from Nigeria) | 67 | ABF26518.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAK**R**EAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | DQ441434.1 | 8/19/2006 |
| | | | | | | | |
| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| VARV minor | Garcia-1966 | 1 | CAA53856.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | X76266.1 | 12/31/1995 |
| VARV minor | Garcia-1966 | 2 | CAB54734.1 | MDGTLFPG**DDDLAI**PATEFFSTKAA**KKPEAKREAIVKAD**GDDNEETLKQRLTNLEKKITNVTTKFEQ**IEKC**CKRNDDVLFRLENHAETLRAAMISLAKKIDVQTGRRPYE | 110 | Y16780.1 | 4/18/2005 |

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| | | | | **Epitope 4** **Epitopes 1A-1D** **Epitope 5** | | | |
| Volepox | Volepox virus | 1 | ABD37608.1 | **KPE**EKR**K**A**V**VKA**E**EEEDDETIKDRLTNLEKKITNVTTKFAQ**IEKC**CKRNDEV | 52 | DQ374627.1 | 7/26/2016 |

The epitopes are highlighted in bold. Deviations of VACV/VARV-typical epitopes are marked in red.

**Table S5** Nucleotide sequences of the OPXV A27 protein homologs available so far in the GenBank, showing mutations which are crucial for amino acid exchanges as well as silent mutations.

| Number | OPXV | Strain/*Isolate* | Source | Epitope 4 | | | | | | Epitope | Complex | 1A-D | | | | | | | | | | | | Epitope 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | D | D | D | L | A | I | K | K | P | E | A | K | R | E | A | I | V | K | A | D | I | E | K | C |
| | | | | GAT | GAC | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | ATA | GAA | AAG | TGT |
| 1 | BPXV | *Aur04* | DQ117952.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | BPXV | *BP4* | DQ117951.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | BPXV | *Ind99* | DQ117954.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 4 | BPXV | *Vij96* | DQ117953.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 5 | BPXV | *Buffalopox virus clone 1* | FJ748495.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 6 | BPXV | *Buffalopox virus clone 2* | FJ748496.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 7 | BPXV | *Pune/09* | GQ443265.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 8 | BPXV | *Pune/H/09* | GQ443266.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 9 | BPXV | *SP69 Gujarat/08* | GQ464367.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 10 | BPXV | *SP70 Hyderabad/08* | GQ464368.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 11 | BPXV | *SP66 Pune/08* | GQ464366.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 12 | BPXV | *Bareilly/99* | HM008906.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 13 | BPXV | *Bareilly/00* | HM008907.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 14 | BPXV | *Bangalore/03* | HM008914.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 15 | BPXV | *Bangalore/09* | HM008910.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 16 | BPXV | *Vij/97* | HM008908.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 17 | BPXV | *Krisna/07* | HM008905.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 18 | BPXV | *Nel/06* | HM008904.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 19 | BPXV | *Pune 1/04* | HM008902.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 20 | BPXV | *Pune 2/04* | HM008903.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 21 | BPXV | *Pune/06* | HM008909.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 22 | BPXV | *Pune/07* | HM008913.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 23 | BPXV | *Karachi 2005* | MG599038.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 24 | BPXV | *SP56 BPXV Izatnagar/08* | GQ464365.1 | ... | ... | ... | ... | ... | .C.(T) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 25 | BPXV | *Pune/milk* | HM008911.1 | ... | ... | ... | ... | ... | .C.(T) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 26 | BPXV | *Hyd 17/04* | HM008912.1 | ... | ... | ... | ... | ... | .C.(T) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

| | | | | **D** | **D** | **D** | **L** | **A** | **I** | **K** | **K** | **P** | **E** | **A** | **K** | **R** | **E** | **A** | **I** | **I** | **K** | **A** | **D** | **I** | **E** | **K** | **C** |
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| | | | | GAT | GA**T** | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | A**T**T | AAA | GCC | GAT | ATA | GAA | AAG | TGT |
| 1 | CMLV | CMS | AY009089.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | CMLV | Cp-1 | X75156.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | CMLV | *M-96* | AF438165.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 4 | CMLV | Cp-1 | AY223496.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 5 | CMLV | NIGER | AY299081.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 6 | CMLV | SAUDI | AY299082.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 7 | CMLV | MAURETANIA | AY299083.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 8 | CMLV | CP-14 | AY299084.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 9 | CMLV | CP-5 | AY299085.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 10 | CMLV | CP-202/95H | AY299086.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 11 | CMLV | CP-1260 | AY299087.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 12 | CMLV | *DEL09* | GQ465930.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 13 | CMLV | *BAR09* | GQ465928.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 14 | CMLV | *JSL09* | GQ465929.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 15 | CMLV | *Al-Ahsaa* | JN805951.1 | --- | --- | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 16 | CMLV | *M-96* | NC_003391.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 17 | CMLV | 0408151v | KP768318.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 18 | CMLV | *BAR09* | GQ465927.1 | ... | ... | ... | ... | ... | ... | ... | .G. (R) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

| | | | | **D** | **D** | **D** | **L** | **A** | **I** | **K** | **K** | **P** | **E** | **A** | **K** | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **D** | **I** | **E** | **K** | **C** |
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| | | | | GAT | GAC | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | ATA | GAA | AAG | TGT |
| 1 | CPXV | GRI-90 | Z99060.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | CPXV | 98/5 | AY223500.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | CPXV | EP-5 | AY223497.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 4 | CPXV | EXP-GR | AY223499.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 5 | CPXV | Revin | AY223498.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 6 | CPXV | EP-1 | AY299040.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 7 | CPXV | EP-3 | AY299043.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 8 | CPXV | EP-5 | AY299042.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 9 | CPXV | EP-7 | AY299044.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 10 | CPXV | EP-8 | AY299045.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 11 | CPXV | 28-00 | AY299046.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 12 | CPXV | FINNLAND | AY299080.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 13 | CPXV | OPV-89-1 | AY299053.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ... | ..C | ... | ... | ... |
| 14 | CPXV | OPV-98-2 | AY299047.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..C | ... | ... | ... |
| 15 | CPXV | OPV-99-1 | AY299048.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 16 | CPXV | RAT-MOSCOW | AY299041.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 17 | CPXV | GRI-90 | X94355.2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 18 | CPXV | Austria 1999 | HQ407377.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 19 | CPXV | Finland_2000_MAN | HQ420893.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 20 | CPXV | Germany_1998_2 | HQ420897.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..C | ... | ... | ... |
| 21 | CPXV | HumGri07/1 | KC813511.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 22 | CPXV | EleGri07/1 | KC813507.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 23 | CPXV | HumAac09/1 | KC813508.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 24 | CPXV | HumGra07/1 | KC813510.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 25 | CPXV | HumKre08/1 | KC813512.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 26 | CPXV | HumLit08/1 | KC813493.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 27 | CPXV | RatAac09/1 | KC813501.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 28 | CPXV | RatGer09/1 | KC813503.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 29 | CPXV | RatKre08/2 | KC813505.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 30 | CPXV | *CEPAD332* | KC592404.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 31 | CPXV | *CEPAD333* | KC592406.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 32 | CPXV | *CEPAD336* | KC592405.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 33 | CPXV | RatPox09 | LN864565.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 34 | CPXV | *Ger/2017/Alpaca2* | LT896732.2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 35 | CPXV | *Ger/2015/Human2* | LT993232.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 36 | CPXV | *Ger/2015/Prairie-dog* | LT993231.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 37 | CPXV | *Ger/2017/Alpaca1* | LT993230.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 38 | CPXV | *Ger/2017/common vole FMEimka* | LT993228.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 39 | CPXV | *Ger/2007/Vole* | LT896722.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ... | ..C | ... | ... | ... |
| 40 | CPXV | *89Lcow* | DQ385058.1 | --- | --- | --- | --- | --- | --- | --G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ... | ..C | ... | ... | ... |
| 41 | CPXV | *A280* | DQ374558.1 | --- | --- | --- | --- | --- | --- | --- | ..A | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ... | ..C | ... | ... | ... |
| 42 | CPXV | Kostroma_2015 | KY369926.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 43 | CPXV | Germany_1971_EP1 | KY463519.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 44 | CPXV | *Ger/2017/Alpaca2* | LT896732.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 45 | CPXV | *Ger/2012/Alpaca* | LT896726.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 46 | CPXV | *Ger/2015/Human1* | LT896720.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 47 | CPXV | *Ger/2015/Cat2* | LT896727.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 48 | CPXV | *Ger/2015/Cat3* | LT896733.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 49 | CPXV | *Ger/2015/Cat4* | LT896731.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 50 | CPXV | *Ger/2014/Cat2* | LT896725.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 51 | CPXV | *Ger/2010/Cat* | LT896729.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 52 | CPXV | *Ger/2010/Racoon* | LT896730.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 53 | CPXV | *Ger 2010 MKY* | LT896721.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..C | ... | ... | ... |
| 54 | CPXV | Brighton | X75158.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 55 | CPXV | Hamburg-1985 | Z99062.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 56 | CPXV | Brighton | AY223502.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 57 | CPXV | EP-2 | AY223504.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 58 | CPXV | EP-4 | AY223501.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 59 | CPXV | OPV-89-3 | AY299058.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 60 | CPXV | OPV-89-5 | AY299052.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 61 | CPXV | OPV-90-1 | AY299049.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 62 | CPXV | OPV-90-2 | AY299056.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 63 | CPXV | OPV-90-3 | AY299070.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 64 | CPXV | OPV-90-4 | AY299071.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 65 | CPXV | OPV-90-5 | AY299069.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 66 | CPXV | OPV-91-1 | AY299051.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 67 | CPXV | OPV-91-3 | AY299050.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 68 | CPXV | OPV-91-4 | AY299064.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 69 | CPXV | OPV-98-1 | AY299059.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 70 | CPXV | OPV-98-3 | AY299075.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 71 | CPXV | OPV-98-4 | AY299076.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |

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| 72 | CPXV | SWEDENI | AY299065.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 73 | CPXV | SWEDENII | AY299067.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 74 | CPXV | NANCY | AY299074.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 75 | CPXV | NW-MAN | AY299055.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 76 | CPXV | OPV-88H | AY299057.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 77 | CPXV | EP-RIEMS | AY299077.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 78 | CPXV | EP-6 | AY299073.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 79 | CPXV | BIBER | AY299078.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 80 | CPXV | CATPOX3 | AY299068.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 81 | CPXV | CATPOX5 | AY299063.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ..T | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 82 | CPXV | NW-CAT | AY299054.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 83 | CPXV | 780 | AY299079.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 84 | CPXV | Brighton Red | AF482758.2 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 85 | CPXV | Brighton Red | AF482758.2 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 86 | CPXV | Germany 91-3 | DQ437593.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 87 | CPXV | Calpox | HQ891540.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 88 | CPXV | France_2001_Nancy | HQ420894.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 89 | CPXV | Germany_1980_EP4 | HQ420895.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 90 | CPXV | Germany_1990_2 | HQ420896.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 91 | CPXV | Germany_2002_MKY | HQ420898.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 92 | CPXV | Norway_1994_MAN | HQ420899.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |

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| 93 | CPXV | RatHei09/1 | KC813504.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 94 | CPXV | HumLan08/1 | KC813492.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 95 | CPXV | HumLue09/1 | KC813494.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 96 | CPXV | HumMag07/1 | KC813495.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 97 | CPXV | HumPad07/1 | KC813496.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 98 | CPXV | JagKre08/2 | KC813498.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 99 | CPXV | MarLei07/1 | KC813499.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 100 | CPXV | MonKre08/4 | KC813500.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 101 | CPXV | HumBer07/1 | KC813509.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 102 | CPXV | BeaBer04/1 | KC813491.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 103 | CPXV | CatBer07/1 | KC813502.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 104 | CPXV | CatPot07/1 | KC813506.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 105 | CPXV | JagKre08/1 | KC813497.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 106 | CPXV | *CEPAD335* | KC592407.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 107 | CPXV | FM2292 | LN864566.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 108 | CPXV | Brighton Red | NC_003663.2 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 109 | CPXV | *A220* | DQ374542.1 | --- | --- | --- | --- | --- | --- | --. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 110 | CPXV | *A247* | DQ374550.1 | --- | --- | --- | --- | --- | --- | --G | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 111 | CPXV | CPXV Amadeus 2015 | LN879483.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 112 | CPXV | *CPXV_1639* | KY549148.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ..T | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 113 | CPXV | *CPXV_Catpox5wv1* | KY549144.1 | ... | ... | ... | ... | ... | ... | ..G | ... | ... | ... | ... | ... | ... | ..T | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |

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| 114 | CPXV | *Norwayfeline* | KY549151.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | ..T | ... | ..C | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 115 | CPXV | *Ger/2014/Cat1* | LT896723.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 116 | CPXV | *Ger/2010/Alpaca* | LT896718.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 117 | CPXV | *Ger/2010/Rat* | LT896728.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 118 | CPXV | *Ger/2013/Alpaca* | LT896719.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 119 | CPXV | *Ger/2014/Human* | LT993226.1 | ... | ... | ... | ..C | ... | ... | ..G | ... | ... | ... | ... | ... | … | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 120 | CPXV | 427 | AY299060.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 121 | CPXV | 428 | AY299062.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 122 | CPXV | 2739 | AY299061.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 123 | CPXV | UK2000_K2984 | HQ420900.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 124 | CPXV | *A009* | DQ374477.1 | --- | --- | --- | --- | --- | --- | --G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 125 | CPXV | *CPXV_K428* | KY549145.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 126 | CPXV | *CPXV_K2739* | KY549149.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 127 | CPXV | *CPXV_K4207* | KY549150.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 128 | CPXV | *CPXV_Catpox3L97* | KY549143.1 | ... | ... | ... | ... | ... | ... | ..G | ... | .A. (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 129 | CPXV | Turkmenia-1974 | Z99063.1 | ... | ... | ... | ... | ... | ... | ... | .G. (R) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 130 | CPXV | *Ger/2015/Cat1* | LT896724.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .T. (V) | ... | ... | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 131 | CPXV | OPV-89-2 | AY299072.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .A. (H) | ... | ... | ... | ... | ... | ..T | ..G (E) | ... | ... | ... | ... |
| 132 | CPXV | 89/4 | AY223503.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | T.. (F) | ... | ... | ..T | ..G (E) | ..C | ... | ... | ... |
| 133 | CPXV | SWEDENIII | AY299066.1 | ... | ... | ... | ... | ... | ... | ... | .GC (S) | .AG (Q) | AG. (R) | CTA (L) | ..C (N) | GCG (A) | A.G (K) | CA. (Q) | T.G (L) | T.A (L) | ..G (K) | CTG (L) | AGG (R) | --- | --- | --- | --- |
| 134 | CPXV | *A279* | DQ374556.1 | --- | --- | --- | --- | --- | --- | --. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T | ... | ..C | ... | ... | .A. (Y) |

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| | | | | GAT | GAC | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GAT | AAA | CAC | GAA | GCA | ACT | GTT | AAA | GCC | GAT | ATC | GAA | AAG | TGT |
| 1 | ECTV | Munich-1 | X75157.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | ECTV | Moscow | AF012825.2 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | ECTV | K-1 (Ect 3) | Z99055.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 4 | ECTV | MP-2 | AY299023.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 5 | ECTV | C99-505 | AY299026.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 6 | ECTV | MP-5 | AY299024.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 7 | ECTV | MP-33221 | AY299025.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 8 | ECTV | SILBERFUCHS | AY299022.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 9 | ECTV | MPV-CC | AY973172.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 10 | ECTV | Moscow | NC_004105.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 11 | ECTV | ERPV | JQ410350.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 12 | ECTV | *NAVAL* | KJ563295.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 13 | ECTV | *Hamptead* | KY554976.1 | ... | ..T | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 14 | ECTV | Ectromelia virus | DQ178390.1 | --- | --- | --- | --- | --- | --- | --. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| | | | | GAT | GAC | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | ATA | GAA | AAG | TGT |
| 1 | HSPV | *MNR-76* | DQ792504.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 2 | HSPV | MNR | KY349117.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

| | | | | **D** | **D** | **D** | **L** | **A** | **I** | **K** | **N** | **P** | **E** | **T** | **K** | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **Y** | **I** | **E** | **K** | **C** |
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| | | | | GAT | GAC | GAT | CTT | GCA | ATT | AAA | AAT | CCA | GAG | ACT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | TAT | ATA | GAA | AAG | TGT |
| 1 | MPXV | Copenhagen | X75155.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | MPXV | Zaire 79 | AY160186.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | MPXV | Sierra Leone 70-0666 | Z99065.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 4 | MPXV | *DRC 07-0093* | JX878416.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 5 | MPXV | CDC#v70-I-187 | AY223481.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 6 | MPXV | CDC#v78-I-3945 | AY223480.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 7 | MPXV | CDC#v79-I-005 | AY223479.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 8 | MPXV | CDC#v97-I-004 | AY223478.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 9 | MPXV | Congo 8 | AY223477.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 10 | MPXV | AP-4 | AY299031.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 11 | MPXV | AP-2 | AY299032.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 12 | MPXV | AP-6 | AY299033.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 13 | MPXV | AP-5 | AY299034.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 14 | MPXV | WRAIR7-61 | AY603973.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 15 | MPXV | *Sierra Leone MPXV-SL-132* | AY741551.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 16 | MPXV | COP-58 | AY753185.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 17 | MPXV | Congo_2003_358 | DQ011154.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 18 | MPXV | Liberia_1970_184 | DQ011156.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 19 | MPXV | USA_2003_039 | DQ011157.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 20 | MPXV | USA_2003_044 | DQ011153.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 21 | MPXV | Zaire_1979-005 | DQ011155.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 22 | MPXV | Zaire-96-I-16 | AF380138.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 23 | MPXV | Zaire 1979-005 | HM172544.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 24 | MPXV | D14L knockout | HQ857563.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 25 | MPXV | V79-I-005 | HQ857562.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 26 | MPXV | Zaire-96-I-16 | NC_003310.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 27 | MPXV | Sudan 2005_01 | KC257459.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 28 | MPXV | DRC Yandongi 1985 | KC257460.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 29 | MPXV | *DRC 06-0950* | JX878407.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 30 | MPXV | *DRC 06-0970* | JX878408.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 31 | MPXV | *DRC 06-1070* | JX878410.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 32 | MPXV | *DRC 07-0120* | JX878418.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 33 | MPXV | *DRC 06-0999* | JX878409.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 34 | MPXV | *DRC 06-1075* | JX878411.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 35 | MPXV | *DRC 06-1076* | JX878412.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 36 | MPXV | *DRC 07-0045* | JX878413.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 37 | MPXV | *DRC 07-0046* | JX878414.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 38 | MPXV | *DRC 07-0092* | JX878415.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |

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| 39 | MPXV | *DRC 07-0104* | JX878417.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 40 | MPXV | *DRC 07-0275* | JX878419.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 41 | MPXV | *DRC 07-0283* | JX878420.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 42 | MPXV | *DRC 07-0286* | JX878421.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 43 | MPXV | *DRC 07-0287* | JX878422.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 44 | MPXV | *DRC 07-0337* | JX878423.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 45 | MPXV | *DRC 07-0338* | JX878424.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 46 | MPXV | *DRC 07-0354* | JX878425.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 47 | MPXV | *DRC 07-0450* | JX878426.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 48 | MPXV | *DRC 07-0480* | JX878427.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 49 | MPXV | *DRC 07-0514* | JX878428.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 50 | MPXV | *DRC 07-0662* | JX878429.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 51 | MPXV | Ivory Coast 2012 | KJ136820.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 52 | MPXV | UTC | KJ642614.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 53 | MPXV | W-Nigeria | KJ642615.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 54 | MPXV | PCH | KJ642616.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 55 | MPXV | Nigeria-SE-1971 | KJ642617.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 56 | MPXV | *Cote d'Ivoire_1971* | KP849470.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 57 | MPXV | MPXV_Nig_2017_297957 | MG693723.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| | | | | GAT | GAC | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | ATA | GAA | AAG | TGT |
| 1 | RaPXV | Rabbitpox virus Uttrecht | Z99059.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | RaPXV | Rabbitpox virus | AY484669.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| | | | | **D** | **D** | **D** | **I** | **A** | **I** | **K** | **K** | **P** | **E** | **K** | **P** | **A** | **K** | **R** | **K** | **V** | **V** | **K** | **A** | **D** | **I** | **E** | **K** | **C** |
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| | | | | GAC | GAT | GAT | ATT | GCA | ATT | **AAA** | **AAA** | **CCA** | **GAA** | **AAA** | **CCA** | **GCT** | **AAA** | **CGT** | **AAA** | **GTA** | **GTT** | **AAA** | **GCG** | **GAT** | | | | |
| | | | KP143769.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | | | | |
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| 1 | RPXV | Raccoonpoxvirus | DQ374628.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

| | | | | **D** | **D** | **D** | **L** | **A** | **I** | **K** | **K** | **P** | **E** | **A** | **K** | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **D** | **I** | **E** | **K** | **C** |
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| | | | | GAT | GAT | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | ATA | GAA | AAG | TGT |
| 1 | TaPXV | Dahomey 1968 | NC_008291.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | TaPXV | Dahomey 1968 | DQ437594.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | TaPXV | Taterapox virus | DQ374634.1 | --- | --- | --- | --- | --- | --- | --. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

| | | | | **D** | **D** | **D** | **L** | **A** | **I** | **K** | **K** | **P** | **E** | **A** | **K** | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **D** | **I** | **E** | **K** | **C** |
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| | | | | GAT | GAC | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | ATA | GAA | AAG | TGT |
| 1 | VACV | Vaccinia virus | M18173.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | VACV | Tian Tan | AF095689.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | VACV | LIVP-1 | Z99057.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 4 | VACV | COP-2 | Z99056.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 5 | VACV | LIVP-2 | Z99058.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 6 | VACV | Wyeth | Z99064.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 7 | VACV | Ankara | U94848.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 8 | VACV | Acambis 3000 MVA | AY603355.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 9 | VACV | BP-1 | AY299016.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 10 | VACV | IHD | AY299017.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 11 | VACV | LEVADITI | AY299018.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 12 | VACV | CVA | AY299019.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 13 | VACV | ELSTREE | AY299020.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 14 | VACV | EP-MARINA | AY299021.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 15 | VACV | Connaught | AY160184.2 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 16 | VACV | LC16m8 | AY678275.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 17 | VACV | LC16mO | AY678277.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 18 | VACV | WR (Western Reserve) | AY243312.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 19 | VACV | Copenhagen | M35027.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 20 | VACV | 3737 | DQ377945.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 21 | VACV | DUKE | DQ439815.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 22 | VACV | Lister clone VACV107 | DQ121394.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 23 | VACV | CVA | AM501482.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 24 | VACV | GLV-1h68 | EU410304.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |

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| 25 | VACV | unknown Sequence 318 from patent US 7645456 | ADC23632.1 | no nt sequence available | | | | | | | | | | | | | | | | | | | | | | | | | |
| 26 | VACV | Acambis clone 3 | AY313848.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 27 | VACV | Acambis clone 2000 | AY313847.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 28 | VACV | unknown Sequence 8 from patent US7807180 | ADS58159.1 | no nt sequence available | | | | | | | | | | | | | | | | | | | | | | | | | |
| 29 | VACV | Dryvax clone DPP12 | JN654979.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 30 | VACV | Dryvax clone DPP9 | JN654976.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 31 | VACV | Dryvax clone DPP10 | JN654977.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 32 | VACV | Dryvax clone DPP11 | JN654978.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 33 | VACV | Dryvax clone DPP13 | JN654980.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 34 | VACV | Dryvax clone DPP15 | JN654981.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 35 | VACV | Dryvax clone DPP16 | JN654982.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 36 | VACV | Dryvax clone DPP17 | JN654983.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 37 | VACV | Dryvax clone DPP19 | JN654984.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 38 | VACV | Dryvax clone DPP20 | JN654985.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 39 | VACV | Dryvax clone DPP21 | JN654986.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 40 | VACV | IHD-W | KC201194.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 41 | VACV | TianTan clone TP3 | KC207810.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 42 | VACV | TianTan clone TP5 | KC207811.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 43 | VACV | TianTan clone TT8 | JX489135.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 44 | VACV | TianTan clone TT9 | JX489136.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 45 | VACV | TianTan clone TT10 | JX489137.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

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| 46 | VACV | TianTan clone TT11 | JX489138.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 47 | VACV | TianTan clone TT12 | JX489139.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 48 | VACV | Brazil Serro 2 | KF179385.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 49 | VACV | L-IVP | KP233807.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 50 | VACV | Dryvax clone DPP25 | KJ125438.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 51 | VACV | Tashkent cloneTKT3 | KM044309.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 52 | VACV | Tashkent cloneTKT4 | KM044310.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 53 | VACV | *WAU86/88-1* | KF866253.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 54 | VACV | WR (Western Reserve) | NC_006998.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 55 | VACV | IOC cloneB141 | KT184690.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 56 | VACV | IOC cloneB388 | KT184691.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 57 | VACV | Cantagalo | KT013210.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 58 | VACV | Lister clone: Lister Butantan | KX061501.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 59 | VACV | Vaccinia virus WR | P11258.3 | no nt sequence available | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 60 | VACV | Vaccinia virus | X57318.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ..T (D) | CGG (R) | ... | ... | ... | CAG (Q) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 61 | VACV | *WR 65-16* | P26312.1 | no nt sequence available | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | **D** | **D** | **D** | **L** | **A** | **I** | **K** | **K** | **P** | **E** | **A** | **K** | **R** | **E** | **A** | **I** | **V** | **K** | **A** | **D** | | **I** | **E** | **K** | **C** |
| | | | | GAT | GAT | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | | ATA | GAA | AAG | TGT |
| 1 | VARV ma. | India-1967 | X67115.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | | ... | ... | ... | ... |
| 2 | VARV ma. | Bangladesh-1975, ssp. major | L22579.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | | ... | ... | ... | ... |

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| 3 | VAR V ma. | Harvey, ssp. major | X65517.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 4 | VAR V ma. | India378 | AY223483.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 5 | VAR V ma. | M-Sur-60 | AY223485.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 6 | VAR V ma. | 6/58 | AY223492.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 7 | VAR V ma. | Aslam | AY223490.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 8 | VAR V ma. | Brazil128 | AY223494.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 9 | VAR V ma. | Brazil131 | AY223495.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 10 | VAR V ma. | Butler | AY223493.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 11 | VAR V ma. | India164 | AY223484.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 12 | VAR V ma. | Khateen | AY223491.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 13 | VAR V ma. | M-A-60 | AY223487.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 14 | VAR V ma. | M-Bl-60 | AY223488.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 15 | VAR V ma. | M-N-60 | AY223489.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 16 | VAR V ma. | M-Sok-60 | AY223486.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 17 | VAR V ma. | TajBarin | AY223482.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 18 | VAR V ma. | India-1967, ssp. major | X69198.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 19 | VAR V ma. | United Kingdom 1947 Higgins (Staffordshire) | DQ441446.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 20 | VAR V ma. | Afghanistan 1970 Variolator 4 | DQ437580.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 21 | VAR V ma. | Bangladesh 1974 (nur islam) | DQ441420.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 22 | VAR V ma. | Bangladesh 1974 (Shahzaman) | DQ441421.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |
| 23 | VAR V ma. | Bangladesh 1974 (Solaiman) | DQ441422.1 | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . | . . . |

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| 24 | VAR V ma. | Bangladesh 1975 v75-550 Banu | DQ437581.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 25 | VAR V ma. | Benin, Dahomey 1968 (v68-59) | DQ441416.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 26 | VAR V ma. | Botswana 1972 (v72-143) | DQ441417.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 27 | VAR V ma. | Botswana 1973 (v73-225) | DQ441418.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 28 | VAR V ma. | Brazil 1966 (v66-39 Sao Paulo) | DQ441419.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 29 | VAR V ma. | China Horn 1948; Sabin Lab July 1948 | DQ437582.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 30 | VAR V ma. | Congo 9 1970 (v74-227 Gispen) | DQ441423.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 31 | VAR V ma. | Congo 1970 v70-46 Kinshasa | DQ437583.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 32 | VAR V ma. | Ethiopia 1972 (Eth16 R14-1X-72 Addis) | DQ441424.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 33 | VAR V ma. | Ethiopia 1972 (Eth17 R14-1X-72 Addis) | DQ441425.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 34 | VAR V ma. | Germany 1958 Heidel-berg | DQ437584.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 35 | VAR V ma. | Guinea 1969 (005) | DQ441426.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 36 | VAR V ma. | India 1953 (Kali-Muthu-M50 Madras) | DQ441427.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 37 | VAR V ma. | India 1953 (New Delhi) | DQ441428.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 38 | VAR V ma. | India 1964 7124 Vellore | DQ437585.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 39 | VAR V ma. | India 1964 7125 Vellore | DQ437586.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 40 | VAR V ma. | Iran 1972 2602 Tabriz | DQ437587.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 41 | VAR V ma. | Japan 1946 (Yamada MS-2(A) Tokyo) | DQ441429.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 42 | VAR V ma. | Japan 1951 (Harper, Mas-terseed) | DQ441430.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 43 | VAR V ma. | Japan 1951 (Stillwell, Masterseed) | DQ441431.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |
| 44 | VAR V ma. | Korea 1947 (Lee, Master-seed) | DQ441432.1 | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |

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| 45 | VARV ma. | Kuwait 1967 (K1629) | DQ441433.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 46 | VARV ma. | Nepal 1973 V73-175 | DQ437588.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 47 | VARV ma. | Pakistan 1969 (Rafig Lahore) | DQ437589.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 48 | VARV ma. | Sierra Leone 1969 (V68-258) | DQ441437.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 49 | VARV ma. | Somalia 1977 (V77-1252) | DQ441438.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 50 | VARV ma. | SOM77_1605_139 | DQ441439.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 51 | VARV ma. | Somalia 1977; V77-2479 | DQ437590.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 52 | VARV ma. | South Africa 1965 (102 Natal, Ingwavuma) | DQ441435.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 53 | VARV ma. | South Africa 1965 (103 T'vaal, Nelspruit) | DQ441436.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 54 | VARV ma. | United Kingdom 1946 Harvey | DQ441444.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 55 | VARV ma. | Sudan 1947 (Juba) | DQ441440.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 56 | VARV ma. | Sudan 1947 (Rumbec) | DQ441441.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 57 | VARV ma. | Sumatra 1970 V70-228 | DQ441442.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 58 | VARV ma. | Syria 1972 V72-199 | DQ437592.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 59 | VARV ma. | Tanzania 1965 kembula | DQ441443.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 60 | VARV ma. | United Kingdom 1946 Hinden (Middlesex) | DQ441445.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 61 | VARV ma. | United Kingdom 1952 Butler | DQ441447.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 62 | VARV ma. | Yugoslavia 1972 V72-164 | DQ441448.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 63 | VARV ma. | Sumatra 1970 V70-222 | DQ437591.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 64 | VARV ma. | India-1967, ssp. major | NC_001611.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 65 | VARV ma. | *PoxSib* | JX080526.1 | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 66 | VAR V ma. | VD21, 17th century | KY358055.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 67 | VAR V ma. | Niger 1969 (001, importation from Nigeria) | DQ441434.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | .A. (H) | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | | | | D | D | D | L | A | I | K | K | P | E | A | K | R | E | A | I | V | K | A | D | I | E | K | C | | | | | | | | |
| | | | | GAT | GAT | GAT | CTT | GCA | ATT | AAA | AAG | CCA | GAG | GCT | AAA | CGC | GAA | GCA | ATT | GTT | AAA | GCC | GAT | ATA | GAA | AAG | TGT | | | | | | | | |
| 1 | VAR V mi. | Garcia-1966 | X76266.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | VAR V mi. | Garcia-1966 | Y16780.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | | | | D | D | D | L | A | I | K | K | P | E | E | K | R | K | A | V | V | K | A | E | I | E | K | C | | | | | | | | |
| | | | | --- | --- | --- | --- | --- | --- | --. | AAA | CCA | GAA | GAA | AAA | CGT | AAA | GCG | GTT | GTT | AAA | GCA | GAA | ATC | GAG | AAA | TGC | | | | | | | | |
| 1 | Vo-lepox | Volepox virus | DQ374627.1 | --- | --- | --- | --- | --- | --- | --. | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| | | | | D | D | D | M | A | I | K | K | P | E | E | P | V | K | R | K | V | V | K | N | K | N | K | H | K | V | V | K | A | D | | |
| | | | | GAT | GAT | GAC | ATG | GCG | ATT | AAA | AAA | CCA | GAA | GAA | CCA | GTT | AAA | CGT | AAA | GTA | GTT | AAA | AAC | AAA | AAT | AAA | CAT | AAA | GTG | GTT | AAA | GCA | GAT | | |
| 1 | SkPX V | SKUNK | AY299088.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2 | SkPX V | WA | NC_031038.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 3 | SkPX V | WA | KU749310.1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

Derivations of VACV/VARV-typical epitopes are marked in red. Matches are indicated by dots.

**Table S6** Mapping of epitope #5 based on 391 complete and partial amino acid sequences from the NCBI GenBank database.

| **Linear A27 epitope aa 68-71** | **OPXV genera** | **Number of DB entries** |
|---|---|---|
| IEKC | VARV major | 67/67 |
| | VARV minor | 2/2 |
| | VACV | 60/61 |
| | BPXV | 26/26 |
| | HSPV | 2/2 |
| | RPXV | 2/2 |
| | CMLV | 18/18 |
| | CPXV | 132/134 |
| | ECTV | 14/14 |
| | MPXV | 57/57 |
| | TaPXV | 3/3 |
| | RCNV | 1/1 |
| | VPXV | 1/1 |
| **(aa 93-96)** | SkPXV | 3/3 |
| **(aa 93-96)** | VACV | 1/61 |
| IEK**Y** | CPXV | 1/134 |
| **C-Terminus truncated** | CPXV | 1/134 |

Differences within the epitope sequence are highlighted.

**Table S7** Mapping of epitope complex #1A-D based on 391 complete and partial amino acid sequences from the NCBI GenBank database.

| **Linear A27 epitope aa 26-39** | **OPXV genera** | **Number of DB entries** |
|---|---|---|
| KKPEAKREAIVKAD | VARV major | 66/67 |
| | VARV minor | 2/2 |
| | VACV | 59/61 |
| | BPXV | 26/26 |
| | HSPV | 2/2 |
| | RPXV | 2/2 |
| | CPXV | 51/134 |
| | TaPXV | 2/3 |
| _KPEAKREAIVKAD | CPXV | 3/134 |
| | TaPXV | 1/3 |
| KKPEAKREAIVKA**E** | CPXV | 64/134 |
| _KPEAKREAIVKA**E** | CPXV | 2/134 |
| KKPEAKREAI**I**KAD | CMLV | 17/18 |
| K**R**PEAKREAI**I**KAD | CMLV | 1/18 |
| K**R**PEAKREAIVKA**E** | CPXV | 1/134 |
| K**N**PE**T**KREAIVKA**Y** | MPXV | 57/57 |
| KKPE**D**K**H**EA**T**VKAD | ECTV | 13/14 |
| _KPE**D**K**H**EA**T**VKAD | ECTV | 1/14 |
| KKPEAK**H**EAIVKAD | VARV major | 1/67 |
| KKPEAK**H**EAIVKA**E** | CPXV | 1/134 |
| KK**Q**EAKREAIVKA**E** | CPXV | 8/134 |
| _K**Q**EAKREAIVKA**E** | CPXV | 1/134 |
| KKPEAKREA**F**VKA**E** | CPXV | 1/134 |
| KKPE**V**KREAIVKA**E** | CPXV | 1/134 |
| _KPE**E**KR**K**A**V**VKA**E** | VPXV | 1/1 |
| KKP**DR**KRE**Q**IVKAD | VACV | 1/61 |
| _KPEAKR**KVVE**KAD | RCNV | 1/1 |
| K**SQRLNAKQLLKLR** | CPXV | 1/1 |
| **TSRSSTGS**A**NPS**A**S** | VACV | 1/61 |
| KKPE**EPV**KR**KVVKNKNKHKV**VKAD **(aa 26-49)** | SkPXV | 3/3 |

Differences within the epitope sequence are highlighted.

## 2.2 CHAPTER II

Submitted to:

PLOS Pathogens

# Characterization of an *in vivo* neutralizing anti-vaccinia virus D8 single chain Fragment variable (scFv) from a human anti-vaccinia virus-specific recombinant immunoglobulin library

Short title: Characterization of an *in vivo* neutralizing anti-D8 scFv

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**ABSTRACT**

Variola virus (VARV) was eradicated by active heterologous immunization using vaccinia virus (VACV), but VARV is still considered to be used as a potential biological weapon. Currently, the community is naïve and in case of an epidemic, a therapeutic support of the vaccination campaign is required. Potent neutralizing antibodies can protect against orthopoxvirus (OPXV) infections. Recombinant human immunoglobulin libraries are a new approach to engineer standardized, pathogen-free, and target optimized recombinant antibody preparations for prophylactic and therapeutic reasons. For the development of OPXV specific recombinant human single chain antibodies (scFvs), the IgG repertoire from four donors vaccinated intracutaneously with live vaccinia virus vaccine was amplified, cloned, and displayed onto M13 phages. The library resulted in a diversity of $\geq 4x10^8$ independent colonies. Different immuno-screening protocols against VACV Elstree revealed a predominant selection of scFv-clones specifically binding to the D8 protein. The obtained scFv-1.2.2.H9 was engineered into the larger human scFv-Fc-1.2.2.H9 and IgG1-1.2.2.H9 formats, to improve its apparent binding affinity and effector function within the human immune response. Similar binding kinetics were shown for scFv-1.2.2.H9 and scFv-Fc-1.2.2.H9 (1.61 nM and 7.68 nM, respectively), whereas, IgG1-1.2.2.H9 had a much higher affinity (43.82 pM). None of the purified recombinant 1.2.2.H9 antibodies were able to neutralize 100 pfu of VACV Elstree *in vitro.* However, after addition of 1% human complement, the VACV Elstree-neutralization abilities of the larger antibody formats scFv-Fc-1.2.2.H9 and IgG1-1.2.2.H9 were significantly improved (0.0776 µM and 0.01324 µM, respectively). In an *in vivo* passive immunization Naval Medical Research Institute (NMRI)-mouse-model, 100 µg purified scFv-1.2.2.H9 and the IgG1-1.2.2.H9 partially protected against the challenge with $4LD_{50}$ VACV Munich 1 as 3/6 mice survived. In contrast, the scFv-Fc-1.2.2.H9 showed no protection

effect. In conclusion, screening of human OPXV-immunoglobulin libraries is a useful tool for the identification of protective recombinant antibodies against OPXV.

**AUTHORS SUMMARY**

Variola virus (VARV) is the most famous species of the family *Poxviridae* and caused more fatalities than all other human infectious diseases taken together. Fortunately, VARV was officially declared eradicated in 1980. However, there are several other zoonotic poxvirus species endangering human health. To obtain protective recombinant antibodies against OPXV, we need to know the target regions of the antibody directed to poxviruses, resulting in a target directed screening of human immunoglobulin libraries. In our study, a human OPXV-immunoglobulin library was developed, using the immunoglobulin G (IgG) repertoire from four previously vaccinated donors. Interestingly, we obtained an antibody, which neutralizes VACV in vitro in the presence of complement and mediates partial protection in mice after VACV infection.

**INTRODUCTION**

Vaccinia virus (VACV) is considered the prototype of the genus *Orthopoxviruses* (Moss, 2006). VACV was used as a heterologous vaccine against variola virus (VARV), the causative agent of smallpox. Vaccination was terminated after eradication of VARV in 1980 leaving an increasing susceptible population (Fenner et al., 1988). While VARV solely infects humans (Fenner et al., 1988; Ladnyi and Breman, 1978), zoonotic poxviruses like cowpox virus (CPXV) and monkeypox virus (MPXV) can also cause severe and sometimes fatal infections (Becker et al., 2009; Campe et al., 2009; Kurth et al., 2008; Ladnyj et al., 1972; Reed et al., 2004; Vaughan et al., 2018; Vorou et al., 2008). While vaccination is generally safe and effective for prevention of smallpox, in well-documented cases of various adverse reactions in individuals, especially in immune-

compromised humans, caused by licensed vaccines (Cono et al., 2003; Fulginiti, 2003; Fulginiti et al., 2003), vaccinia immune globulin (VIG) have been used for treatment (Feery, 1976; Hopkins et al., 2004; Hopkins and Lane, 2004; Kempe, 1960). Nevertheless, VIG prepared from human donors bear the risk of quality variances between batches (Cono et al., 2003) and, even so it is reduced, a risk for the transmission of pathogenic agents (Sawyer, 2000). Currently, the availability of an immune-serum donor population is scarce.

Two antigenic distinct forms are present in VACV (Smith et al., 2002). The intracellular mature virus (MV) is the most abundant infectious form in *Orthopoxvirus* responsible for host-to-host transmission. Extracellular enveloped virus (EV) consists of an additional envelope and is thought to be important for dissemination within the host (Blasco and Moss, 1992; Boulter and Appleyard, 1973; Smith et al., 2002). Targets for neutralizing and protective antibodies were identified for MV surface proteins A13, A17, A27, D8, H3, L1, A28, and EV surface proteins B5 and A33 (Hsiao et al., 1999; Ichihashi and Oie, 1996; Lin et al., 2000; Rodriguez et al., 1985; Wallengren et al., 2001; Wolffe et al., 1995). One linear epitope, which is very conserved among OPXVs, was mapped at the C-terminus of the A13 (aa residues 59-69) (Xu et al., 2011). Moreover, six linear epitopes were mapped on the A27 protein of OPXVs (epitope #4: aa region 9-14, epitope complex #1A-D: between aa 26 and 39 and epitope #5: aa region 68-71) (Ahsendorf et al., 2019). Other studies discovered four epitope groups on the A27 protein of VACV (group I: aa residues 21-40; group II: discontinuous; group III: aa residues 81-100 and group 4: aa residues 91-110) (Kaever et al., 2016). Anti-B5 mAbs detected a conformational epitope (aa residues 22-130) (Chen et al., 2006) as well as two further ones localized to the SCR1–SCR2 border, and in the stalk region (Aldaz-Carroll et al., 2005). Hitherto, five conformational antigenic sites were identified on the D8 protein (Czerny et al., 1994) reacting

with neutralizeing antibodies VACV only in the presence of complement (Matho et al., 2012).

The 32 kDa protein D8 is a type 1 membrane protein and plays a role in the adsorption of the poxvirus to the host cell (Hsiao et al., 1999; Maa et al., 1990). The atomic structure revealed a carbonic anhydrase fold with a central positively charged crevice binding to chondroitin sulfate (CS) E on cell surfaces (Hsiao et al., 1999; Maa et al., 1990; Matho et al., 2014; Matho et al., 2012). Sequence alignments of D8 orthologs suggest a structural conservation of this binding site (Matho et al., 2012). A hexameric arrangement of D8 on the viral particle is proposed, mediated as a trimeric self-association of disulfide bonded homodimers, which might increase the avidity of D8 to CS (Matho et al., 2014). VACV D8L knock out mutants exhibited reduced infectivity in a BALB/c mouse model (Rodriguez et al., 1992) but replicated efficiently in cell culture (Niles and Seto, 1988). Using an optimized D8 DNA vaccine approach in a BALB/c mouse model, high titers of neutralizing antibodies were induced, which were protective against a subsequent challenge with VACV WR (Sakhatskyy et al., 2006). The characterization of a panel of murine monoclonal antibodies revealed four distinct antigenic groups on the D8 surface. Most effective were antibodies blocking the chondroitin sulfate CS-E interaction sites at K41, R44, K108, and R220 (Matho et al., 2014). In addition, D8 seems to possess a high and a low affinity binding region within the central crevice for CS-E and CS-A, respectively (Matho et al., 2018).

Phage display provides a robust technique to isolate monoclonal antigen binding fragments, which can then be converted into other larger molecules or full-size antibodies (Hoogenboom et al., 1998). Schmaljohn et al. (Schmaljohn et al., 1999) constructed a Fab phage display library from peripheral blood of one human donor. Here, we isolated the peripheral blood mononuclear cells of four donors immunized previously with Dryvax® and amplified the genetic information of IgG heavy and light chains using phages. We

describe the selection, engineering, and full *in vitro* as well as *in vivo* characterization of an anti-D8 antibody derived from a human IgG based phage display library.

## RESULTS

### Immunization, library construction, and characterization

The titer of circulating anti-VACV IgG in the peripheral blood of four volunteers was measured by ELISA. The determined titers ranged between $1.024x10^4$ and $4.096x10^4$ /ml serum. Volunteer 2, previously unvaccinated, revealed the lowest titer, whereas volunteer 1 vaccinated several times showed the highest anti-VACV titer (Fig 1).

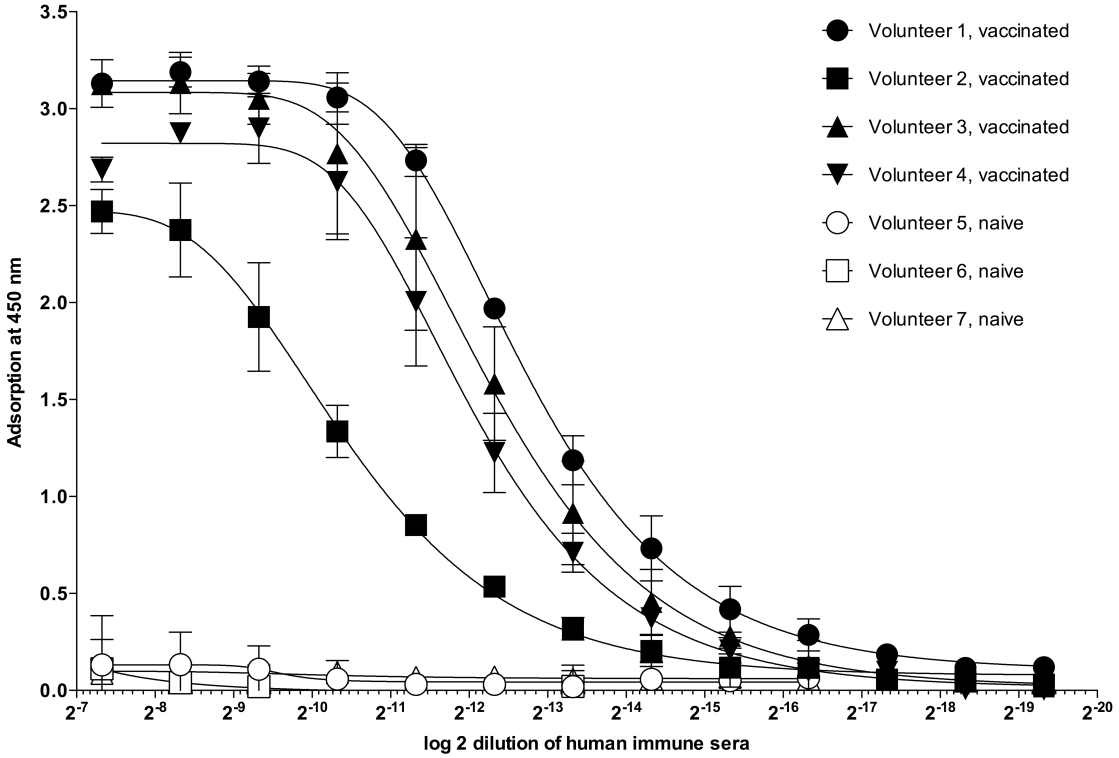



**FIG 1** Circulating anti-VACV IgG in sera of four volunteers was measured by indirect ELISA. Blood samples were either taken 20 days (volunteer 1 and 2) or 28 days (volunteer 3 and 4) after immunization with Dryvax® (Wyeth Laboratories, Inc., USA). Three volunteers (volunteer 5 to 7) were not vaccinated. Sera were titrated in two-fold serial dilutions.

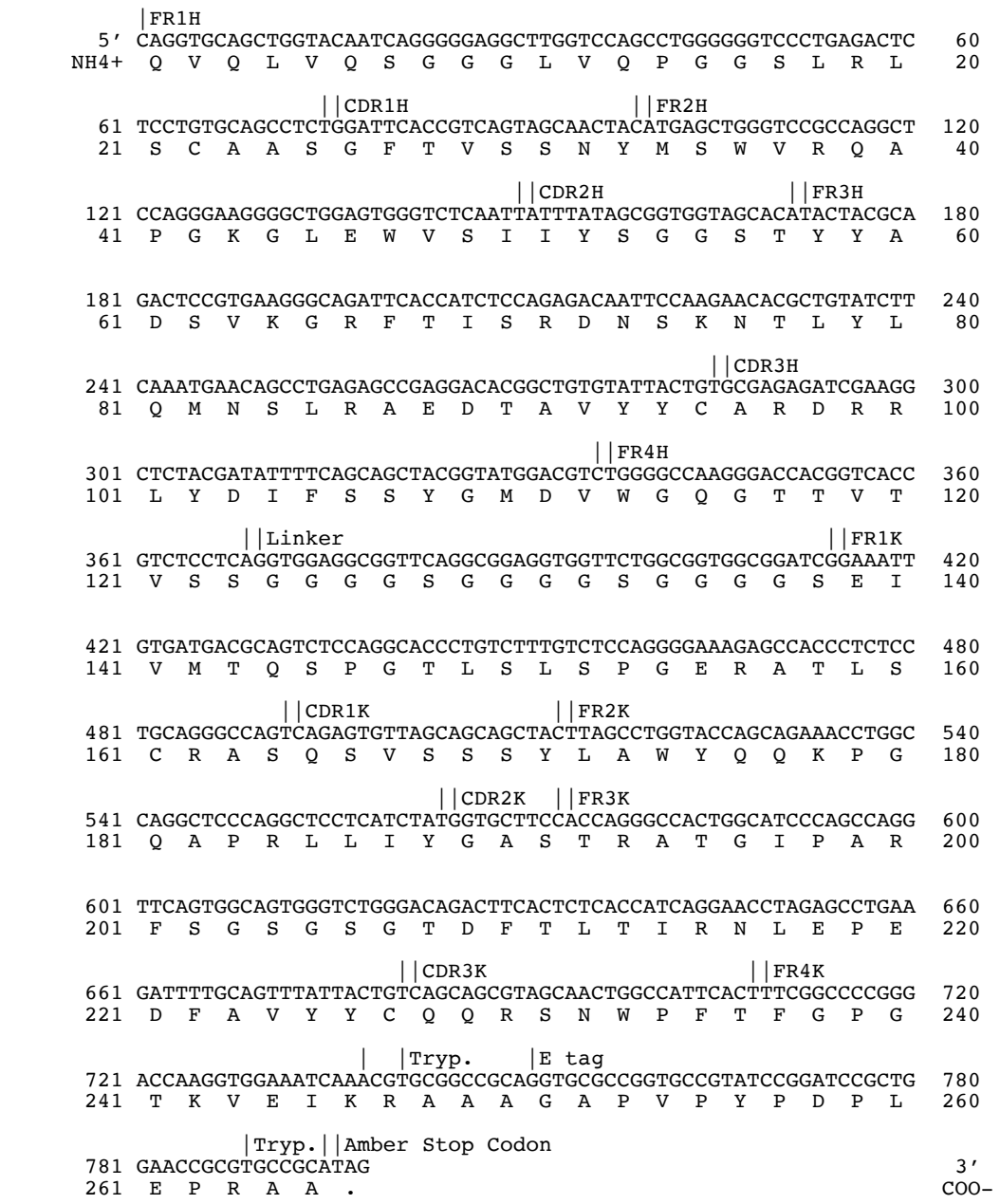
Neutralizing antibodies in the sera of the immunized individuals were determined through plaque reduction neutralization test using VACV Elstree. The sera showed neutralizing titers of $1.6x10^2$ to $3.2x10^2$ /ml.

To construct the scFv library, RT-PCR was performed using total RNA of at least $10^7$ cells per volunteer. It was possible to amplify a specific product with every primer combination for every sample (Fig S1 to S3). After pooling of the related 650 bp products and amplification of the variable regions with linker overhangs (Fig S4), the scFvs were joined by SOE-PCR (Fig S5). Subsequent to the ligation of scFv into pCANTAB5E, 40 transformations resulted in $\geq 4x10^8$ independent colonies.

**Selection of Vaccinia virus specific scFv**

Specific antibodies were selected for four rounds of enrichment. After each round, 176 individual *E. coli* HB2151 colonies were isolated for the production of soluble antibodies in a microtiter well format. No specifically binding scFvs were observed after the first selection round. The second selection round revealed one clone with a 25 times higher absorption over the background. The clone was designated as 1.2.2.H9. Two clones both from the third and fourth round of selection had absorptions with at least four times over background. In addition, the fourth round revealed one scFv, 1.4.1.C4, with an absorption of around 33 times over background. ScFv-1.2.2.H9 and 1.4.1.C4 were sequenced and according to the deduced amino acid residues classified to the human VH3/D3/JH6-VKIII/JK3 (1.2.2.H9) (Fig 2A) and VH1/D2/JH6-VKIII/JK2 (1.4.1.C4) families (Fig 2B).

A


|FR1H
5′ CAGGTGCAGCTGGTACAATCAGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTC 60
NH4+ Q V Q L V Q S G G G L V Q P G G S L R L 20
||CDR1H ||FR2H
61 TCCTGTGCAGCCTCTGGATTCACCGTCAGTAGCAACTACATGAGCTGGGTCCGCCAGGCT 120
21 S C A A S G F T V S S N Y M S W V R Q A 40
||CDR2H ||FR3H
121 CCAGGGAAGGGGCTGGAGTGGGTCTCAATTATTTATAGCGGTGGTAGCACATACTACGCA 180
41 P G K G L E W V S I I Y S G G S T Y Y A 60
181 GACTCCGTGAAGGGCAGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTT 240
61 D S V K G R F T I S R D N S K N T L Y L 80
||CDR3H
241 CAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATCGAAGG 300
81 Q M N S L R A E D T A V Y Y C A R D R R 100
||FR4H
301 CTCTACGATATTTTCAGCAGCTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACC 360
101 L Y D I F S S Y G M D V W G Q G T T V T 120
||Linker ||FR1K
361 GTCTCCTCAGGTGGAGGCGGTTCAGGCGGAGGTGGTTCTGGCGGTGGCGGATCGGAAATT 420
121 V S S G G G G S G G G G S G G G G S E I 140
421 GTGATGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCC 480
141 V M T Q S P G T L S L S P G E R A T L S 160
||CDR1K ||FR2K
481 TGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGC 540
161 C R A S Q S V S S S Y L A W Y Q Q K P G 180
||CDR2K ||FR3K
541 CAGGCTCCCAGGCTCCTCATCTATGGTGCTTCCACCAGGGCCACTGGCATCCCAGCCAGG 600
181 Q A P R L L I Y G A S T R A T G I P A R 200
601 TTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGGAACCTAGAGCCTGAA 660
201 F S G S G S G T D F T L T I R N L E P E 220
||CDR3K ||FR4K
661 GATTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCCATTCACTTTCGGCCCCGGG 720
221 D F A V Y Y C Q Q R S N W P F T F G P G 240
| |Tryp. |E tag
721 ACCAAGGTGGAAATCAAACGTGCGGCCGCAGGTGCGCCGGTGCCGTATCCGGATCCGCTG 780
241 T K V E I K R A A A G A P V P Y P D P L 260
|Tryp.||Amber Stop Codon
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261 E P R A A . COO–

B

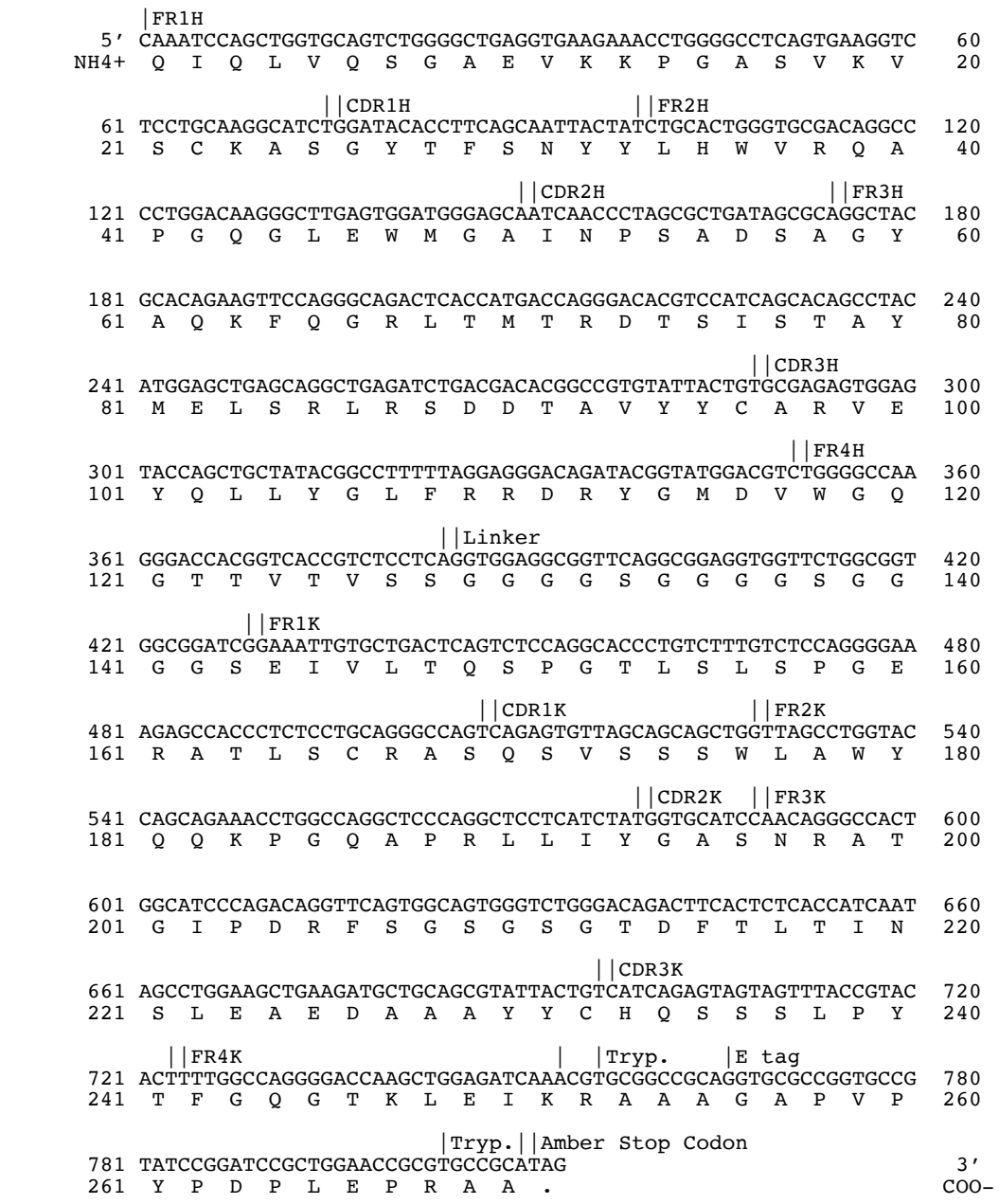


**FIG 2** Deduced amino acid sequence of variable domains of heavy and light chains of the anti-VACV 1.2.2.H9 (A) and 1.4.1.C4 (B).

Large-scale production and immune affinity purification were successful only for the scFv-1.2.2.H9 as the concentration of scFv-1.4.1.C4 was always very low.

**Binding of scFv-1.2.2.H9, scFv-Fc-1.2.2.H9, and IgG1-1.2.2.H9 by ELISA**

The $EC_{50}$ was measured using an indirect ELISA with two-fold serial dilutions in

triplicate starting with 10 µM of the respective antibody (Fig 3) and calculated according to Michaelis-Menten (Michaelis and Menten, 1913).

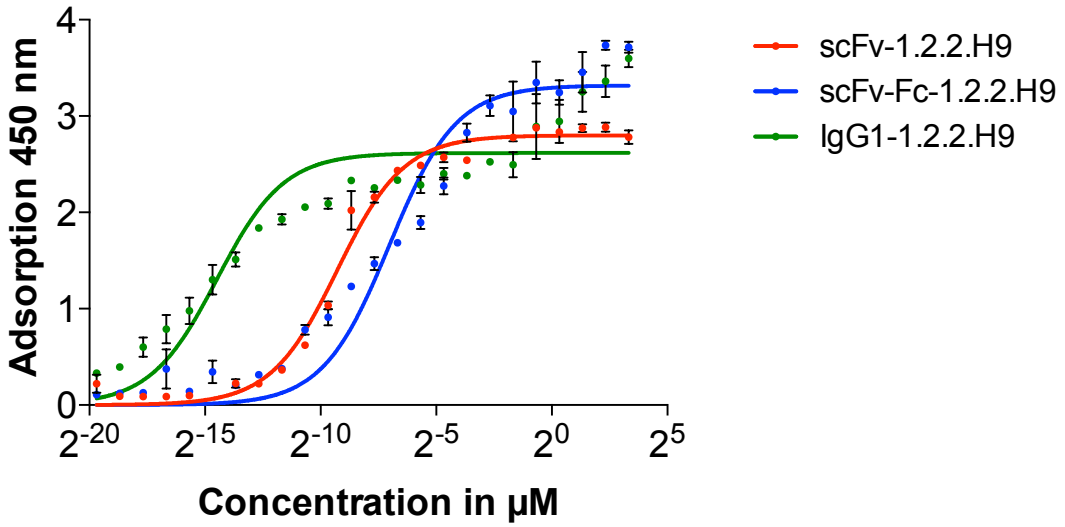


**FIG 3** Indirect ELISAs with scFv-1.2.2.H9, scFv-Fc-1.2.2.H9 and IgG1-1.2.2. H9 on 2 µg/ml VACV Elstree in triplicates were used to calculate the $EC_{50}$. The titration conducted in two-fold serial dilutions. The starting concentrations were 10 µM of purified antibodies.

The $EC_{50}$ of the scFv-1.2.2.H9 to VACV Elstree was 1.61 nM. This corresponds to values of $v_{max}$ of 3.015 and $K_m$ of 0.1285 µg/ml. The affinity of the scFv-Fc-1.2.2.H9 was determined with 7.68 nM, which corresponds to $v_{max} = 3.319$ and $K_m = 0.2369$ µg/ml. The lowest $EC_{50}$ of 43.82 pM was calculated for the IgG1-1.2.2.H9 molecule size. This equals $v_{max} = 2.617$ and $K_m = 6.57$ ng/ml.

Distinct capture abilities were observed with the scFv-1.2.2.H9 for $\geq 1.5625 \times 10^3$ pfu/ml VACV Elstree. No specific binding to scFv-1.2.2.H9 and the used detector anti-MVA-polyclonal rabbit Ab were seen with other viruses tested (Fig 4).

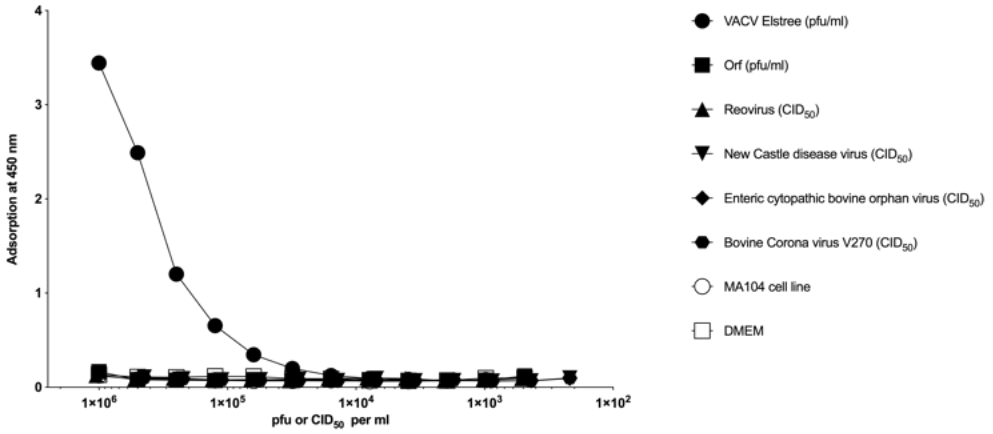


**FIG 4** Capture ELISA with 25 µg/ml 1.2.2.H9 coated onto 96-well microtiter plate. Different virus strains received from cell cultured material were tested: $10^6$ pfu/ml Vaccinia virus Elstree (VACV), $10^6$ pfu Parapoxvirus Orf D1701 (Orf), $10^{6.75}$ $CID_{50}$/ml bovine Corona virus V270 (BCV), $10^{7.25}$ $CID_{50}$/ml Reovirus (Reo), $10^{7.25}$ $CID_{50}$/ml New castle disease virus (ND), and $10^{7.25}$ $CID_{50}$/ml enteric cytopathic bovine orphan virus (ECBO). Negative controls were MA104-cell line and DMEM. The titration was conducted in two-fold serial dilutions. Specific binding was detected using anti-MVA-polyclonal rabbit Ab (1:5000).

**Competitive ELISA for epitope detection**

To reveal the epitope recognized by the scFv-1.2.2.H9, an inhibition ELISA with murine monoclonal antibodies was performed. Therefore, microtiter plates were coated with 2 µg/ml VACV. Purified scFv (Fig 5a) or mAb (Fig5b) (Ab1) adjusted to a starting concentration of 100 µg/ml were added in two-fold serial dilutions (100 µl/well). After incubation and five washing steps, purified scFv/mAb (Ab 2) was incubated under same conditions.

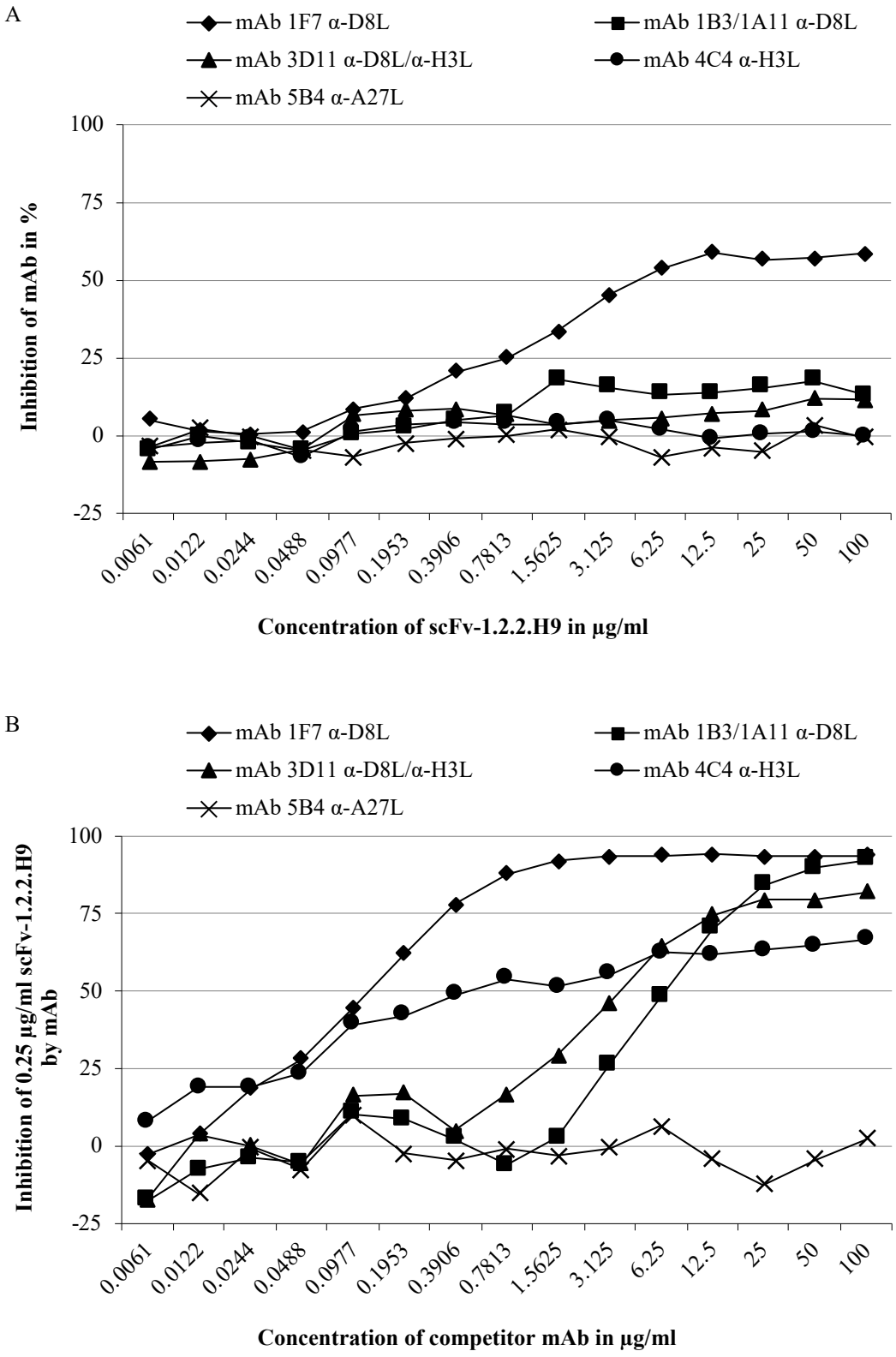


**FIG 5** Inhibition of mAb in binding to their target by scFv-1.2.2.H9 with a starting concentration of 100 µg/ml (A) and *vice versa* (B). Microtiterplates were coated with 2 µg/ml VACV Elstree. The results demonstrate the average from three independent assays. A reduction in the photometer adsorption ($OD_{450nm}$) of ≥50% indicated that two tested mAbs bind to identical or closely related antigenic sites.

The scFv-1.2.2.H9 was able to block the mAb 1F7/2F9 with up to 58%, whereas the scFv-1.2.2.H9 was blocked by the mAbs 1F7/2F9, 1B3/1A11, 3D11/2G7, and 4C4/2B6 in different concentrations. The mAb 1F7/2F9 inhibited the scFv-1.2.2.H9 with at least 0.1953 µg/ml and a maximum of 94%. A concentration of at least 0.7813 µg/ml mAb 4C4/2B6 was sufficient in the same assay. The highest concentration of 12.5 µg/ml was needed to block the scFv-1.2.2.H9 by mAbs 3D11/2G7 and 1B3/1A11. The target of 1.2.2.H9 was assigned to the VACV D8 epitope #2B (Czerny et al., 1994).

### *In vitro* neutralization

The classical plaque reduction neutralization test (PRNT) was performed in triplicates with a starting-concentration of 10 µM of the respective 1.2.2.H9 molecule. The scFv-1.2.2.H9 with and without human complement as well as the scFv-Fc-1.2.2.H9 and IgG1-1.2.2.H9 without addition of human complement showed no neutralization. However, after addition of 1% human complement both molecules with an Fc part neutralized with 0.0776 µM and 0.01324 µM, respectively (Fig 6).

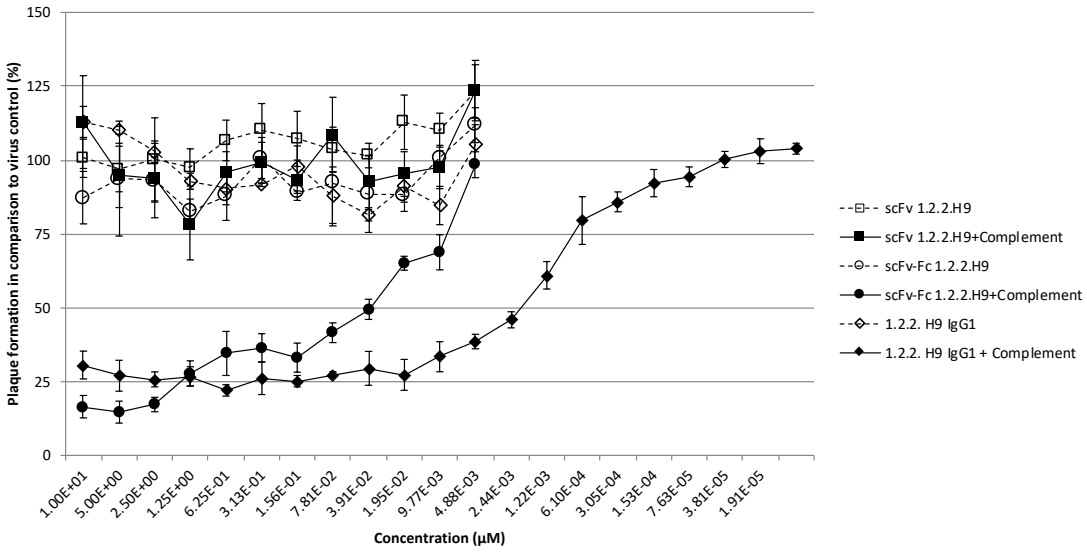


**FIG 6** The PRNT was performed in triplicates with a starting-concentration of 10 µM of the respective 1.2.2.H9 molecule. The scFv-1.2.2.H9 with and without complement

as well as the scFv-Fc-1.2.2.H9 and IgG1-1.2.2.H9 without addition of human complement showed no neutralization. However, after addition of 1% human complement both molecules with an Fc part neutralized with 0.0776 µM and 0.01324 µM, respectively.

### *In vivo* neutralization

The *in vivo* neutralizing ability of the antibodies were investigated using an NMRI mouse challenge model. All 1.2.2.H9 molecules were investigated and a polyclonal anti-MVA rabbit immune sera (pAb) served as positive control. As negative control groups mice were injected with either PBS or anti-EHV1 6B11 mAb (kindly provided by Hermann Meyer, Bundeswehr Institute of Microbiology, Munich, Germany). Six groups consisting of six mice were injected intra-peritoneal with the respective antibody or control 24h before challenge with 4LD50 VACV Munich 1. Weight was monitored daily. Of mice receiving the scFv-1.2.2.H9 or IgG1-1.2.2.H9, always three were protected and three were sacrificed at days 7, 10, and 13 or on days 10 and 11 post challenge, respectively. Five out of six mice of the scFv-Fc-1.2.2.H9 group were sacrificed on days 8-11 post challenge. One animal in the pAb positive control group had to be sacrificed at day 9, the remaining five animals survived the challenge until termination of the experiment at day 28. All animals of the two negative control groups (PBS and anti-EHV1 6B11 mAb) were sacrificed between day 6 and 11.

The least square-means of weight of the surviving and sacrificed animals are shown in Fig 7. Compared to the surviving animals, all sacrificed animals showed a significant reduction of weight development during the post challenge time period except the animals of pAb group. This is explained by the fact that at the beginning of the experiment this group showed a weight gain followed by a marked decrease in weight development.

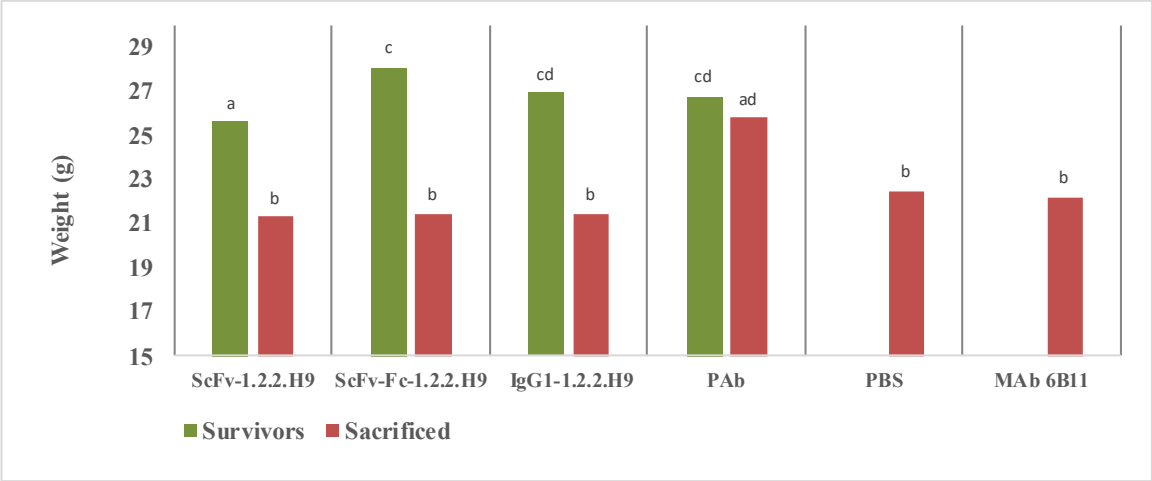


**FIG 7** Least squares means for weight (g) for the effect of survival ability of different antibody-treatment groups. Different alphabets (a, b, c) illustrate the significant differences between the least squares means of different factor levels ($P<0.05$).

The development of the infection-induced weight loss is reflected in the survivability of the animals of the different treatment groups, illustrated by Kaplan-Meier survival analyses in Figs 8 and 9.

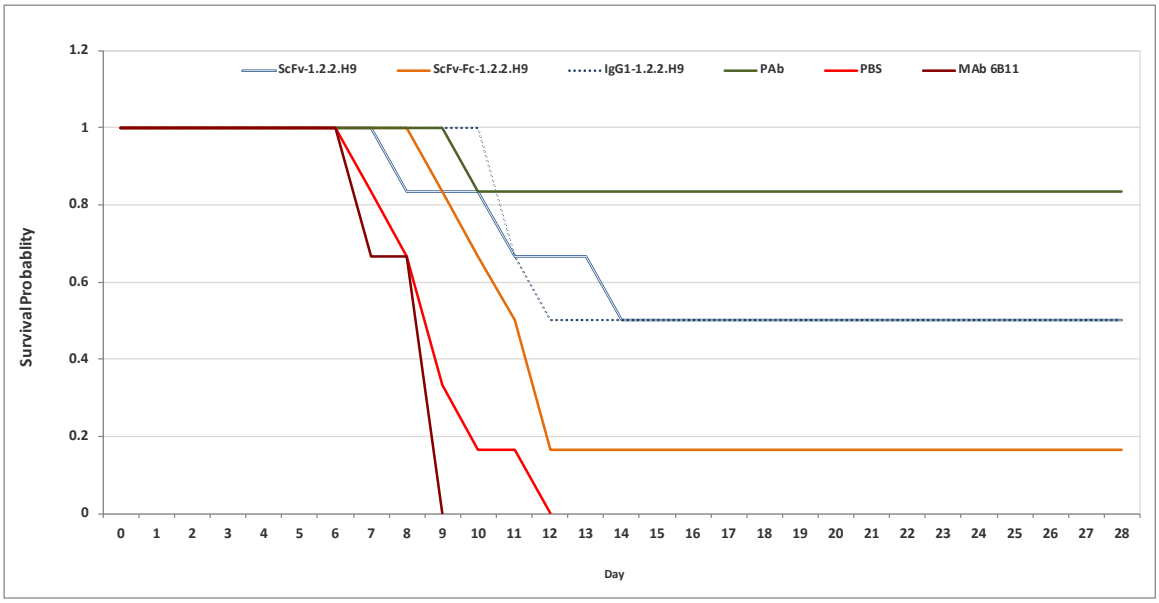


**FIG 8** Survival rate of mice during the passive immunization experiment.

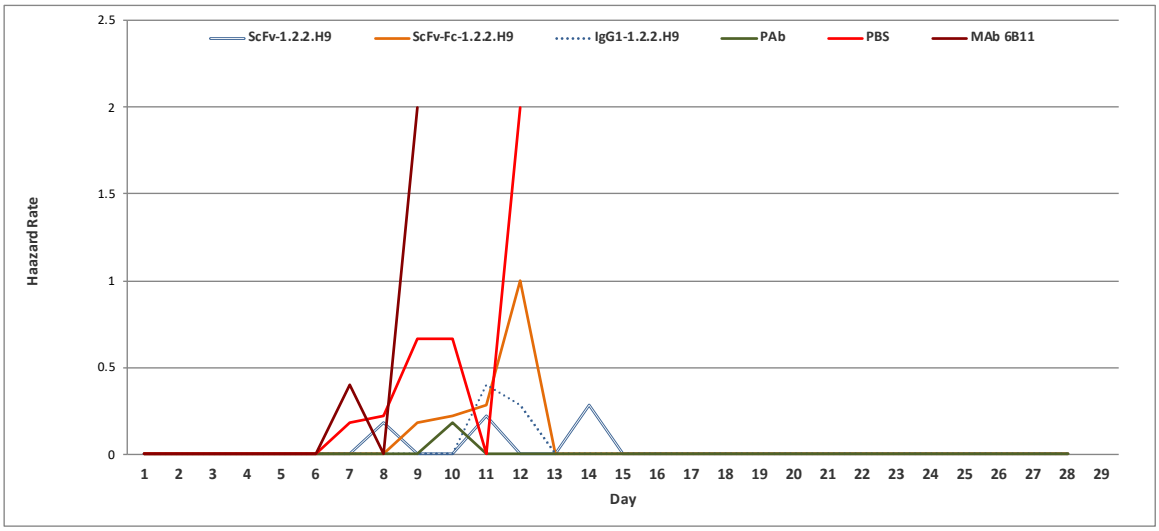


**FIG 9** Hazard rate derived from the non-parametric survival function estimated with the Kaplan-Meier method.

At the end of the experiment (day 28 post challenge), heart, liver, spleen, lung, brain, and kidney of all animals were harvested and examined for viral loads by real-time PCR (Figure 10).

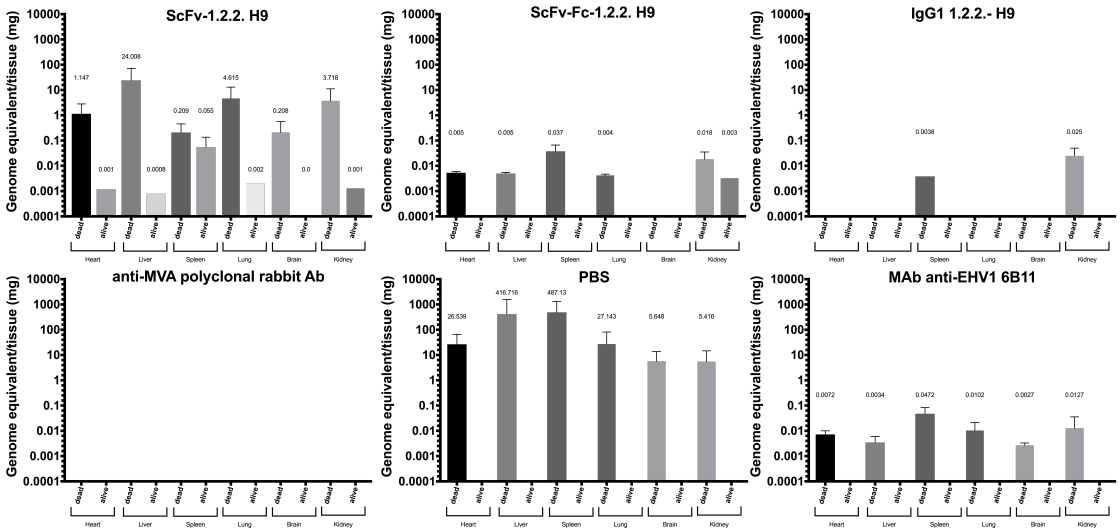


**FIG 10** Concentration of the VACV DNA in different organs of mice after challenge of VACV Munich1.

The viral loads showed differences between surviving and dead animals as well as between the different antibody-treatments. The highest viral load of the scFv-1.2.2.H9-inoculated surviving mice was found in the spleen, followed by lung, kidney, heart, and liver. Sacrificed animals showed a higher viral load in liver, followed by lung, kidney, heart, brain, and spleen. In scFv-Fc-1.2.2.H9-inoculated sacrificed mice a higher viral load was observed in spleen and kidney, followed by heart, liver, and lung, while the surviving mouse showed viral particles solely in the kidney. The IgG1-1.2.2.H9-inoculated sacrificed mice had detectable viral loads only in kidney and spleen. No virus DNA was found in the organs of mice inoculated with the pAb. In the case of PBS and the anti-EHV1 mAb the highest viral load was found in the spleen, followed by kidney, lung, heart, liver, and brain.

**DISCUSSION**

In this study the IgG repertoire from four donors vaccinated intracutaneously with live vaccinia virus vaccine was amplified, cloned, and displayed onto M13 phages to develop OPXV specific recombinant human scFv antibodies. The antibodies generated in this way are safer and applicable in human therapy. This study demonstrates the neutralizing activity against VACV using only one monoclonal scFv, as the smallest human antibody derived molecule. Schmaljohn et al. (Schmaljohn et al., 1999) were the first who established a recombinant human Fab-library and selected specific binding molecules to a number of VACV proteins. Two Fabs bound to a 35 kDa protein, while one Fab was precipitated with a 34 kDa protein. Those Fabs were able to neutralize *in vitro*. While our library with higher diversity was constructed from peripheral B lymphocytes of four different volunteers, others selected B5 specific recombinant Fabs from a phage display library from immunized chimpanzees (Chen et al., 2006) as B cells were isolated from bone marrow 11 weeks after immunization. A majority of 80-90% of antigen-specific

plasma cells resides from day 45 in the bone marrow and can be detected for at least one year after immunization (Slifka et al., 1995). To avoid an invasive intervention to the study volunteers, we preferred to collect blood samples and isolate peripheral blood lymphocytes. There, the amount of specific B cells can be determined with about 20% (Schmaljohn et al., 1999). Therefore, we expect a high amount of VACV unspecific antibodies within the library, which can be disadvantageous in the selection procedure. However, after a selection procedure on full VACV particles, specific binding fragments were obtained.

The selected scFv-1.2.2.H9 was engineered to a full-size human IgG to improve the binding affinity and effector function. The VACV-neutralizing abilities of the scFv-Fc-1.2.2.H9 and the IgG1-1.2.2.H9 were improved by the addition of 1% human complement *in vitro*, while the addition of complement had no effect on the scFv-1.2.2.H9, because of the lacking Fc-region. The complement system is a complex of the innate immune response, consisting of over 30 proteins (Dunkelberger and Song, 2010). Activation of complement leads to a cascade of enzymatic reactions, resulting in the so-called membrane attack complex, which forms a pore into the cell membrane followed by cell lysis (Sarma and Ward, 2011). Furthermore, complement got its name by its complementing effect on the antibodies, by linking them over their Fc-region to get a larger construct (Dunkelberger and Song, 2010). Other authors also characterized VACV neutralizing abilities of anti-D8 mAbs only in the presence of complement, because complement is needed to increase the footprint of those mAbs (Matho et al., 2012). These complement-dependent findings were also confirmed by other mAbs directed against the VACV A27 (Ahsendorf et al., 2019; Kaever et al., 2016) and B5 (Benhnia et al., 2009a; Benhnia et al., 2009b) proteins.

Several studies confirmed the feasibility of protection against OPXV infections using monoclonal antibodies targeting neutralizing epitopes of the EV and MV (Benhnia et al.,

2009a; Czerny and Mahnel, 1990; McCausland et al., 2010; Ramirez et al., 2002). *In vivo*, the scFv-1.2.2.H9 and the IgG1-1.2.2.H9 were able to protect 50% of mice from a lethal challenge with VACV Munich 1, whereby the scFv-Fc-1.2.2.H9 was not able to neutralize the virus. However, the viral loads of the scFv scarified animals were much higher than these ones, found in the scFv-Fc-1.2.2.H9-group. Moreover, due to the small numbers of six mice per antibody-group, we cannot conclude that the scFv-Fc-1.2.2.H9 protects less than the scFv- and the IgG1-1.2.2.H9. As mentioned above, in vaccinia neutralization the importance of complement especially the Fc receptors (FcγR) is proven. Human IgG subclasses showed a similar affinity to the mouse FcγR as to the corresponding receptors in humans. Moreover, human IgG binds mouse FcγR with similar affinities as mouse IgG (Dekkers et al., 2017). Interestingly, complement- and FcγR-deficient mice showed an enhanced susceptibility to infections (Huber et al., 2001; Suresh et al., 2003). Thus, we assume, that the two additional mice, that died more in the scFv-Fc-1.2.2.H9 group might have a lack of FcγR. Furthermore, the mice of the negative control groups died earlier. The viral loads detected in their organs were much higher than those found in the scFv-Fc-1.2.2.H9 immunized sacrificed animals. In addition, the sacrificed animals of all groups showed a significant weight reduction, except only one mouse that died in the pAb control group. The weights of the survivors of the IgG1-1.2.2.H9 and the pAb treatment groups were not significantly different. However, in comparison with the IgG1-1.2.2.H9 and the pAb groups, the scFv-1.2.2.H9 and the scFv-Fc-1.2.2.H9 group showed a slight reduction and increase in body weight, respectively.

Another important point in the development of recombinant antibodies is the accurate mapping of their epitopes. The target of the scFv-1.2.2.H9 was assigned to the 32kDa D8 protein of VACV, which is part of the MV, the most common infectious form (Smith and Vanderplasschen, 1998). The function of the conserved D8 protein is the adsorption of virus to the host cell surface due to its binding to chondroitin sulfate (Hsiao et al., 1999;

Maa et al., 1990). The reciprocal blocking effect of scFv-1.2.2.H9 and the mAb 1F7/2F9 led to the conclusion that the target of scFv-1.2.2.H9 is the same or a part of the conformational epitope #2B region, recognized by mAb 1F7/2F9 (Czerny et al., 1994). Further western blotting analysis of the unselected hyperphage pool revealed the possibility to select scFv against other VACV proteins e.g. using single protein directed panning methods. The goal in passive and therapeutic protection might be the combination of EEV and IMV specific monoclonal antibodies, which can block a poxvirus infection at different replication stages (Hooper et al., 2000; Lustig et al., 2005). Well-characterized monoclonal antibodies produced in GMP controlled cell culture systems or plants for example have the advantage of always the same quality and functionality. Moreover, the data on antigenic sites for cross-reacting or monospecific neutralizing antibodies are of high relevance for target directed screening of human immunoglobulin libraries to generate specifically engineered human recombinant antibodies, which might help in controlling any future outbreaks of zoonotic orthopoxviruses.

## MATERIAL AND METHODS

### Immunization and lymphocyte preparation

Four human volunteers were immunized via scarification with Dryvax® (Wyeth Laboratoires, Marietta, USA) according to the manufacturer's directions with a two-pronged needle. Volunteer 1 was vaccinated five years ago whereas Volunteer 2 was naive. Volunteers 3 and 4 have been immunized more than ten years ago. After 20 (Volunteer 1 and 2) or 28 (Volunteer 3 and 4) days post immunization, approximately 500 ml peripheral blood was collected, and respective sera were tested in duplicates for the presence of circulating anti-vaccinia virus (VACV) IgG in an ELISA. Sera from three unvaccinated volunteers (volunteer 5 to 7) were used as negative serum controls and to determine the unspecific background. Plates were coated either with 2 µg/ml VACV or BSA (as

irrelevant protein). For the calculation of the antibody titer specific for VACV, the adsorption measured on VACV was corrected with the corresponding value determined on BSA. The cut-off value was calculated according to Frey et al. (Frey et al., 1998) for each respective dilution:

$$\text{Cut off} = \overline{X} + SD \times f$$

with

$\overline{X}$ = mean of independent control sera

SD = standard deviation of independent control sera

$f$ = 3.372 for confidence level 95% (Frey et al., 1998).

Peripheral blood mononuclear cells were isolated using Ficoll-Paque PLUS density gradient (GE Bioscience, Freiburg, Germany). Total RNA was extracted from at least $10^7$ cells per volunteer with the RNeasy MiniKit (Qiagen, Hilden, Germany) followed by cDNA synthesis using oligohexamers ($pdN_6$) (Invitrogen, Karlsruhe, Germany) as manufacturers´ instructions.

**Library construction**

The amplification of the variable region of IgG-heavy and κ- and λ-light chains was performed with degenerated primer set (BACK-primers) binding to the first 23 bp of framework region (FR) 1 of the variable regions and primers binding to the first constant regions either of human IgG1 to 4 or κ- and λ-light chains (FOR-primers, Table S1). In parallel, the quality and integrity of the cDNA was monitored with a primer pair amplifying 788 bp of human Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, (Hurteau and Spivack, 2002)). A 50 µl PCR reaction consisted of 1-2 µl cDNA, 1x PCR buffer (7.5 mM Tris HCl pH 9, 0.2 mM $MgCl_2$, 5 mM KCl, 2 mM $(NH4)_2SO_4$; Biotools),

0.4 µM of one BACK-primer, 0.4 µM of one FOR-primer, 10 mM of each dNTP, and 2 U DNA polymerase (Biotools). Amplification was performed with 10 min denaturation at 95ºC, 25 cycles of 94ºC for 1 min, annealing at 58ºC for 1 min, elongation at 72ºC for 2 min, and final elongation for 10 min. The length and purity of the products were visualized by 1% agarose gelelectrophoresis. The PCR-products with a size of about 650 bp were purified with the DNA Clean & Concentrator-5-kit (Zymo Research Europe, Glasgow, Scotland).

In the following second semi-nested PCR, the variable regions of heavy and light chains were amplified. The purified corresponding PCR-products of each volunteer were pooled to equal amounts, of which 50 ng was used for amplification with Phusion polymerase and the 5xGC-buffer (Finnzymes, Espoo, Finland). In case of the variable region of the heavy chains, the BACK-primer set used in the first PCR was combined with primers binding to FR4 (Table S2). Those primers were extended with 20 bp of an overlapping sequence coding for the $(G_4S)_3$-linker. The complementary part is coded by an overhang in the primers annealing to FR1 of the light chains. The reactions were incubated for 30 s at 98ºC, followed by 30 cycles at 98ºC for 30 s, 60ºC for 30 s, 72ºC for 30 s, and final elongation for 10 min. The variable regions with their overhangs were gel-purified (MinElute-Kit; Qiagen, Hilden, Germany).

The formation of single chain Fragment variable (scFv) occurred with splicing-by-overlap-extension. In the first step, 300 ng of mixed to equal amounts of heavy and light chain variable regions were joined in the absence of primers. In a second step, 5 µl of the connected products were re-amplified with outer primers including restriction sites for *Sfi*I (3'-GTC CTC GCA ACT GC*G GCC CAG CCG GCC* ATG GCC-HuVH BACK-5') and *Not*I (3'-GAG TCA TTC TCG ACT T*GC GGC CGC*-HuJκ/λ FOR-5'). The scFvs were gel-extracted and cleaved first by *Sfi*I (NEB, Frankfurt a. M., Germany) and then by *Not*I (NEB, Frankfurt a. M., Germany). The fragments were ligated into the phagemid

pCANTAB5E (GE Biosciences, Freiburg, Germany) and electroporated into *Escherichia coli*, TG1. Colonies were grown overnight either on Bioassay dishes (NUNC, Germany) or in 8 cm diameter petri dishes (Sarstedt, Germany) for titration on 2×TYG-A (tryptone 16 g/l, yeast 10 g/l, NaCl 5 g/l, glucose 2%, ampicillin 100 µg/ml) agar (15 g/l). Colonies were scraped into 5 ml 2×TYG-A-15% glycerol and stored at -80°C.

**Cells and viruses**

The permanent monkey kidney cell line MA104 cultured in minimum essential medium (MEM) and supplemented with 7% fetal calf serum was used to propagate the VACV strains Elstree and Munich1 (M1) (for references see (Czerny and Mahnel, 1990). Infectivity titers were determined on 24-well plates (Nunc, Wiesbaden, Germany) and calculated as plaque forming units (pfu/ml). For plaque reduction neutralization test, Vero cells cultured in MEM and supplemented with 5% fetal calf serum were used and maintained in the same way as MA104.

**Gradient purification of Vaccinia viruses Elstree and Munich1**

Vaccinia viruses Elstree as well as Munich1 (M1) were grown in MA104 cells for one day and harvested by pelleting at 13,700×g for 2h. The pellets were resolved with 1 mM Tris-HCl, pH 9.0, and sonicated/freeze-thawed three-times to lyse the cells. Cell debris and nuclei were removed by centrifugation (2,200×g, 10 min). The supernatant was layered onto a 36% (w/v) sucrose cushion prepared in 1 mM Tris-HCl, pH 9.0. After ultracentrifugation in a SW28-rotor (Beckman Coulter GmbH, Krefeld, Germany) at maximum 112,700×g for 90 min the pellet was resolved with 1 mM Tris-HCl, pH 9.0 and applied onto a 60%/40%/20% (w/w in 1 mM Tris-HCl, pH 9.0) sucrose discontinuous gradient and again ultra-centrifuged at maximum 40,018×g for 90 min in a SW40-rotor (Beckman Coulter GmbH, Krefeld, Germany). The visibly white viral bands were

collected and washed with 1 mM Tris-HCl, pH 9.0 to remove residual sucrose solution (maximum 11,1160×g for 1h). Finally, the pellets were resolved in 1 mM Tris-HCl, pH 9.0 and the protein concentrations were determined (Lowry et al., 1951).

**ScFv selection using purified Vaccinia virus Elstree**

The scFv-phage library was panned using VACV Elstree in four rounds. Phages were rescued by infection of log phase *E. coli* TG1. The first phage rescue was performed from $6x10^9$ bacteria cells, which were cultured in 1 l 2×TYG-A. The cells were grown to an optical density at 600 nm (OD600nm) of 0.4-0.5. M13K07ΔpIII (Hyperphage, Progen, Heidelberg, Germany) and a multiplicity of infection (MOI) of 30 were used to infect 150 ml of the bacterial suspension. The incubation was stationary at 37°C for 30 min followed by shaking with 250 rpm (Sartorius Certomat® BS-1, Goettingen, Germany) under the same conditions. The infected cells were harvested by centrifugation (137,000xg/ 4°C/ 20 min) and resuspended in 1 l 2×TY-A-K (kanamycin 70 µg/ml). For further replication and phage production, the infected cells were incubated under gentle shaking at 30°C over night (Sartorius Certomat® BS-1, Goettingen, Germany). Phages were precipitated two times by the addition of 1/5 volume 20 % polyethylene glycol 8000/2.5 M sodium chloride (20% PEG/2.5M NaCl). The phages were resuspended in 1 ml PBS/15% glycerol. Aggregates were removed by high-speed centrifugation (10,000xg/ 4°C/ 1 min), and the supernatant stored at 4°C over night. For the successive selection rounds, $10^9$ bacteria cells were grown in 100 ml media. Twenty milliliters of the bacteria solution were infected with M13K07 MOI 30 (NEB, Frankfurt a. M., Germany). Phage packaging was performed in 200 ml 2×TY-A-K.

Unselected phages were used in a western blotting assay on VACV Elstree gradient to determine the range of VACV proteins detected by the phage repertoire. The visualization occurred with HRP/anti-M13 monoclonal conjugate (GE Healthcare, Freiburg,

Germany).

For phage selection, one well of a 96-well MaxiSorb plate (Thermo Fisher Scientific, Langenselbold Site, Germany) was coated with 10 µg VACV Elstree diluted in 150 µl carbonate-bicarbonate buffer (pH 9.6) at 37°C for 4h and stored at 4°C over night. The well was washed three times using a Tecan-washer (Tecan, Männedorf, Switzerland) for standardized washing procedure. The virus coated and an additional empty well were blocked with 2% skimmed milk powder (SMP) and 10% FCS in PBS/0.1% Tween at 37°C for 2h. Approximately 1 to $5\times10^{12}$ cfu phages diluted in 150 µl PBS/2% SMP/10% FCS/0.05% Tween were incubated in the pre-blocked well at room temperature for 1h. The blocking solution of the coated well was replaced by the pre-blocked phages. Incubation occurred at room temperature for 2h. Unbound phages were removed by washing ten-times in the first panning round followed by a fifteen-times washing procedure using PBS/0.1% Tween in the successive rounds. Bound phages were eluted by enzymatic cleavage with 200 µl trypsin solution (10 µg/ml PBS) and an incubation period of 30 min at 37°C. Ten microliter were added to 1 ml previously prepared log phase *E. coli* HB2151 while the remaining solution was used to infect 10 ml log phase *E. coli* TG1. The cells were streaked on 2×TYG-A plates and incubated at 30°C over night. Phage input and output titers were calculated for each panning round as colony forming units. Following each panning round, 176 *E. coli* HB2151 clones were pre-cultured in 150 µl 2×TYG-A in a Multiple Well Plate 96-Well (Sarstedt, Nümbrecht, Germany).

**Screening of randomly selected scFv producing HB2151 clones in indirect ELISAs**

In order to produce antibody fragments without pIII fusion, 0.5 µl of the *E. coli* HB2151 pre-cultures were transferred into 100 µl 2×TYG(0.1%)-A and incubated at 30°C for 4 h. The expression was induced by the addition of IPTG to a final concentration of 2 mM

dissolved in 50 µl 2×TY-A by gentle shaking at 30°C overnight (Sartorius Certomat® BS-1, Goettingen, Germany). The cells were pelleted (137,000xg/ 4°C/ 20 min) and the supernatants were applied in an ELISA for pre-screening. Wells of two Maxisorb microtiter plates were coated either with 100 µl of 2 µg/ml VACV Elstree or BSA as negative control. All subsequent steps were performed at room temperature. The plates were washed three times and blocked for 2h with 300 µl/well PBS/2% SMP/0.1% Tween 20. The block solution was renewed with 50 µl. On each plate 50 µl scFv-supernatant was added. The monoclonal antibody (mAb) 5B4/2F2 binding to epitope 1A (Ahsendorf et al., 2019; Czerny et al., 1994) of VACV A27 was used as positive control whereas the Parapoxvirus Orf specific mAb 3C5 (Czerny et al., 1997) was applied as negative control for the coated virus. The wells were washed five times with PBS/0.1% Tween after 2h of incubation. Bound scFvs were detected with an HRP conjugated anti-E tag polyclonal antibody (Abcam, Cambridge, UK) diluted 1:5000, while the mAbs were detected with a polyclonal goat anti-mouse antibody (Dako, Hamburg, Germany) diluted 1:5000. After ten times washing, 100 µl/well of TMB (3, 3', 5, 5'-tetramethylbenzidine) substrate was added and the covered plates were incubated for 20 min. The reaction was stopped with 50 µl/well of 1 M HCl and the absorbance was measured at 450 nm.

Plasmids of ELISA positive colonies were isolated from 5 ml media using the MiniPrep Kit (Qiagen, Hilden, Germany). The genes encoding the variable regions of the heavy (VH) and light (VL) chains were sequenced using vector specific forward primers S1 (5'-CAA CGT GAA AAA ATT ATT ATT CGC-3') and R1 (5'-CCA TGA TTA CGC CAA GCT TTG GAG CC-3') and reverse primers S6 (5'-GTA AAT GAA TTT TCT GTA TGA GG-3') and R2 (5'-CGA TCT AAA GTT TTG TCG TCT TTC C-3'). The sequences were analyzed with the DNAStar program (SeqMan Pro and MegAlign. Version 12.0. DNASTAR. Madison, WI). The deduced amino acid sequences were used to

classify the presumed family and germline origin by search of IMGT/V-QUEST (Brochet et al., 2008; Giudicelli et al., 2011).

**Purification of selected scFvs**

Two monoclonal scFvs with high ELISA values were produced in one liter. The culture was centrifuged at 1500×g at 4°C for 15 min. The supernatant was precipitated on ice on a tumbler with the same volume of saturated solution of ammonium sulfate for 1h. Thereafter, the resuspension was centrifuged with 1500×g at 4°C for 10 min. The pellet was resuspended in 5 ml 1 M Tris-HCl, pH 8.0. The periplasmatic fraction was collected by addition of 20 ml ice-cold 1×TES (200 mM Tris-HCl, 500 mM Ethylenediaminetetraacetic Acid, 500 mM Sucrose, pH 8.0) to the bacterial pellet. A total of 33 ml of 1/5×TES was added. The suspension was incubated on ice while shaking for at least 30 min. $MgSO_4$ was added to a final concentration of 5 mM. Centrifugation conducted with 1500×g at 4°C for 10 min. The supernatant contained the periplasmatic fraction of the scFv.

Both filter-sterilized and to pH 7.0 to 8.0 adjusted fractions were purified with an anti-E tag column (GE-Healthcare, Freiburg, Germany). The column was equilibrated with binding buffer (20 mM phosphate buffer, 0.005% $NaN_3$ pH 7.0). The samples were applied and washed with binding buffer. After removing all unbound proteins, the scFv was eluted with 0.1 M glycine buffer pH 3.0 into neutralization buffer (0,1M Tris, 0.005% $NaN_3$ pH 8.2) (10:1). The protein concentration was determined after dialyzing against PBS (Lowry et al., 1951).

**Competitive ELISA for epitope detection**

An inhibition ELISA with murine monoclonal antibodies was used to reveal the epitope recognized by the specific binding scFv. The associated epitopes of the mAbs on the

VACV proteins are described in Table 1. Flat-bottom 96-well microtiter plates (Nunc MaxiSorp) were coated with 2 µg/ml VACV in carbonate/bicarbonate buffer (pH 9.6; 100 µl/well). After blocking with 2% skimmed milk and 10% fetal calf serum in PBS, purified scFv or mAb (Ab 1) adjusted to a starting concentration of 100 µg/ml were added in two-fold serial dilutions (100 µl/well). The maximum extinction with 100 µg/ml was monitored in one well. Incubation was performed at room temperature for 2h. After five washing steps with PBS, purified scFv/mAb (Ab 2) was incubated under same conditions. The concentrations of Ab 2 were determined empirically to ensure sufficient saturation of all free epitopes. The maximal extinction of Ab 2 was measured in an additional well coated with virus. The detection of the Ab 2 occurred either with goat pAb to E tag (HRP) (1:2000) or goat anti-mouse IgG peroxidase conjugate developed in goat (1:2000) (whole molecule; Sigma Aldrich, Taufkirchen, Germany) at room temperature for 1h. After, five washing times with PBS, the developing solution (3, 3', 5, 5' tetramethylbenzidine; Abcam, Cambridge, UK) was added. The reaction was stopped by 1 N hydrochloric acid. The OD-values were measured by a photometric plate reader (TECAN, Männedorf, Switzerland) at a wavelength of 450 nm. Reduction of the photometer extinction of detected challenge antibodies by competing antibodies was calculated as:

$$\%_{(inhibition)} = (1 - \frac{OD_{450nm}(Ab1Ab2)}{OD_{450nm}(Ab2)}) \times 100$$

An inhibition of at least 50% was regarded as significant blocking effect.

**Table 1** Monoclonal antibodies (mAb) used in an inhibition ELISA for the identification of the target of scFv 1.2.2.H9.

| Epitope ID | MAb | Isotype | Virus strain used for MAb production |
|---|---|---|---|
| 2A | 1B3/1A11 | IgG2a | VACV M1 |

| | | | |
|---|---|---|---|
| 2B | 1F7/2F9 | IgG2b | ECTV M1 |
| 2D | 3D11/2G7 | IgG2a | CPXV KR2 Brighton |
| 2G | 4C4/2B6 | IgG2a | CPXV KR2 Brighton |

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**Engineering of specific binding scFv to human scFv-Fc and IgG1 molecules**

Specific binding scFv were converted into scFv-Fc and IgG format. The addition of the second and third constant region of a human IgG1 enables studies on further effector mechanisms within the immune system. The binding affinities to VACV and neutralization abilities were compared.

The pCANTAB5E, possessing the genetic information of the selected scFvs, and the vector pCMX2.5 were cleaved using the restriction enzymes *Nco*I and *Not*I. 1 µg of each plasmid was first incubated at 37°C for 1h with *Nco*I and after heat inactivation incubated with *Not*I under the respective buffer conditions. The vector pCMX2.5 was dephosphorylated while the scFv was gel-extracted. Ligation occurred at 16°C overnight, followed by transformation into *E. coli* DH5α. Colonies were randomly selected and the successful ligation was confirmed by sequencing of isolated plasmids. HEK293T cells (approximately $7.5\times10^5$ cells) were seeded in growth medium (Dulbecco's modified Eagle's medium (DMEM) containing 5% (v/v) fetal calf serum (FCS) and 1% penicillin/streptomycin (PS) into 6-well culture plates (Sarstedt, Germany) and were grown to reach a confluence of 75 to 80% for transfection after 24 h. A total of 20 µl of a 1 mg/ml PEI (polyethylenimine, linear, 25 kDa, Polysciences) solution was diluted in 125 µl DMEM. In parallel, 2 µg of purified pCMX2.5-scFv-Fc was diluted in 150 µl DMEM. PEI and DNA dilutions were combined and incubated for 15-30 min at RT to allow formation of PEI-

DNA complexes. This suspension was dispersed over the cells and incubated overnight. The medium was changed to DMEM/4% FCS/1% PS for primary production of scFv-Fc into the culture supernatant. Immunoaffinity purification of the fragments occurred by Protein G column.

For expression of IgG1, Fv fragments were cloned into IgG expression vectors pCSL3k (light chain) and pCSH1c (heavy chain). The VH and VL were PCR amplified using primers TS_UDH9VH_*Bss*HII_f (5'-CACAG*GCGCGC*ACTCCCAGGTGCAGCTGG-TACA-3') and TS_UDH9VH_*Nhe*I_r (5'-TGGT*GCTAG*CTGAGGAGACGGTGAC-CGT-3') for the VH and TS_UDH9VL_*Age*I_f (5'-AAGC*ACCGGT*GAAATT-GTGATGACGCAG- 3') and TS_UDH9VL_*Bsi*WI_r (5'-CCAC*CGTACG*TTTGAT-TTCCACCT-3') for the VL. Cloning of VH and VL was done as described previously (Steinwand et al., 2013).

Monocistronic heavy and light chain vectors were co-transfected into HEK293-6E cells for transient expression (Durocher et al., 2002). In brief, heavy and light chain vectors were mixed with a molar ration of 1:1 and HEK293-6E cells at densities of 1.8-2×$10^6$ cells/ml transfected using PEI as described previously (Jager et al., 2013). Forty-eight hours after transfection, one volume of medium including 1 % Tryptone N1 (TekniScience) was added for feeding. Supernatant was harvested 5 days after transfection. Immunoglobulins were purified by protein A affinity chromatography using the ProfiniaTM Affinity Chromatography Protein Purification System (Bio-Rad, Munich) according to the manufacturer's description.

**SDS-PAGE and Western blotting for the detection of the target virus protein**

For Western blot analyses, 5 µg of gradient purified VACV Elstree and 5 µg of the purified recombinant D8 protein were fractionated by vertical 12% sodium dodecyl sulfate

(SDS)-polyacrylamid gel electrophoresis (Laemmli, 1970) and subsequently transferred to nitrocellulose membranes. Immunodetection was performed by standard techniques using 10 µg scFv after a blocking step in PBS/2% SMP/10% FCS at room temperature for 2h. Goat pAb to E tag (HRP) (1:500, Abcam, UK), and HRP color-developing reagent (Bio-Rad, Heidelberg, Germany) were used for visualization. For the verification of the virus gradient integrity an anti-MVA-polyclonal rabbit antibody (1:500) was used and visualized with polyclonal goat anti-rabbit IgG conjugated with HRP (Sigma-Aldrich, Taufkirchen, Germany). The protein sizes were estimated with a concurrent protein standard (Bio-Rad, München, Germany). The parapoxvirus Orf D1701 was chosen as virus negative control and an anti-Orf-polyclonal sheep Ab (1:500) was used for its verification.

**Enzyme-linked immunosorbent assay (ELISA)**

For quantification of the binding affinities, the purified scFv, scFv-Fc, and IgG1 were titrated in triplicates in two-fold serial dilutions starting with 100 µM in an ELISA. Plates were coated with 2 µg/ml gradient purified VACV Elstree. The background was determined with BSA and subtracted from the respective dilution measured on VACV Elstree. The affinity was calculated from the average adsorption of the triplicates using the Michaelis-Menten kinetics (Michaelis and Menten, 1913) and GraphPad Prism 6 for Mac (La Jolla California, USA).

The capture abilities of the scFv were tested in a sandwich ELISA. Plates were coated with 100 µl of 25 µg/ml scFv at 37°C for 4h followed by 4°C overnight. Blocking was performed after eight washings with PBS/0.1% Tween. The blocking solution was removed by an additional eight times washing. The chosen virus samples, composed of cell culture supernatants, were titrated in two-fold serial dilutions and incubated at 37°C for 1h. The tested viruses were reovirus ($5\times10^{5.625}$ $CID_{50}$/ml), Newcastle disease virus

($5\times10^{5.25}$ $CID_{50}$/ml), Orf D1701 ($10^4$ pfu/ml), enterocytopathogenic bovine orphan virus ($5\times10^{5.25}$ $CID_{50}$/ml), bovine corona virus V270 ($5\times10^{5.25}$ $CID_{50}$/ml) and VACV Elstree ($10^4$ pfu/ml). Negative controls were DMEM and the MA104 cell line in DMEM. After washing ten times, the detection occurred with anti-MVA-polyclonal rabbit sera in a prior determined dilution of 1:5000. Binding of the detector was visualized with anti-rabbit IgG (whole molecule) peroxidase conjugate (Sigma-Aldrich, Taufkirchen, Germany) pre-diluted at 1:2000 and TMB substrate.

### *In vitro* Plaque Reduction Neutralization Test (PRNT)

To assess the neutralization abilities of scFv, scFv-Fc, and IgG1, a confluent monolayer of Vero cells was grown in 24-well culture plates. Antibodies were adjusted to 10 µM and titrated in triplicates in 2-fold serial dilution against approximately 50 pfu of VACV Elstree in MEM (PAN-BIOTECH GmbH, Aidenbach, Germany) and incubated at 37°C for 1h with or without 1% human complement (Sigma Aldrich, Taufenkirchen, Germany). The virus-antibody (-complement) mixture was then added to the cells and again incubated at 37°C for 1h under 5% $CO_2$. The supernatant was removed and replaced with 0.5 ml of 2.5% FCS-0.5% methylcellulose in MEM ensuring plaque formation within 48h. As virus positive control and an equal volume of MEM with or without complement was added to 50 pfu VACV Elstree. The neutralization positive control was anti-MVA-polyclonal rabbit sera. Fixation and staining of cells were done using 1.5% crystal violet in 8.5% ethanol/25% formaldehyde. Plaques were counted visually and a reduction of plaque number of ≥50% compared to the virus control was considered as significant virus neutralization.

### *In vivo* neutralization

All animal experiments were approved by Lower Saxony State Office for Consumer Protection and Food Safety under the number 33.9-42502-04-15/1967 and in compliance with German animal welfare laws.

To examine the neutralization abilities of scFv, scFv-Fc, and IgG1, six groups of six female NMRI mice were passively immunized intraperitoneally (i.p.) with 300 µl containing 100 µg of the corresponding antibody in PBS. Two groups of mice served as negative control receiving either PBS or an anti-equine-herpesvirus mAb. One group served as antibody-positive-control group receiving a polyclonal anti-MVA antibody. Animals were challenged i.p. with 4 $LD_{50}$ VACV Munich 1 24h later. Mice were monitored for survival and signs of illness. Moreover, weight, and ear temperature of each mouse were measured daily. Mice with a body weight loss of > 30% were euthanized. The impact of treatment on weight was analyzed using the mixed procedure of SAS with the following model:

$$y_{ijkm} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + b\left(G_{ij}\right) + \gamma_{ijk} + \varepsilon_{ijkm}$$

where yijk is the observation for weight, µ is the general mean, $\alpha_i$ is the effect of treatment, βj is the fixed effect of survival ability, $\alpha\beta_{ij}$ is the fixed effects of interactions between treatment and survival ability, $G_{ij}$ is the starting individual weights, $\gamma_{ijk}$ is the random effect of repeated measurement and e represent the random error.

Statistical analysis of the survived mice was carried out by the Kaplan–Meier method by using the LIFETEST procedure of SAS System 9.3 (SAS Institute Inc., USA) and using the following model:

$$\hat{S}(t) = \prod_{j:t_j \leq t} \left[1 - \frac{d_j}{n_j}\right] for\ t_1 \leq t \leq t_k$$

Where $\hat{S}(t)$ is the survivor function and t is the lifetime of mice. For each $_j$: $t_j \geq t$, let $t_1 < t_2 < \ldots < t_k$ representing the different event times. $n_j$ is the number of individuals at risk just prior to $t_i$, and $d_j$ is the number of individuals that die at time $t_j$.

Tests of equality across strata were used to explore whether significant differences between different antibody-treatment group existed. Hazard rates were derived from the non-parametric survival function estimated with the Kaplan-Meier method.

At the end of the experiment (day 28 after challenge), heart, liver, spleen, lung, brain, and kidney were harvested and examined for infectious particles. DNA was purified from the organs using QIAamp DNA Blood Mini Kit and QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the to the manufacturer's instructions. Real-time PCR was performed on a LightCycler 480 (Roche, Mannheim, Germany) using the LightCycler 480 Probes Master Kit (Roche, Mannheim, Germany) to amplify the D8 fragment of VACV. The reaction volume contained 10 µl Light Cycler 480 Probes Master mix, 1 µl of each 5 pmol/µl D8L_forward: 5´-CATATTCATTGGGGAGAAACC-3´ and D8L_reverse: 5´-GCGATTGAAGACGTTAGACTAA-3´, 1 µl of 4 pmol/µl D8L_probe: 5´-TTCTGGA-TAGTGGTTGGTTTCGACTCA-3' and 2 µl HPLC as well as 5 µl of the DNA template. The LightCycler was programmed as follow: first ten minutes pre-incubation at 95°C, then 40 cycles of 95°C/30 sec, 58°C/45 sec and 72°C/60 sec followed by a final cooling step at 40°C for 10 min.

**ACKNOWLEDGEMENTS**

We thank Caroline Bierschenk and Sebastian Schimkowiak for their excellent technical assistance. Moreover, we thank Ahmed Abd El Wahed for the critical review of the manuscript.

## SUPPLEMENTAL MATERIAL

### Supplemental figures

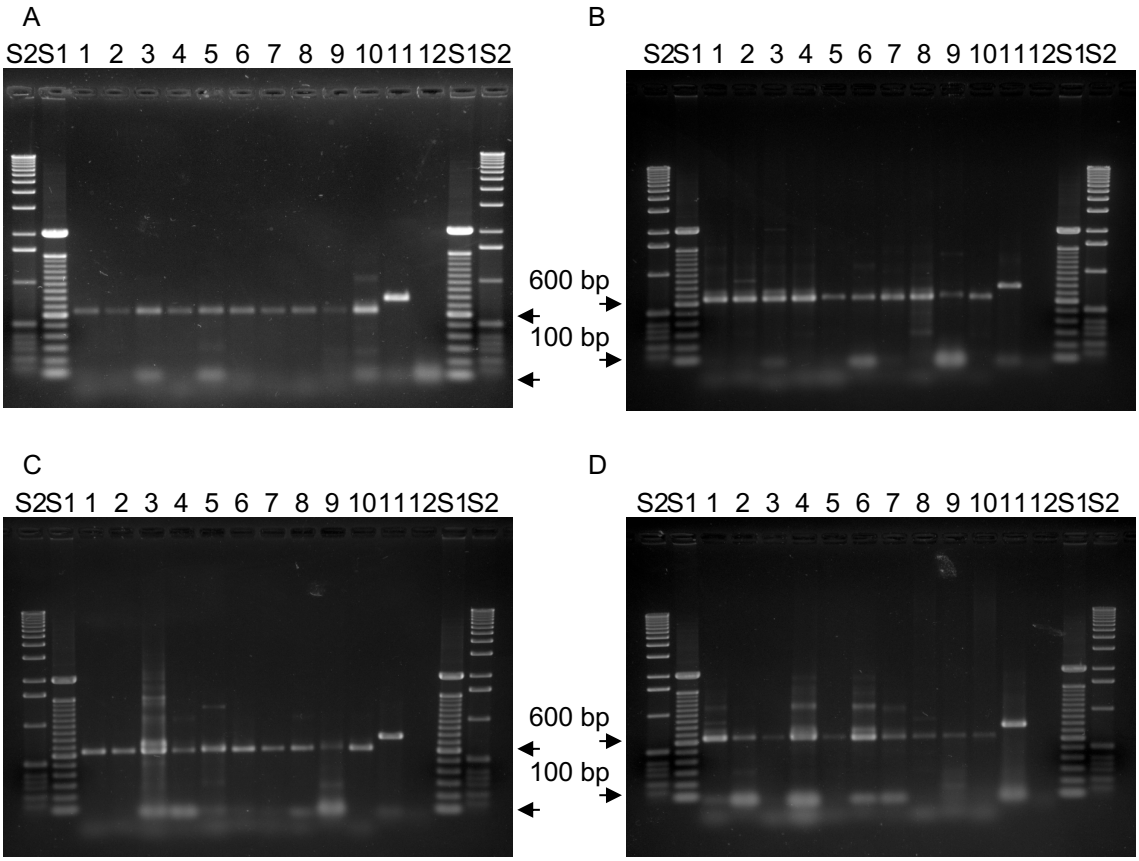


**FIG S1** Amplification of the 650 bp fragments of the IgG heavy chains from the cDNA synthesized from four different B-lymphocyte donors (A to D). The fragments consisted of the variable and parts of the constant regions. Primers amplifying GAPDH (11) were chosen to monitor the quality of the cDNA. S1 and S2 were the 1 kb and 100 bp ladders respectively. The products occurred from the combination of HuIgG1-4CH1FOR with HuVH1aBACK (1), HuVH1bBACK (2), HuVH1cBACK (3), HuVH1d-7BACK (4), HuVH2aBACK (5), HuVH3aBACK (6), HuVH4aBACK (7), HuVH5aBACK (8), HuVH6aBACK (9), and HuVH7aBACK (10). The no-template control as negative control is applied in lane 12.

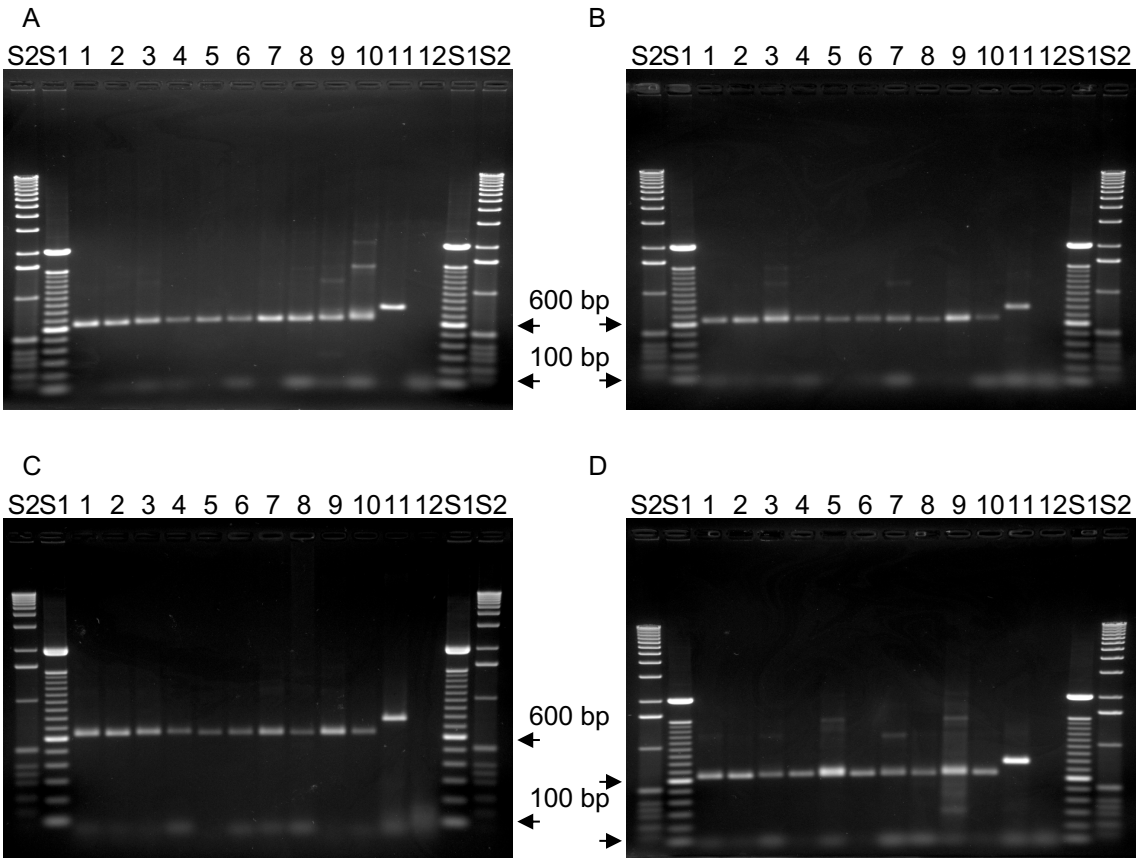


**FIG S2** Amplification of the 650 bp fragments of the κ light chains from the cDNA synthesized from four different B-lymphocyte donors (A to D). The fragments consisted of the variable and parts of the constant regions. Primers amplifying GAPDH (11) were chosen to monitor the quality of the cDNA. S1 and S2 were the 1 kb and 100 bp ladders respectively. The products occurred from the combination of HuCκFOR with HuVκ1aBACK (1), HuVκ1bBACK (2), HuVκ2aBACK (3), HuVκ2bBACK (4), HuVκ3aBACK (5), HuVκ3cBACK (6), HuVκ4aBACK (7), HuVκ5aBACK (8), HuVκ6aBACK (9), and HuVκ6aBACK (10). The no-template control as negative control is applied in lane 12.

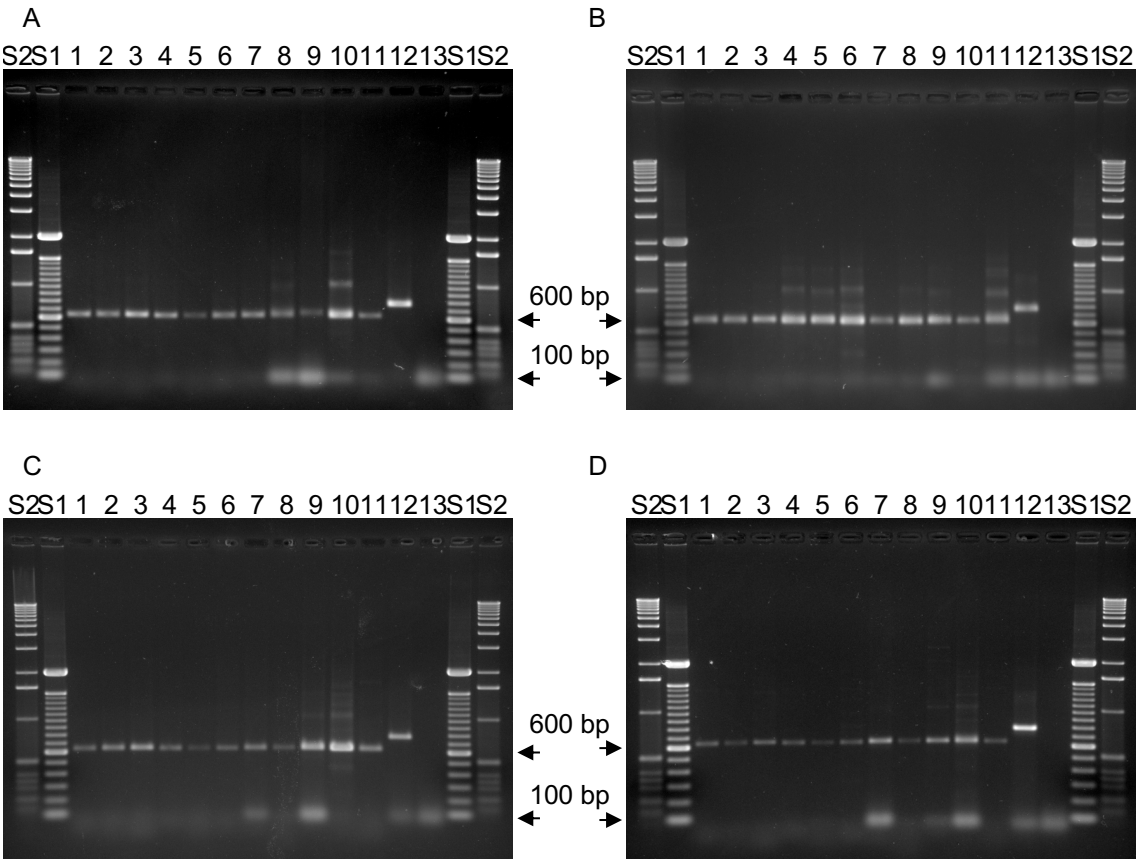


**FIG S3** Amplification of the 650 bp fragments of the λ light chains from the cDNA synthesized from four different B-lymphocyte donors (A to D). The fragments consisted of the variable and parts of the constant regions. Primers amplifying GAPDH (12) were chosen to monitor the quality of the cDNA. S1 and S2 were the 1 kb and 100 bp ladders respectively. The products occurred from the combination of HuCλ2FOR and HuCλ7FOR in equal amounts with HuVλ1aBACK (1), HuVλ1bBACK (2), HuVλ2BACK (3), HuVλ3aBACK (4), HuVλ3bBACK (5), HuVλ4a-9BACK (6), HuVλ4bBACK (7), HuVλ5BACK (8), HuVλ6BACK (9), HuVλ7-8BACK (10), and HuVλ10BACK (11). The no-template control as negative control is applied in lane 13.

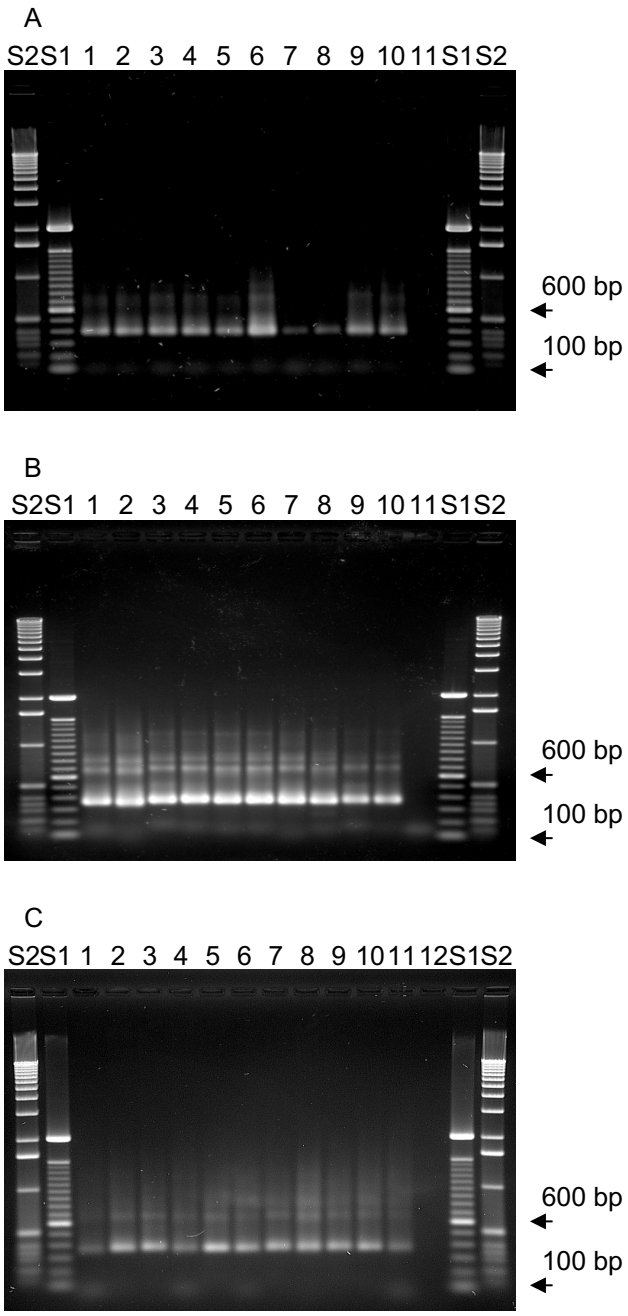


**FIG S4** Amplification of the variable regions of the heavy chains (A), κ light chains (B), and λ light chains (C) from pooled 650 bp samples (1-10, and 11 for the λ variable regions). The fragments consisted of the variable region and overlapping parts coding for the $(G_4S)_3$-linker. S1 and S2 were the 1 kb and 100 bp ladders respectively. The no-template control as negative control is applied in lane 11, and 12 for the λ variable regions respectively. The products were gel-purified and taken as template for splicing by overlap extension-PCR.

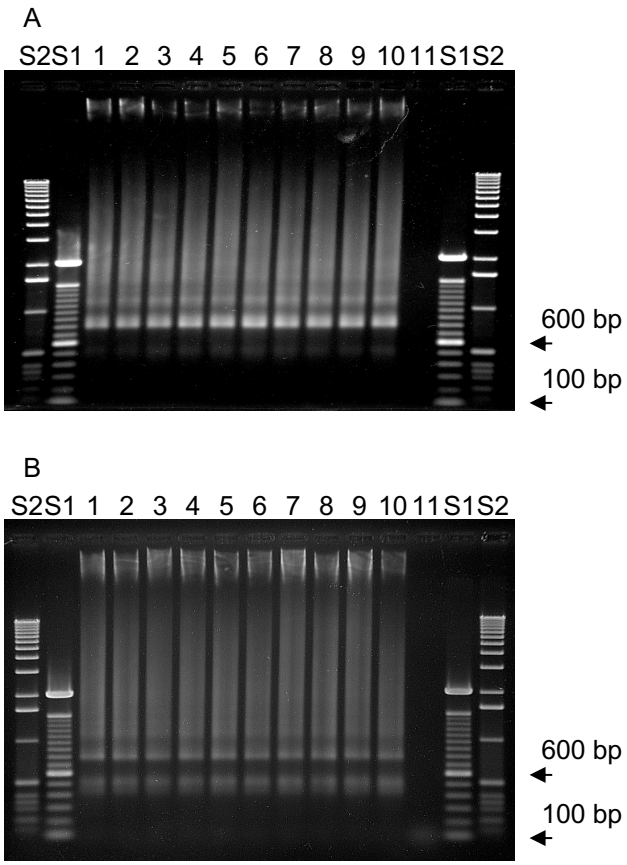


**FIG S5** Amplification of full scFv including restriction sites for *Sfi*I and *Not*I. The pooled variable regions of the heavy chains were either connected with the variable regions of κ light chains (A), or λ light chains (B). After SOE-PCR (splicing by overlap extension) the products were divided into 10 templates (1-10) for the re-amplification and connection of restriction sites. S1 and S2 were the 1 kb and 100 bp ladders respectively. The no-template control as negative control is applied in lane 11. The products were gel-purified and restricted before ligation into pCANTAB5E.

## Supplemental tables

**Table S1** Degenerate primer used for the amplification of variable- and part of the constant region of the heavy IgG-chains and κ- and λ-light chains respectively. The expected product size was 650 bp.

| Primer | Sequence 5'→3' |
|---|---|
| HuIgG1-4CH1FOR | GTC CAC CTT GGT GTT GCT GGG CTT |
| HuVH1aBACK | CAR RTG CAG CTG GTG CAG TCT GG |
| HuVH1bBACK | CAG GTY CAG CTK GTG CAG TCT GG |
| HuVH1cBACK | SAG GTC CAG CTG GTA CAG TCT GG |
| HuVH1d-7BACK | CAR RTS CAG CTG GTG CAR TCT GG |
| HuVH2aBACK | CAG RTC ACC TTG ARG GAG TCT GG |
| HuVH3aBACK | SAG GTR CAG CTG GTG GAG TCT GG |
| HuVH4aBACK<br>HuVH4CBACK | CAG STG CAG CTG CAG GAG TCS GG |
| HuVH5aBACK | GAR GTG CAG CTG GTG CAG TCT GG |
| HuVH6aBACK | CAG GTA CAG CTG CAG CAG TCA GG |
| HuVH7aBACK | CAG GTS CAG CTG GTG CAA TCW GG |
| HuCκFOR | AGA CTC TCC CCT GTT GAA GCT CTT |
| HuVκ1aBACK | RAC ATC CAG WTG ACC CAG TCT CC |
| HuVκ1bBACK | GMC ATC CAG TTG ACC CAG TCT CC |
| HuVκ2aBACK | GAT RTT GTG ATG ACY CAG WCT CC |
| HuVκ2bBACK | GAT RTT GTG ATG ACW CAG TCT CC |
| HuVκ3aBACK | GAA ATW GTG WTG ACR CAG TCT CC |
| HuVκ3cBACK | GAA ATT GTR WTG ACA CAG TCT CC |
| HuVκ4aBACK | GAC ATC GTG ATG ACC CAG TCT CC |
| HuVκ5aBACK | GAA ACG ACA CTC ACG CAG TCT CC |
| HuVκ6aBACK | GAA ATT GTG CTG ACT CAG TCT CC |
| HuVκ6bBACK | GAT GTT GTG ATG ACA CAG TCT CC |
| HuCλ2FOR | TGA AGA TTC TGT AGG GGC CAC TGT CTT |
| HuCλ7FOR | AGA GCA TTC TGC AGG GGC CAC TGT CTT |
| HuVλ1aBACK | CAG TCT GTG CTG ACT CAG CCA CC |
| HuVλ1bBACK | CAG TCT GTG YTG ACG CAG CCG CC |
| HuVλ2BACK | CAG TCT GCC CTG ACT CAG CCT GC |
| HuVλ3aBACK | TCC TAT GWG CTG ACW CAG CCA CC |
| HuVλ3bBACK | TCT TCT GAG CTG ACT CAG GAC CC |
| HuVλ4a-9BACK<br>HuVλ9BACK | CWG CCT GTG CTG ACT CAG CCM CC |
| HuVλ4bBACK | CAG CYT GTG CTG ACT CAA TCR |
| HuVλ5BACK | CAG SCT GTG CTG ACT CAG CCR |
| HuVλ6BACK | AAT TTT ATG CTG ACT CAG CCC CA |
| HuVλ7-8BACK | CAG RCT GTG GTG ACY CAG GAG CC |
| HuVλ10BACK | CAG GCA GGG CTG ACT CAG CCA CC |

**Table S2** Degenerate primer used for the amplification of variable regions with overlapping parts coding for the $(G_4S)_3$-linker. The expected product sizes were 420 bp for the variable region of the heavy chains and 380 bp for the light chains. The BACK-primers used for the PCR of the heavy chain variable region were the same shown in table S1.

| Primer | Sequence 5'→3' |
|---|---|
| Linker-HuJH1-2 | AGA ACC ACC TCC GCC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC CAG GGT GC |
| Linker-HuJH3 | AGA ACC ACC TCC GCC TGA ACC GCC TCC ACC TGA AGA GAC GGT GAC CAT TGT CC |
| Linker-HuJH4-5 | AGA ACC ACC TCC GCC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC CAG GGT TC- |
| Linker-HuJH6 | AGA ACC ACC TCC GCC TGA ACC GCC TCC ACC TGA GGA GAC GGT GAC CGT GGT CC |
| HuJκ1FOR | ACG TTT GAT TTC CAC CTT GGT CCC |
| HuJκ2FOR | ACG TTT GAT CTC CAG CTT GGT CCC |
| HuJκ3FOR | ACG TTT GAT ATC CAC TTT GGT CCC |
| HuJκ4FOR | ACG TTT GAT CTC CAC CTT GGT CCC |
| HuJκ5FOR | ACG TTT AAT CTC CAG TCG TGT CCC |
| Linker-HuVκ1a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGR ACA TCC AGW TGA CCC AGT CTC C |
| Linker-HuVκ1b | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG MCA TCC AGT TGA CCC AGT CTC C |
| Linker-HuVκ2a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG ATR TTG TGA TGA CYC AGW CTC C |
| Linker-HuVκ2b | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG ATR TTG TGA TGA CWC AGT CTC C |
| Linker-HuVκ3a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG AAA TWG TGW TGA CRC AGT CTC C |
| Linker-HuVκ3c | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG AAA TTG TRW TGA CAC AGT CTC C |
| Linker-HuVκ4a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG ACA TCG TGA TGA CCC AGT CTC C |
| Linker-HuVκ5a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG AAA CGA CAC TCA CGC AGT CTC C |
| Linker-HuVκk6a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG AAA TTG TGC TGA CTC AGT CTC C |
| Linker-HuVκ6b | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGG ATG TTG TGA TGA CAC AGT CTC C |
| Hu Jλ1FOR | ACC TAG GAC GGT GAC CTT GGT CCC |
| Hu Jλ2-3FOR | ACC TAG GAC GGT CAG CTT GGT CCC |
| Hu Jλ4-5FOR | ACC TAA AAC GGT GAG CTG GGT CCC |
| Hu Jλ7FOR | ACC GAG GAC GGT CAG CTG GGT GCC |
| Linker-HuVλ1a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC AGT CTG TGC TGA CTC AGC CAC C |
| Linker-HuVλ1b | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC AGT CTG TGY TGA CGC AGC CGG C |
| Linker-HuVλ2 | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC AGT CTG CCC TGA CTC AGC CTG C |

| | |
|---|---|
| Linker-HuVλ3a | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGT CCT ATG WGC TGA CWC AGC CAC C- |
| Linker-HuVλ3b | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGT CTT CTG AGC TGA CTC AGG ACC C |
| Linker-HuVλ4a-9 | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC WGC CTG TGC TGA CTC AGC CMC C |
| Linker-HuVλ4b | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC AGC YTG TGC TGA CTC AAT CRG C |
| Linker-HuVλ5 | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC AGS CTG TGC TGA CTC AGC CRT C |
| Linker-HuVλ6 | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGA ATT TTA TGC TGA CTC AGC CCC A |
| Linker-HuVλ7-8 | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC AGR CTG TGG TGA CYC TGG AGC C |
| Linker-HuVλ10 | GTT CAG GCG GAG GTG GTT CTG GCG GTG GCG GAT CGC AGG CAG GGC TGA CTC AGC CAC C |

## 2.3 CHAPTER III

Paper planned for:

PLOS ONE

# Characterization of an anti-vaccinia virus F13 single chain Fragment variable from a human anti-vaccinia virus-specific recombinant immunoglobulin library

Running title: Characterization of an anti-F13 scFv

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**ABSTRACT**

Vaccinia virus (VACV) is belonging to the genus *Orthopoxvirus* of the family *Poxviridae*. There are four different forms of infectious virus particles: intracellular mature virus (IMV), intracellular enveloped virus (IEV), cell-associated enveloped virus (CEV) and extracellular enveloped virus (EEV). The F13 protein occupies the inner side of the CEV and the EEV- and the outer side of the IEV-membranes. It plays an important role in the wrapping progress and the EEV production.

We constructed a human single chain fragment variable (scFv) library with a diversity of $\geq 4x10^8$ using peripheral blood from four vaccinated donors. One anti-F13 scFv was isolated and characterized after three rounds of panning. In western blotting assays, the scFv 3E2 reacted with the recombinant $F13_{VACV}$ protein with reduction of binding under denatured and reduced conditions. Two antigenic binding sites (139-GSIHTIKTLGVYSDY-153 and 169-AFNSAKNSWLNL-188) of scFv 3E2 were mapped using a cellulose membrane encompassing 372 15-mere peptides with 12 overlaps covering the whole F13 protein. No neutralization capabilities were observed either in presence or absence of complement.

In conclusion, the construction of recombinant immunoglobulin libraries is a promising strategy to isolate specific scFvs to enable the study of the host-pathogen interaction.

**INTRODUCTION**

Vaccinia virus (VACV), a member of the genus *Orthopoxvirus* (OPXV) of the family *Poxviridae* (Moss, 2006), was used as the vaccine, which led to the eradication of smallpox in 1979 (Fenner et al., 1988). While Variola virus (VARV) solely infects humans, OPXV with zoonotic potential, like cowpox virus (CPXV) and monkeypox virus (MPXV), can also cause severe and sometimes fatal infections (Becker et al., 2009; Campe et al., 2009; Kurth et al., 2008; Ladnyj et al., 1972; Reed et al., 2004; Vaughan et

al., 2018; Vorou et al., 2008). There are public concerns of bioterrorism using poxviruses as biological weapons (Henderson, 1999). Therefore, the investigation on poxvirus replication and infectivity is still necessary.

There are four different types of infectious virus particles: intracellular mature virus (IMV), intracellular enveloped virus (IEV), cell-associated enveloped virus (CEV) and extracellular enveloped virus (EEV) (Roberts and Smith, 2008; Smith et al., 2002). The majority of the particles are IMV virions (>90%), which are responsible for the transmission of the virus between hosts and are generated within cytoplasmic factories from crescents precursor cells (Moss, 2012; Smith et al., 2003). Some IEV particles get out of the factories and receive a double layer of intracellular membrane by the *trans*-Golgi network (TGN) or the early endosomes (Hiller and Weber, 1985). The outer membrane of IEV fuses with the plasma membrane of the cell (Geada et al., 2001). The particles stay connected to the cell surface named CEV, while the detached ones termed EEV (Payne, 1980; Smith et al., 2002). The CEV as well as the EEV are responsible for a rapid virus spread within the host (Moss, 2012). Each of these forms has a unique antigen occupancy and distribution on its surface (Benhnia et al., 2009b; Davies et al., 2005; Hsiao et al., 1999; Ichihashi and Oie, 1996; Kaever et al., 2016; Matho et al., 2017, 2018; Matho et al., 2015; Moss, 2006, 2011; Rodriguez et al., 1985; Smith et al., 2002; Wolffe et al., 1995). For instance, the major envelope protein of EEV particles is the 37 kDa F13 non-glycosylated membrane protein (Hiller et al., 1981; Hirt et al., 1986), which is encoded by the ORF F13L gene and consists of 372 aa (Grosenbach and Hruby, 1998; Hirt et al., 1986). The F13 protein has no transmembrane domain, but it is palmitylated at cysteine residues 185 and 186 (Grosenbach et al., 1997), which are located within the TGN membrane (Schmutz et al., 1995). The F13 plays an important role in the membrane association, the virion wrapping progress and the EEV production (Borrego et al., 1999; Husain and Moss, 2001).

Although smallpox is eradicated, there is a rising interest in neutralizing antibodies as well as antiviral drugs, because of the fear of bioterrorism (Breman and Henderson, 1998; Grosenbach et al., 2011). The generation of highly diverse species-specific human antibody libraries by using the phage display technique (Schmaljohn et al., 1999) is a powerful technology. Target-specific human single-chain variable antibody fragments (scFvs) can be even used as a treatment because they are able to penetrate the cell due to their low molecular weight (Farajnia et al., 2014).

In this study, we constructed an anti-F13$_{VACV}$ scFv antibody retrieved from a human immunoglobulin library isolated from an OPXV vaccinee. The specificity, binding affinity and virus neutralization capacities of the F13 scFv were compared to that of a monoclonal antibody.

## RESULTS

### Selection of an anti-F13 specific scFv antibody

Selection of F13 specific antibodies was conducted over three rounds of panning. After each round, 176 individual *E. coli* HB2151 colonies were isolated for the production of soluble antibodies in a microtiter well format. No specific binding of scFv was observed after the first and second selection rounds. The third selection round revealed one clone with an adsorption three times over background designated as 3E2. The scFv 3E2 was sequenced and classified to the human VH3/D2/JH3-VK3/JK4 families (Figure 1).

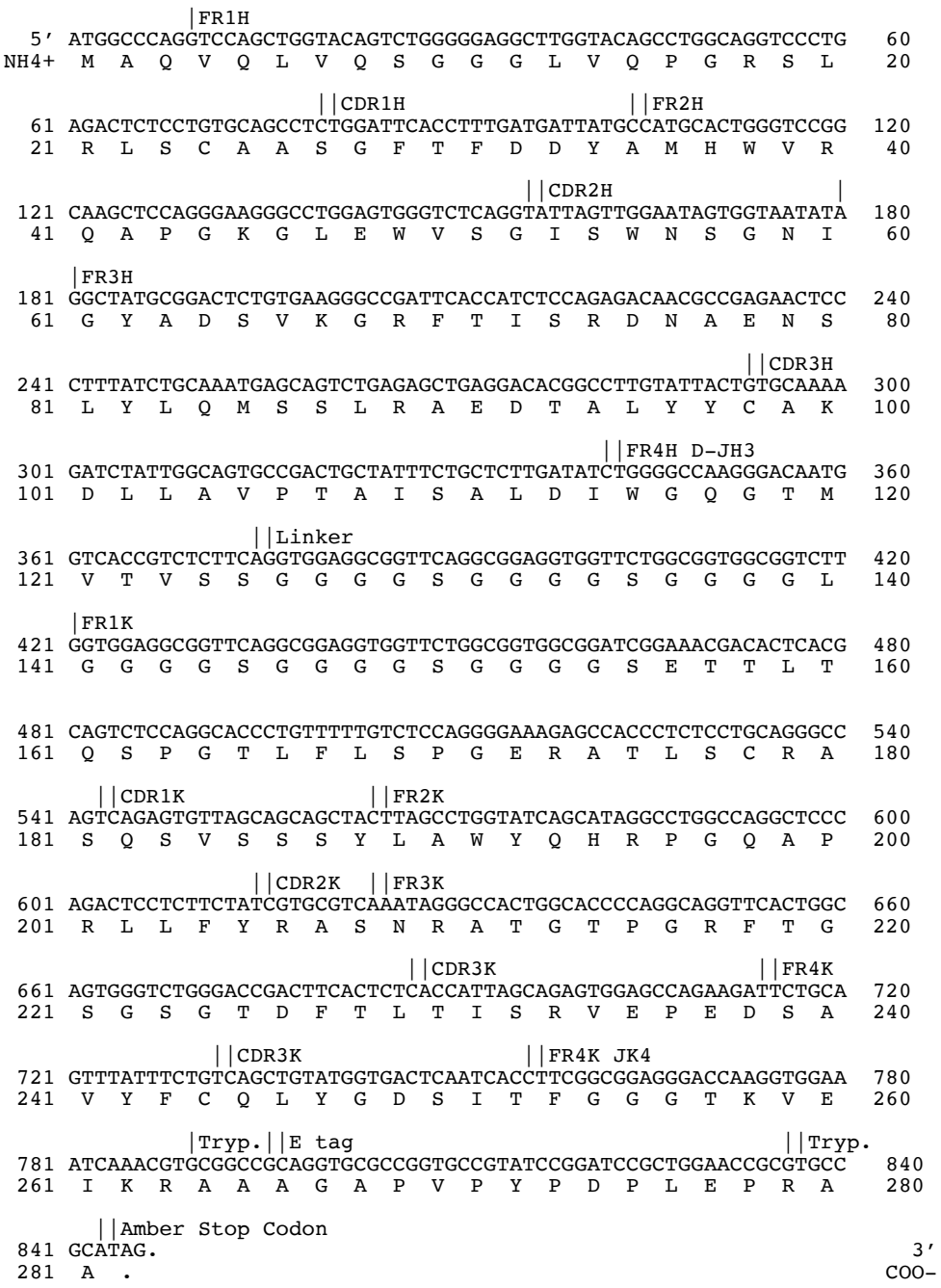



**FIG 1** Genome sequence (upper lines) and amino acid sequence (lower lines) of variable domains of heavy and light chain of the scFv 3E2. The variable region consists of four framework regions (FR1-4) and three hypervariable complementarity-determining regions (CDR1-3). Trypsin-sensitive sites (Tryp.) are needed for the elution of bound phages by enzymatic cleavage with trypsin.

**Specificity and binding affinity studies**

The specificity of the antibodies was measured in an indirect ELISA with two-fold serial dilutions of the scFv starting with 200 µg/ml using various recombinant antigens and

viruses (recombinant F13 and A27 proteins of VACV, VACV Elstree and Munich1). The binding affinities were calculated according to Michaelis-Menten (Michaelis and Menten, 1913) (Figure 2).

The scFv 3E2 did not bind to VACV Elstree, VACV Munich1 and the recombinant A27, however, a Michealis-Menten constant ($K_m$) of 10.97 ng/ml and a maximal velocity ($v_{max}$) of 1.25 were recorded against the F13 protein (Figure 2A).

A

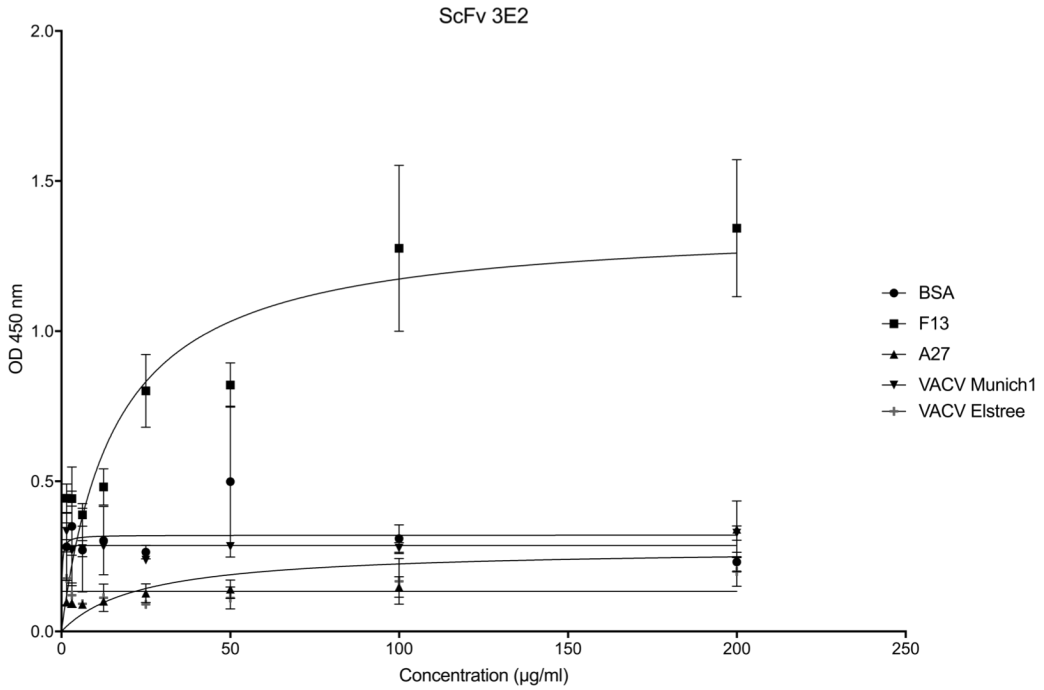



**FIG 2A** Binding affinities of the scFv 3E2 as measured in an indirect ELISA using recombinant F13, A27 proteins of VACV, VACV Elstree, Munich1 or BSA. The scFv 3E2 showed a reaction with the recombinant F13 protein. However, the Ab neither bound to VACV Elstree, VACV Munich1 nor to BSA and A27, the latter used as negative controls.

For comparison, the mAb 15B6 showed a $K_m$ of 0.006724 ng/ml and a $v_{max}$ of 3.54 against the recombinant F13 protein, a $K_m$ of 0.7522 ng/ml and a $v_{max}$ of 1.35 towards VACV-Elstree and a $K_m$ of 0.5108 ng/ml and a $v_{max}$ of 1.19 towards VACV-M1. The mAb 15B6 did not bind to the recombinant A27 (Figure 2B).

B

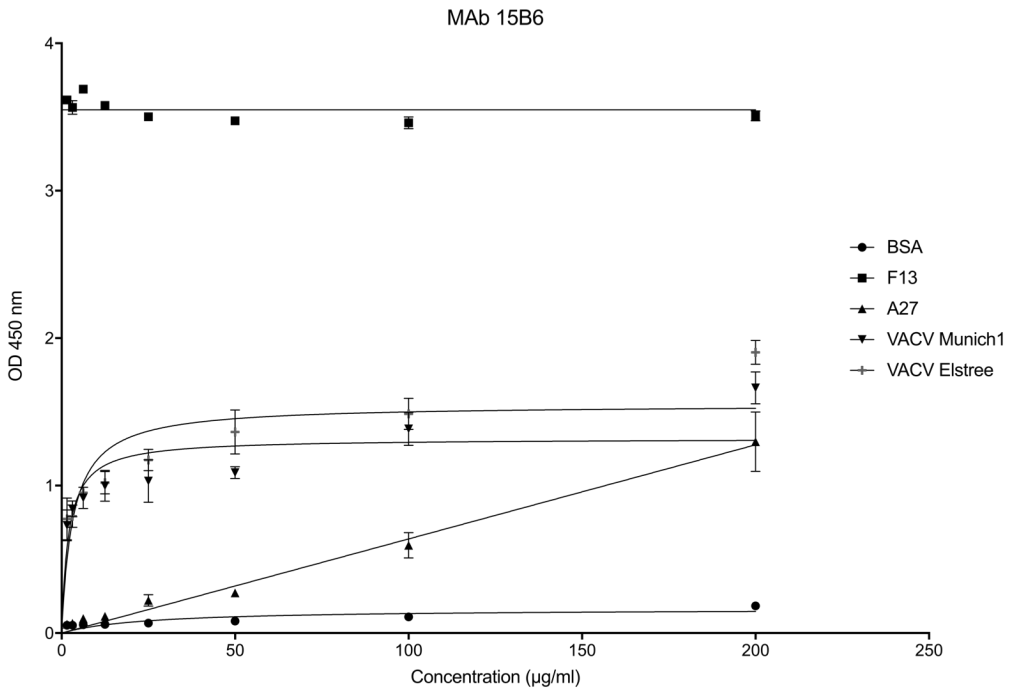



**FIG 2B** Binding affinities of the mAb 15B6 as measured in an indirect ELISA using recombinant F13, A27 proteins of VACV, VACV Elstree, Munich1 or BSA. The mAb 15B6 showed a reaction with the recombinant F13 protein, VACV Elstree and Munich1, while no binding was observed with the recombinant A27 protein and with BSA.

Western blotting analysis revealed the binding of the scFv 3E2 on the recombinant F13 protein of VACV under denaturing and reducing conditions (Figure 3A), while no reaction was observed with the VACV Elstree, VACV Munich1 and the A27 protein. As antibody positive control, the mAb 5B4/2F2, directed against the A27 protein, was used and showed reactivities with VACV Elstree, VACV Munich1 and the recombinant A27 protein of VACV (Figure 3B).

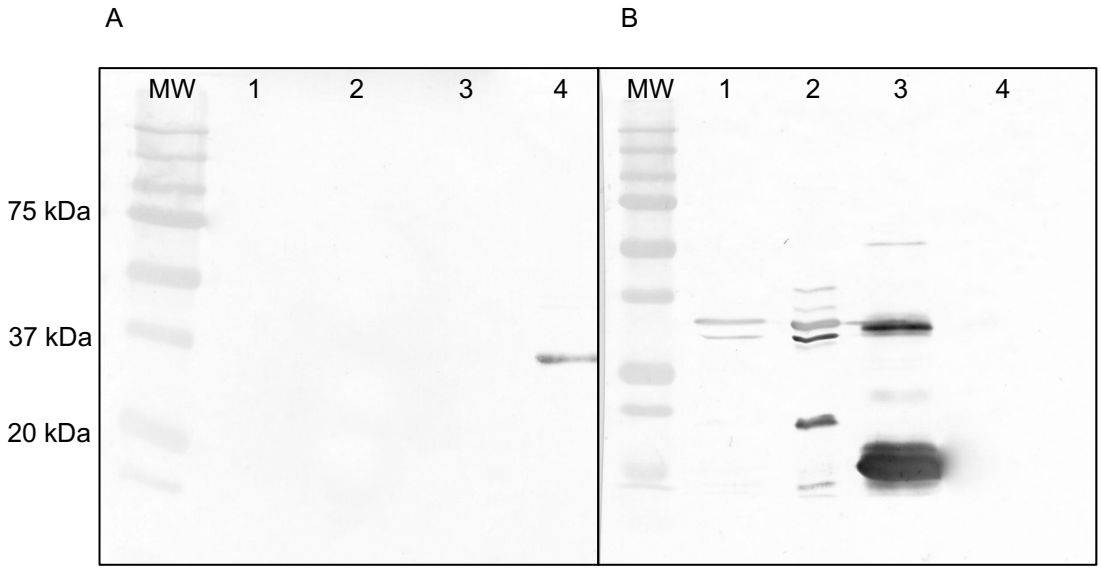
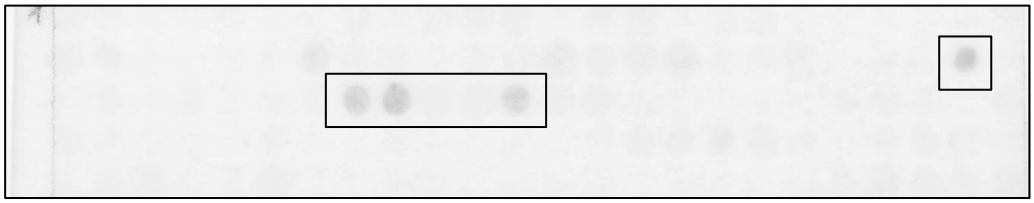



**FIG 3** Western blotting analysis of the scFv 3E2 (A) and the mAb 5B4/2F2 (B) under reducing and denaturing conditions. The scFv 3E2 bound to its epitope on the recombinant F13 protein (4), however not to VACV Elstree (1), VACV Munich1 (2) or to the A27 protein (3) using as negative control. The mAb 5B4/2F2 showed a reaction to VACV Elstree (1), VACV Munich1 (2) and to the recombinant A27 protein of VACV (3).

**Epitope mapping using a peptide membrane and truncated recombinant F13 fragment**

A cellulose membrane, containing 372 15-mere peptides with 12 overlapping amino acids covering the whole F13 protein, was used for epitope mapping. The scFv 3E2 reacted to two different target regions, i.e. 139-GSIHTIKTLGVYSDY-153 and 166-TFKAFNSAKNSWLNLCSAACCLPVSTA-192 (Figure 4).

A



Peptide #47 GSIHTIKTLGVYSDY

Peptide #56 TFK**AFNSAKNSWLNL**
Peptide #57 **AFNSAKNSWLNL**CSA
Peptide #60. **LNL**CSAACCLPVSTA

B

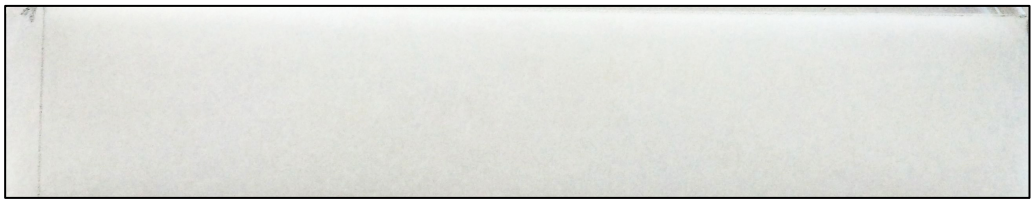


**FIG 4** A cellulose membrane, containing 372 15-mere peptides covering the whole F13 protein was used for epitope mapping. The scFv 3E2 (A) reacted with four peptide spots (No. 47, 56, 57 and 60). Two targets were mapped (139-GSIHTIKTLGVYSDY-153 and 166-TFKAFNSAKNSWLNLCSAACCLPVSTA-192). The minimal sequence essential for binding for the second epitope area was 169-AFNSAKNSWLNL-188. The secondary Ab, used as negative control (B), did not bind to any spots.

The strongest signal was recorded for the area 169-AFNSAKNSWLNL-188. For the mAb 15B6, an epitope with the sequence 202-VFFTDSPEHLLGYSRDLDTDVVID-225 was identified (Figure 5), whereby the minimal sequence essential for binding was 211-LLGYSR-216.

A

| | |
|---|---|
| Peptide #68 | VFFTDSPEH**LLGYSR** |
| Peptide #69 | TDSPEH**LLGYSR**DLD |
| Peptide #70 | PEH**LLGYSR**DLDTDV |
| Peptide #71 | **LLGYSR**DLDTDVVID |

B

**FIG 5** A cellulose membrane, containing 372 15-mere peptides covering the whole F13 protein was used for epitope mapping. MAb 15B6 (A) recognized four peptide spots (No. 68-71). The epitope was mapped to 202-VFFTDSPEHLLGYSRDLDTDVVID-225. The minimal sequence essential for binding is 211-LLGYSR-216. The secondary Ab, used as negative control (B), did not bind to any spots.

To further confirm the epitope mapping results, western blotting analysis was performed. A truncated F13 fragment (#F1) starting at aa 197 with a total length of 532 bp was constructed (Figure 6A) to cover the identified epitope by the mAb 15B6 (Figure 6B).

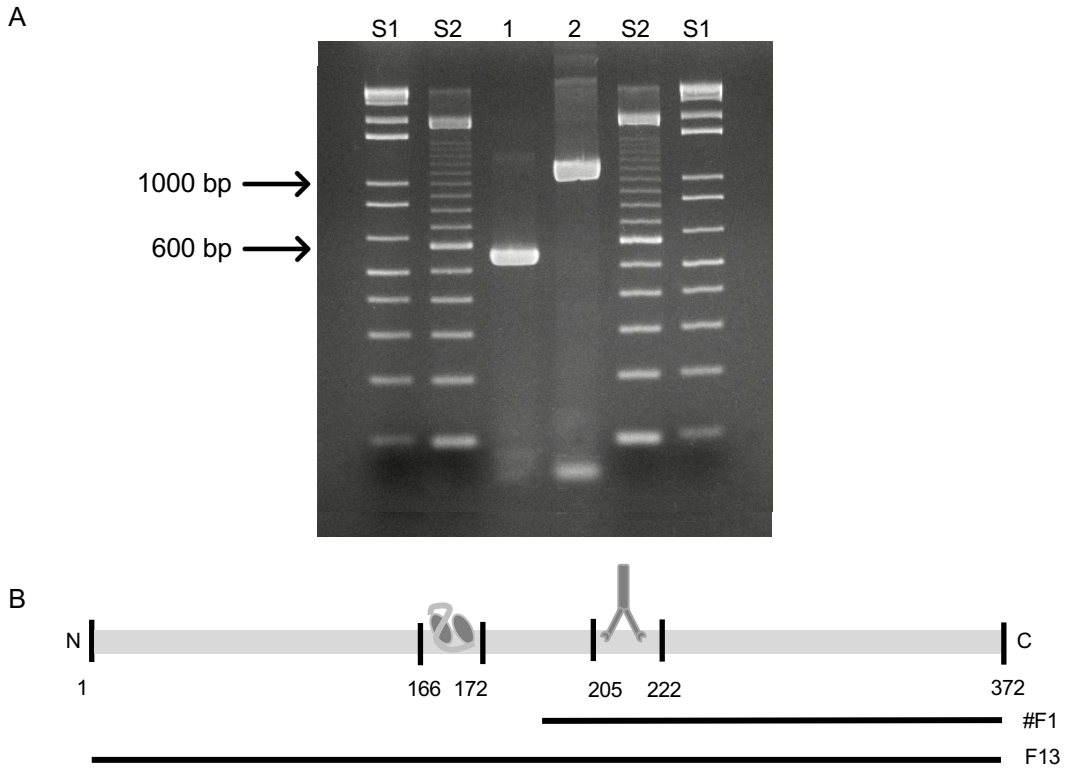



**FIG 6** A: Amplification of the 532 bp truncated fragment #1 of F13L$_{VACV}$ (1) and the 1119 bp F13L gene (2). S1 and S2 are 1 kb and 100 bp ladders, respectively. B: Schematic illustration of the F13 protein and the truncated fragment #F1. The epitope of the mAb 15B6 is located on the #F1, as well as on the whole F13 protein, while the epitope of the scFv 3E2 is located only on the whole F13 protein.

The epitope mapping was verified by western blotting analysis, whereby the mAbs 15B6 and 5B4/2F2 as well as an anti-his tag Ab were used as controls (Figure 7). The scFv 3E2 only bound to the recombinant F13 protein (3) showing a band at 37 kDa (Figure 7A), whereas the mAb 15B6 showed a reaction with the recombinant fragment (2) at 22 kDa as well as with the whole F13 protein (3) and the VACV Elstree (1) at 37 kDa (Figure 7B). The mAb 5B4/2F2 recognized a 14 kDa on the recombinant A27 protein (4) and on the VACV Elstree (1) (Figure 7C). The anti-his-tag bound to all recombinant proteins and therefore confirmed the purity of the proteins. (Figure 7D).

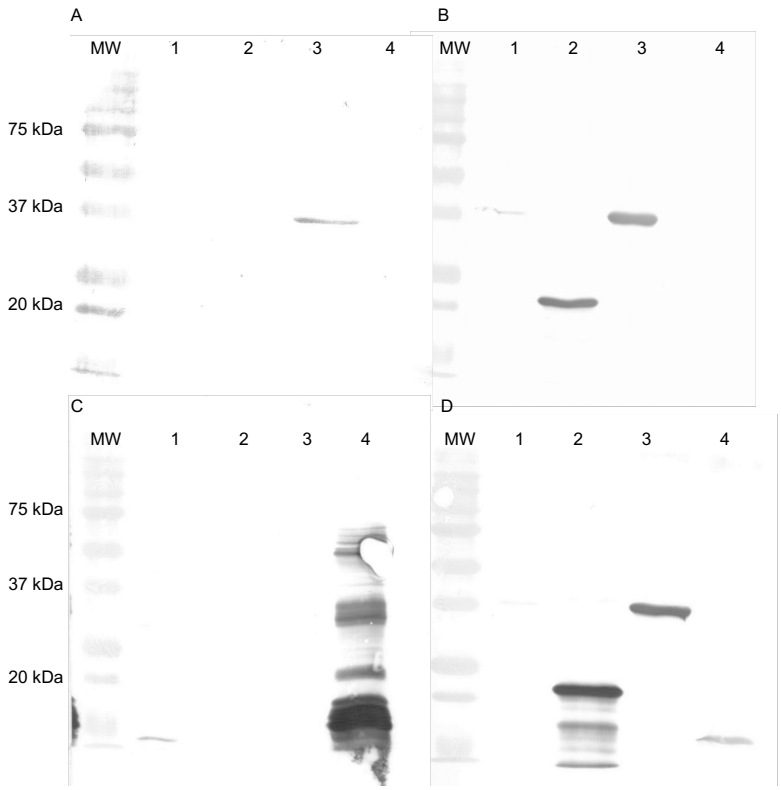



**FIG 7** Western blotting analysis using VACV Elstree (1), #F1 (2), F13 (3) and A27 (4) proteins. The scFv 3E2 (A) detected the whole F13, while the mAb 15B6 (B) showed a reaction on the VACV Elstree, #F1 and the whole F13 protein. The control mAb 5B4/2F2 (C) was used and detected VACV Elstree and the recombinant A27 protein. The his-tag Ab (D) served as positive control and bound to all recombinant proteins. MW was Precision Plus Protein Standard (Bio-Rad).

**Confocal microscopy**

A confluent monolayer of Vero cells was infected with 100 pfu of VACV Munich1 for 3h. After fixing and permeabilization of the cells, the primary Abs diluted in PBS supplemented with 10% FCS were incubated for 2.5h. The secondary Abs diluted in PBS containing 10% FCS were incubated followed by cell nuclei staining with Hoechst' reagent. Cells were incubated at room temperature for 1h. Fluorescence was examined under a confocal laser scanning microscope equipped with a Plan-Apochromat 63x/1.40 Oil DIC objective (LSM 800, Zeiss).

Penetrating the cell through permeabilization was recognized for the following: scFv 3E2 (Figure 8A), the mAb 15B6 (Figure 8B), the mAb 5B4/2F2 (Figure 8C), an anti-envelope rabbit immune serum (Figure 8D), a mixture of the scFv 3E2 and the mAb 5B4/2F2

(Figure 8E), a mixture of the mAbs 15B6 and 5B4/2F2 (Figure 8F), a mixture of the scFv 3E2 and the mAb 15B6 (Figure 8G), a mixture of scFv 3E2 and rabbit anti-envelope serum (Figure 8H) and a mixture of mAb 15B6 and rabbit anti-envelope serum (Figure 8I). Various fluorescence signals were recorded. The scFv 3E2 (Figure 8A) showed the weakest signal, while the mAb 5B4/2F2 (Figure 8C) was the strongest. Interestingly, the mAb 15B6 and the rabbit anti-envelope immune serum bound to the same spot (Figure 8I). Figure 8J showed the cell culture control without VACV infection.

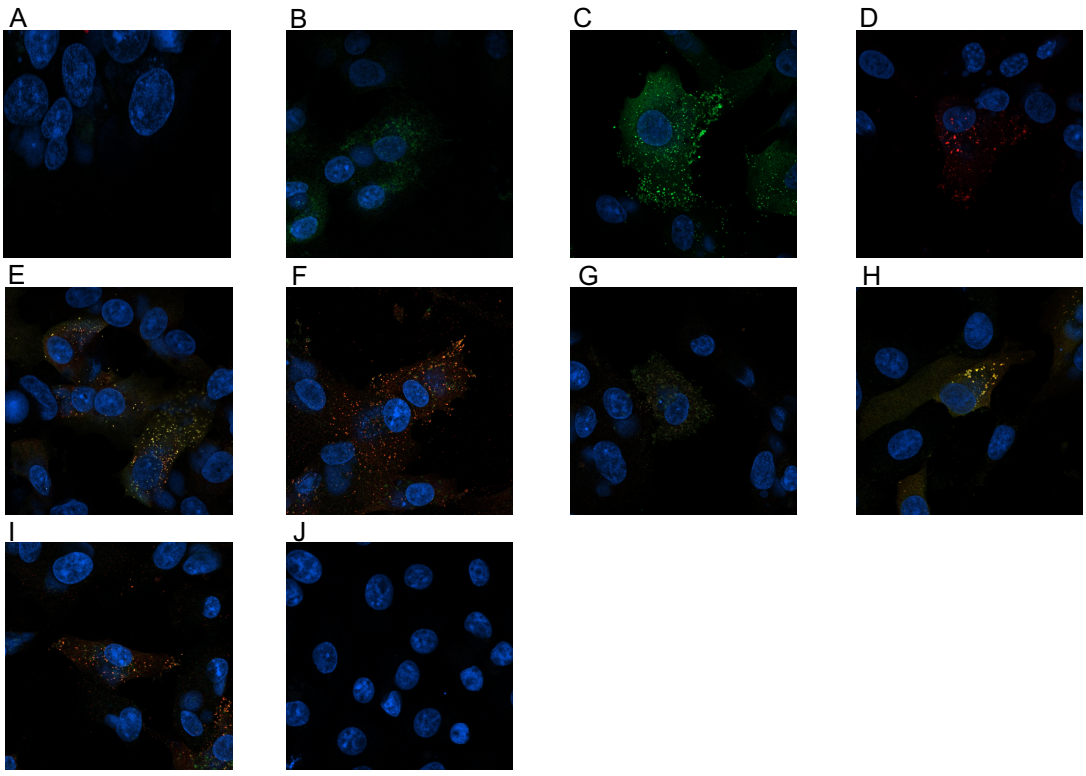



**FIG 8** Confocal microscopy results of the scFv 3E2 (A), the mAb 15B6 (B), the mAb 5B4/2F2 (C), an anti-envelope rabbit immune serum (D), a mixture of the scFv 3E2 and the mAb 5B4/2F2 (E), a mixture of the mAbs 15B6 and 5B4/2F2 (F), a mixture of the scFv 3E2 and the mAb 15B6 (G), a mixture of scFv 3E2 and rabbit anti-envelope serum (H) and a mixture of mAb 15B6 and rabbit anti-envelope serum (I). All were able to penetrate the cell through permeabilization to different extents. J is the negative control without VACV infection.

**Neutralization abilities *in vitro***

The classical plaque reduction test (PRT) was performed in triplicates with a starting-concentration of 200 µg/ml of the antibodies. The VACV-Elstree was neither neutralized

by the scFv 3E2 (Figure 9A) nor by the mAb 15B6 (Figure 9) either in the presence or absence of 1% complement.

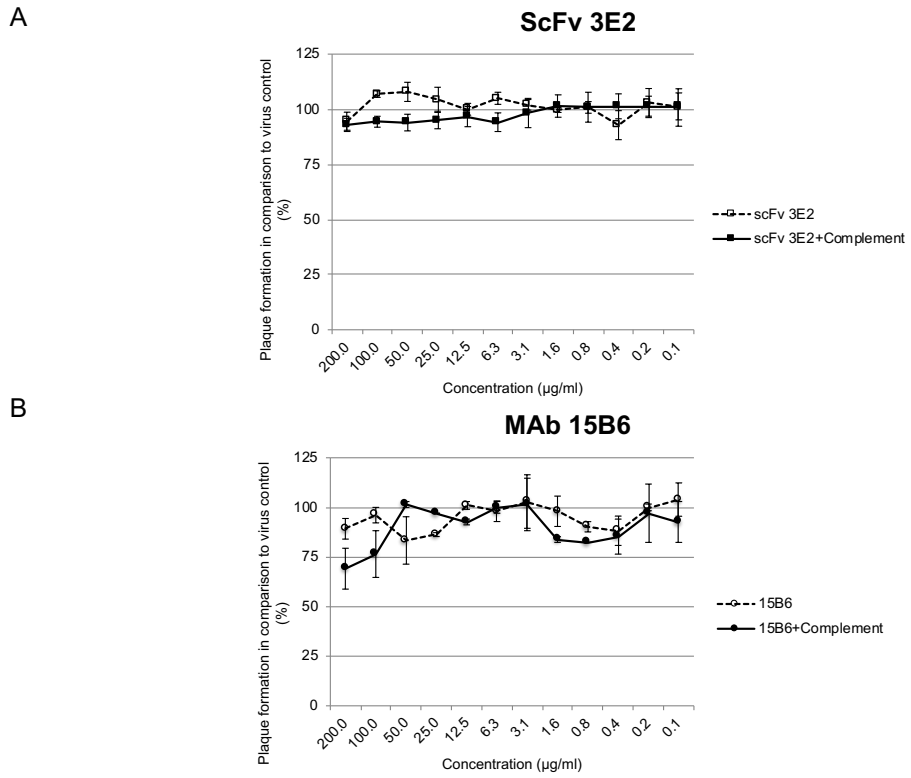



**FIG 9** In vitro Plaque Reduction Neutralization Test. The scFv 3E2 (A) and the mAb 15B6 (B) with and without complement did not neutralize the VACV Elstree.

## DISCUSSION

In this study, we characterized a purified human anti-F13$_{VACV}$ scFv, which was isolated from a human anti-OPXV-immunoglobulin library by phage display. The epitope of the scFv 3E2 was assigned to the 37 kDa F13 protein, which is the most abundant membrane protein of the EEV (Hiller et al., 1981; Hirt et al., 1986) and located within the TGN membrane (Schmutz et al., 1995). F13 plays an important role in the wrapping progress and therefore in the EEV production. After EEV release, F13 is left on the inner side of the EEVs (Husain et al., 2003; Roos et al., 1996). In western blotting assays, the scFv 3E2 detected the recombinant F13 protein under denaturing and reducing conditions. However, the scFv 3E2 did not bind to its epitope on the whole VACV, which was expected due to the inner location of the protein (Roos et al., 1996; Schmutz et al., 1995).

These results were verified by ELISA, in which the scFv 3E2 bound to neither VACV Elstree nor VACV Munich1, too. Moreover, in confocal microscopy study, the scFv 3E2 showed a weak signal only. The stronger binding of the mAb 15B6 to the virus can be explained by the fact, that the mAb contains two antigen binding sites in contrast to the scFv 3E2.

For epitope mapping, a cellulose membrane, spanning 372 15-mere peptides with 12 overlapping amino acids covering the whole F13 protein was used. The scFv 3E2 reacted with two epitopes differently located on the F13 primary structure (139-GSIHTIK-TLGVYSDY-153 and 169-AFNSAKNSWLNL-188). From another study it is known, that one antibody detected two almost identical epitopes of *Clostridium difficile* (Frey and Wilkins, 1992). However, in the case of the scFv 3E2 target regions, no similarities between both regions were found. In western blotting analysis, the scFv 3E2 binding resulted in a weaker band compared to the control mAb 15B6. Moreover, denaturation with SDS and heating reduced the binding of the scFv 3E2. Therefore, the scFv 3E2 is conformationally dependent. A similar result of a conformationally dependent herpes simplex mAb has been observed before (Krawczyk et al., 2011). Other discontinuous epitopes over a larger aa range were mapped on the D8 protein of VACV (Matho et al., 2014). For the mAb 15B6 the epitope sequence of 211-LLGYSR-216 was identified. In predictions of the secondary structure of the F13 protein, ß-turns (Petersen et al., 2010) were evident within the target region. Beta-turns generally lead to a high antigenicity (Chou and Fasman, 1979). Although several studies confirmed the neutralization abilities of mAbs against OPXVs (Benhnia et al., 2013; Benhnia et al., 2009a; Czerny and Mahnel, 1990; McCausland et al., 2010; Ramirez et al., 2002), the scFv 3E2 did not have any neutralizing activity, because of the inner localization of the F13 protein (Schmutz et al., 1995). However, scFvs can penetrate the cell, in contrast to full size immunoglobulins, because of their small size (Holliger and Hudson, 2005; Yokota et al., 1992). In conclusion, the

construction of recombinant scFv phage libraries is a promising strategy to generate engineered, target-specific human recombinant antibodies, which might help for controlling any future outbreak of zoonotic OPXV infections.

## MATHERIAL AND METHODS

### Cells and viruses

The permanent monkey kidney cell line MA-104 cultured in minimum essential medium (MEM) and supplemented with 7% fetal calf serum was used to propagate the VACV strains Elstree and Munich 1 (Czerny and Mahnel, 1990). Infectivity titers were determined and calculated as plaque forming units (pfu/ml). Vero cells cultured in MEM and supplemented with 5% fetal calf serum were used for plaque reduction tests.

Virus preparations were purified and concentrated by sucrose gradient centrifugation as described previously (Czerny et al., 1994; Joklik, 1962). Protein contents of the samples were determined by the method of Lowry et al. (Lowry et al., 1951).

### Monoclonal and polyclonal antibodies

For this study, the A27-specific murine mAb anti-VACV 5B4/2F2 (epitope #1A) (Czerny et al., 1994; Czerny and Mahnel, 1990) and the rat mAb 15B6 directed against the VACV envelope protein F13 (Galfre and Milstein, 1981; Schmelz et al., 1994) were used. The mAb 15B6, used as F13 potitive control, was kindly made available by Jacomina Krijnse Locker. To evaluate the preparation of the protein purifications, an anti-his-tag antibody (Qiagen, Hilden, Germany) was used. Moreover, polyclonal rabbit hyper-immune serum against purified $A27_{VACV}$ (Czerny et al., 1994; Czerny and Mahnel, 1990), used in confocal experiment, was purified on Protein G sepharose columns (HiTrap™ 5 ml Protein G HP, Sigma Aldrich, USA), dialyzed against PBS and sterilized by centrifugation at

20,238 x g. Protein contents of all antibodies preparations were determined according to the method of Lowry et al. (Lowry et al., 1951).

**Immunization, lymphocyte preparation and library construction**

Four human volunteers were immunized via scarification with Dryvax® (Wyeth Laboratoires, Marietta, USA) according to the manufacturer's instructions as described before (Diesterbeck et. al, submitted). 20-28 days post vaccination, about 500 ml blood was collected, the peripheral blood mononuclear cells were isolated using Ficoll-Paque PLUS density gradient (GE Bioscience, Freiburg, Germany), followed by RNA extraction (RNeasy MiniKit (Qiagen, Hilden, Germany)) and by cDNA synthesis using oligohexamers ($pdN_6$) (Invitrogen, Karlsruhe, Germany) as directed by the manufacturers. To construct the scFv library, RT-PCR was performed using total RNA of at least $10^7$ cells per volunteer as described before (Diesterbeck et. al, submitted).

**Construction and purification of F13**

For the amplification of the F13L gene and its truncated sub-fragment, primers were selected by using a published sequence (GenBank accession number: NC_006998.1). The restriction enzymes *Bam*HI and *Hind*III were introduced to both ends of the primers. The reserve primer "F13 (C-terminal)" 5' – GAT CAA GCT TTT AAA TTT TTA ACG ATT TAC - 3', and the following forward primers were used to amplify the full size F13 protein and the truncated fragment #1: "F13 forward (N-terminal)": 5' - GAT CGG ATC CAT GTG GCC ATT TGC ATC GG - 3', "fragment #1 (N-terminal)": 5' –GAT CGG ATC CAG AAT CCT ATA GGT GGA GTG- 3'. The PCR reactions were set as follows: initial denaturation at 95°C/10 min, 35 cycles of 94°C/1 min, primer annealing at 50.6°C/1 min and 72°C/2 min, and a final extension step of 72°C/10 min.

The PCR products were ligated into the pSC-A-amp/kan PCR cloning vector (StrataClone, Agilent Technologies, Germany) and transformed into chemically competent *E. Coli*. Ligation and transformation were performed according to the manufacturer. Plasmids of positive colonies were isolated from 5 ml LB media using the MiniPrep Kit (Qiagen, Hilden, Germany). The genes were sequenced with an ABI Prism 3100 Analyzer (Applied Biosystems Deutschland GmbH, Darmstadt, Germany). Sequences were analyzed with the DNAStar program (SeqMan Pro and MegAlign. Version 12.0. DNASTAR. Madison, WI) and with BLAST (Altschul et al., 1990).

Plasmids were ligated into the expression vectors pQE80L and pQE81L (Qiagen, Hilden, Germany), electroporated into One Shot® TOP10 Electrocomp™ *E. coli*. (Invitrogen™, Karlsruhe, Germany) and were grown in LB media with 1 mM Ampicillin at 37°C until $OD_{600nm}$=0.6. The expression was induced with 1 mM IPTG at 37°C for 5h, while shaking at 200 rpm (Sartorius Certomat® BS-1, Goettingen, Germany). Cells were pelleted (4,500 rpm/ 20 min) and resuspended in TBS buffer containing 7.7 mM Tris pH 7.5/150 mM NaCl before sonicated (100% on ice/ 20 min). Cell debris was removed by centrifugation (2,100 rpm/15 min). Lysate was pelleted at 7,000 rpm for 1h and resuspended in suspension buffer containing 12.5% phosphate buffer/1% 2 M imidazole stock/23% of 43.5% glycerol stock at 4°C over night. Afterwards, the lysates were mixed with an equal volume of 16 M urea stock and incubated by gently shaking at 4°C for 1h. Lysates were clarified at 17,000 g at 4°C for 45 min. Supernatants were compounded with Ni-NTA agarose (Qiagen, Hilden, Germany) and incubated under gently shaking at 4°C overnight. The mixtures were loaded onto 5 ml Ni-NTA columns (Qiagen, Hilden, Germany). The purifications were done according to the manufacturer instructions by using a binding buffer pH 8.0 containing 12.5% phosphate buffer/1% 2 M imidazole stock/23% of 43.5% glycerol stock/50% 16M urea stock and an elution buffer containing 12.5% phosphate buffer/5% 2 M imidazole stock/23% of 43.5% glycerol stock/50% 16M urea stock. After

overnight dialysis against PBS the protein concentrations were determined by Lowry protein assay as described previously (Lowry et al., 1951).

**Selection on purified recombinant F13 protein**

To get specific anti-F13 scFv antibodies, the constructed human anti-OPXV-scFv phage library was three times panned applying purified recombinant F13 protein of VACV Munich 1. The exact screening procedure was performed as previously described (Diesterbeck et. al, submitted).

**Plasmid isolation and sequencing of positive colonies**

To produce antibody fragments without pIII fusion, 0.5 µl of the *E. coli* HB2151 precultures were transferred into 100 µl 2×TYG (0.1%)-A and incubated at 30°C for 4 h. The expression was induced by the addition of IPTG to a final concentration of 2 mM dissolved in 50 µl 2×TY-A by gentle shaking at 30°C overnight (Sartorius Certomat® BS-1, Goettingen, Germany). The cells were centrifuged (137,000xg/ 4°C/ 20 min) and the supernatants were applied in an ELISA for pre-screening as described before (Diesterbeck et. al, submitted). The positive plasmid was isolated from 5 ml media using the MiniPrep Kit (Qiagen, Hilden, Germany). With an ABI Prism 3100 Analyzer (Applied Biosystems Deutschland GmbH, Darmstadt, Germany), the genes encoding the variable regions of the heavy (VH) and light (VL) chains were sequenced. Therefore, the vector specific forward primer R1 (5'-CCA TGA TTA CGC CAA GCT TTG GAG CC-3') and the reverse primer R2 (5'-CGA TCT AAA GTT TTG TCG TCT TTC C -3') were applied to the sequence reaction. The sequence was analyzed with the DNAStar program (SeqMan Pro and MegAlign. Version 12.0. DNASTAR. Madison, WI). Moreover, the amino acid sequence was used to classify the presumed family and germline origin by search of IMGT/V-QUEST (Brochet et al., 2008; Giudicelli et al., 2011) and

IMGT/DomainGapAlign. Moreover the structure of the scFv was analyzed and a 3D model was calculated by using the Phyre[2] server (Kelley and Sternberg, 2009) and VMD 1.9.1 software (Humphrey et al., 1996).

**Upscale production and purification of selected scFv**

Production of the scFv yielding the highest ELISA value was scaled up to one liter and the antibody was purified as described before (Diesterbeck et. al, submitted). Finally, the protein concentration was determined by Lowry protein assay as described previously (Lowry et al., 1951).

**SDS-PAGE and Western blotting**

For western blotting analyses, 5 µg/slot of gradient purified VACV Elstree, gradient purified VACV Munich1, purified recombinant A27 protein and of the purified recombinant F13 protein were fractionated by vertical 12% sodium dodecyl sulfate (SDS)-polyacrylamid gel electrophoresis (Laemmli, 1970) and subsequently transferred to nitrocellulose membranes (Vorou et al., 2008). The blocking step was performed by a mixture of 3% BSA in TBS at room temperature for 2h. Purified Abs (50 µg/ml) were added to the membranes and incubated at room temperature for 2h. Immunodetection followed with horseradish peroxidase-conjugated anti-IgG antisera (1:250) for 2.5h and horseradish peroxidase color-developing reagent (Bio-Rad, Heidelberg, Germany; 375 µg/ml). Between each step, the membranes were washed with TBS three times for 10 minutes. The $M_r$ of stained viral proteins was estimated with a concurrent protein standard (Bio-Rad, Munich, Germany).

**Enzyme-linked immunosorbent assay (ELISA)**

Microtiter plates were coated with either of 2 µg/ml VACV Elstree, 2 µg/ml VACV Munich1, 15 µg/ml $F13_{VACV}$, 5 µg/ml $A27_{VACV}$, and with 2 µg/ml BSA. After blocking, antibodies were added in two-fold serial dilutions starting with a concentration of 200 µg/ml. Incubation was performed at 37°C for 3h. After five washings with PBS detection of the second antibody occurred either with goat pAb to E tag (HRP) (1:2000), anti-mouse IgG (whole molecule) or anti-rat IgG (whole molecule) peroxidase conjugate developed in goat (1:2000) at 37°C for 1h. After ten times washings with PBS, the developing solution composed of tetramethylbenzidine (TMB) was added. The reaction development was stopped using hydrochloric acid. The photometric reading (Spectra II, SLT Labinstruments GmbH, Germany) was performed at 450 nm. The binding affinity was calculated from the average adsorption of three independent assays using the Michaelis-Menten behavior (Michaelis and Menten, 1913) using GraphPad Prism version 6.00 for Mac (La Jolla California, USA).

**Epitope mapping by SPOT synthesis on nitrocellulose membranes**

A total of 372 amino acids representing whole F13 protein were synthesized directly on derivatized cellulose membranes in form of 15-mere peptides with 12 aa overlaps. The synthesis on derivatized cellulose membranes was performed as described before (Frank, 1992). The protein binding assay as well as the subsequent membrane regeneration were executed as previously described (Beutling et al., 2008).

**Cell infection and confocal microscopy**

A confluent monolayer of vero cells was cultivated on glass coverslips placed in 24-well tissue culture plates. Then, 100 pfu (100 µl/well) of VACV Munich1 was added to each well. After incubation at 37°C for 3h, the virus-mixture was replaced with fresh medium containing 2.5% FCS. Cells were incubated at 37°C for about 18h, followed by the

removal of the medium. Cells were fixed on glass coverslips with 4% paraformaldehyde in PBS at room temperature for 20 min. Thereafter, 0.2% Triton X-100 in PBS was added and incubated at room temperature for 5 min for permeabilization of the cells. After three washing-steps with PBS, cells were blocked with PBS supplemented with 10% FCS (200 µl/well) at room temperature for 30 min. Subsequently the blocking solution was removed and 100 µg/ml of the primary Abs diluted in PBS supplemented with 10% FCS (250 µl/well) were incubated at 4°C for 2h, followed by further 30 min at room temperature. After three washing-steps with PBS, both the Hoechst' reagent (1:5000; 100 µl/well) to stain the cell nuclei and the secondary Abs (1:1000 goat anti-mouse Alexa Fluor 488 or goat anti-rabbit Alexa Fluor 568 (Abcam, Cambridge, UK) diluted in PBS containing 10% FCS (250 µl/well)) were added and incubated at room temperature for 1h. After three washing steps with PBS, the glass coverslips were mounted in Mowiol/DABCO. Fluorescence was examined on a confocal laser scanning microscope equipped with a Plan-Apochromat 63x/1.40 Oil DIC objective (LSM 800, Zeiss).

### *In vitro* Plaque Reduction Neutralization Test (PRT)

To assess the neutralization abilities of selected antibodies, a confluent monolayer of Vero cells was grown in 24-well tissue culture plates. The PRT was performed as described before (Ahsendorf et al., 2019). Plaques were counted by visual inspection. Neutralization was determined as ≥50% plaque reduction compared to the virus control.

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## 2.4 AUTHOR'S CONTRIBUTIONS

- **Chapter I:**

  **Species-specific conservation of linear antigenic sites on VACV A27 protein homologues in the evolution of OPXV**

  Conceptualization: C.-P.C.; Investigation: **H.P.A.**, L.L.G., K.H.E., R.L.R. and L.E.H.; Methodology: A.A.E.W., R.L.R., C.S.-H. and C.-P.C.; Project administration: C.-P.C.; Software: S.-K.H.; Supervision: A.A.E.W., C.S.-H. and C.-P.C.; Validation: **H.P.A.**, L.L.G., K.H.E., A.A.E.W., S.-K.H., R.L.R., U.B., M.B., C.S.-H., L.E.H. and C.-P.C.; Visualization: **H.P.A.**, L.L.G., K.H.E., S.-K.H., R.L.R., L.E.H. and C.-P.C.; Writing–original draft: **H.P.A.** and C.-P.C.; Writing–review and editing: **H.P.A.**, L.L.G., K.H.E., A.A.E.W., S.-K.H., R.L.R., U.B., M.B., C.S.-H., L.E.H. and C.-P.C.

- **Chapter II:**

  **Characterization of an *in vivo* neutralizing anti-VACV D8 scFv from a human anti-VACV-specific recombinant library**

  Conceptualization: C.-P.C. and U.S.D.; Investigation: U.S.D., **H.P.A.** and A.F.; Methodology: C.-P.C., U.S.D. and T.S.; Project administration: C.-P.C.; Supervision: C.-P.C. and U.S.D.; Validation: U.S.D., **H.P.A.**, A.F., T.S. and C.-P.C.; Visualization: U.S.D., **H.P.A.** and A.R.S.; Software: A.R.S.; Writing - Original Draft Preparation: U.S.D.; Writing - Review and Editing: U.S.D., **H.P.A.**, A.F., T.S. and A.R.S.

- **Chapter III:**

  **Characterization of an anti-VACV F13 scFv from a human anti-VACV-specific recombinant library**

  Conceptualization: C.-P.C.; Investigation: **H.P.A.** and M.W.; Methodology: C.-P.C., **H.P.A.**, M.W., S.-K.H. and U.S.D.; Project administration: C.-P.C.; Supervision: C.-P.C.; Validation: **H.P.A.**, M.W. and C.-P.C.; Visualization: **H.P.A.** and M.W.; Writing - Original Draft Preparation: **H.P.A.**; Writing - Review and Editing: **H.P.A.** and U.S.D.

# 3 GENERAL DISCUSSION

## 3.1 DISCUSSION

VARV, the causative agent of smallpox, is supposed to have caused more fatalities than all other human diseases together (McFadden, 2005). However, through strict vaccination programs, the non-zoonotic VARV has been officially eradicated since 1979 (Fenner et al., 1988). Due to cessation of vaccination after smallpox eradication, the majority of the population does not have protective immunity, since the worldwide vaccination program has been stopped in 1979. As a consequence, there is a growing fear about the use of VARV or zoonotic cross-reactive OPXVs as potential biological weapons (Henderson, 1999; Rimoin et al., 2010). Moreover, recent outbreaks of MPXV (Ladnyj et al., 1972; Reed et al., 2004; Vaughan et al., 2018) as well as the presence of CPXV in the environment (Becker et al., 2009; Campe et al., 2009; Howard et al., 2008; Kurth et al., 2008; Vogel et al., 2012; Vorou et al., 2008) have encouraged corporations to develop therapeutic drugs. Another treatment option, either as pre- or post-exposure prophylaxis are highly efficient inhibiting anti-OPXV antibodies which prompted us to construct an OPXV-human immunoglobulin library, to generate target-specific neutralizing recombinant antibodies. As is known, vaccination results in the induction of neutralizing antibodies against different envelope proteins (D8, A27, H3, A17, L1, A33 and B5) (Aldaz-Carroll et al., 2005; Hsiao et al., 1998, 1999; Matho et al., 2015; Moss, 2012, 2016; Rodriguez et al., 1985; Vogel et al., 2012). So, we use the immunogenic D8 protein as a target for the library-screening. This conserved protein is involved in the virus attachment by binding to GAG chondroitin sulfate (Hsiao et al., 1999; Maa et al., 1990). Screening yielded one scFv designated 1.2.2.H9. First, to further improve the avidity and effector function (Moutel et al., 2009), the scFv-1.2.2.H9 was also engineered into the larger human scFv-Fc-1.2.2.H9 and IgG1-1.2.2.H9 formats. Similar binding affinities were shown

by scFv-1.2.2.H9 and scFv-Fc-1.2.2.H9, whereas, IgG1-1.2.2.H9 was much more efficient. As expected, a multivalent IgG consisting of two variable domains has a higher avidity than a monovalent antibody with only one antigen binding site. Moreover, due to the engineered Fc-part, complement can bind to these two bigger molecules (Burton, 2002), resulting in an effective *in vitro* VACV-neutralization in the presence of complement. There are more than 30 proteins involved in the complement system, which is activated by a cascade of reactions. The Fc-region of the antibody is used to activate the complement system, which is a member of the innate immune system (Burton, 2002). This finding is also confirmed by other authors, suggesting that complement is indeed needed to enhance the D8 footprint (Matho et al., 2012).

The obtained scFv-1.2.2.H9 was able to protect 50% of mice from a lethal challenge with VACV Munich1, however, no VACV-neutralization could be observed *in vitro*. So, there might be some factors *in vivo*, improving the neutralization. The bound scFv antibodies work hand in hand with the cellular immunity by labeling the virus for destruction. As mentioned above, OPXV can occur in different forms with different proteins and functions. The enveloped CEV and EEV virion particles are important for rapid cell-to-cell spread (Blasco and Moss, 1992; Moss, 2012; Payne, 1980; Roper et al., 1998; Smith et al., 2002), which is an essential parameter in an infection experiment. Therefore, its size is advantageous for the scFv, as the smallest antibody derived molecule, consisting only of the variable (V) regions of the heavy (H) and light (L) chain connected by a short peptide linker. It is known that scFvs are able to penetrate the cell within 10-30 minutes (Bird et al., 1988; Yokota et al., 1992). So, the scFv-1.2.2. H9 could pass the CEVs and EEVs and block the cell-to-cell spread by binding to its epitope. Moreover, another difference of the neutralization efficiency of the scFv between *in vitro* and *in vivo* could be the differentiation of the extracellular matrix. Since D8 is responsible for the attachment by binding to GAG chondroitin sulfate (Chung et al., 1998; Hsiao et al., 1999; Lin et al.,

2000; Moss, 2016), the extracellular matrix may have an effect on the anti-D8-antibody-binding. If the antibody has bound to the D8, there are still four other proteins left for the attachment (A27, H3, A26 and L1) and therefore for the dissemination of the virus (Chiu et al., 2007; Chung et al., 1998; Foo et al., 2009; Hsiao et al., 1999; Lin et al., 2000; Moss, 2016). So, we decided to investigate the A27 protein, as a member of a protein-complex, for further studies. To obtain a good neutralizing drug, it seems to be important, to include many targets, in order to block the infection steps, such as attachment and fusion. In neutralization assays, targeting the A27 protein of VACV, which is also involved in the attachment (Howard et al., 2008), we found out, that the VACV neutralization with anti-A27 mAbs *in vitro* was complement-dependent as well. Other studies, also demonstrated the complement-dependence of A27 mAbs for the VACV neutralization (Kaever et al., 2016), as well as like the complement-improved neutralization of the D8 antibodies as described before. Kaever et al. could not neutralize VACV without complement (Kaever et al., 2016), while in our studies three out of six mAbs neutralized VACV without complement. However, this neutralization could be improved with the addition of complement suggesting, that complement is ameliorating the inhibition of virus attachment. Next to the attachment, the virion wrapping also is important for the cell-to-cell spread of the virus. Therefore, we decided to screen the existing library against the F13 protein, which plays an important role in the virion wrapping and therefore in the EEV production and spread of the virus (Roper and Moss, 1999). However, the obtained scFv 3E2 failed to neutralize VACV, which can be explained by the inner localization of the F13 protein (Hiller and Weber, 1985; Schmutz et al., 1995). Interestingly, these findings could be confirmed by western blotting assays as well as by ELISAs. Here the scFv 3E2 was only able to react with its corresponding epitope on the recombinant F13 protein. On the VACV however, no binding was observed. However, in FACS analyzes (data not shown), the scFv 3E2 was able to bind to permeabilized VACV infected cells, which also is in

agreement with the results.

Furthermore, it is suggested that there might be an interaction between the F13 and the A27 protein, because of their functions in the virion wrapping (Schmutz et al., 1995). Testing the anti-A27 mAb 5B4/2F2 together with the anti-F13 scFv 3E2 *in vitro*, there are no improvements in the neutralization efficiency compared to the mAb 5B4/2F2 alone. So, these two antibodies used together did not display any synergistic efficacy. Our confocal studies demonstrated, that the epitopes of the two tested anti-F13 antibodies (scFv 3E2 and mAb 15B6) and anti-A27 mAb 5B4/2F2 were close to each other. Interestingly, doubling the amount of the mAb 5B4/2F2 in western blotting assays, indicated an additional reaction both with the recombinant F13 protein and with its truncated fragment #1. In our A27 fine-mapping studies, we mapped the epitope of the mAb 5B4/2F2 between aa residues 32-39 (REAIVKAD). Figure 5 shows the sequence of VACV Copenhagen F13 protein (accession number: P20638.1). There are 3 aa residues highlighted 240-AIV-242, which correspond with the target region of the mAb 5B4/2F2 directing to epitope #1A.

```
  1-MWPFASVPAGAKCRLVETLPENMDFRSDHLTTFECFNEIITLAKKYIYI-  49
 50-ASFCCNPLSTTRGALIFDKLKEASEKGIKIIVLLDERGKRNLGELQSHC- 98
 99-PDINFITVNIDKKNNVGLLLGCFWVSDDERCYVGNASFTGGSIHTIKTL-147
148-GVYSDYPPLATDLRRRFDTFKAFNSAKNSWLNLCSAACCLPVSTAYHIK-196
197-NPIGGVFFTDSPEHLLGYSRDLDTDVVIDKLRSAKTSIDIEHLAIVPTT-245
246-RVDGNSYYWPDIYNSIIEAAINRGVKIRLLVGNWDKNDVYSMATARSLD-294
295-ALCVQNDLSVKVFTIQNNTKLLIVDDEYVHITSANFDGTHYQNHGFVSF-343
344-NSIDKQLVSEAKKIFERDWVSSHSKSLKI                     -372
```

**FIG 5** Amino acid sequence of F13 protein (accession number: P20638.1). Fragment #1 (green) starts at aa 197. The marked aa residues are supposed to react with the mAb 5B4/2F2.

In fine-mapping studies using SPOT membranes, we could confirm, that there are only a few aa needed to detect the target regions. Interestingly, the aa sequence 35-IVKA-38 was the most important factor for binding the mAb 5B4/2F2. Based on these findings, we took a closer look at all of the 381 A27 sequences which are available online and figured

out, that the epitope-complex #1 (26-KKPEAKREAIVKAD-39) was hypervariable and therefore responsible for conserved species-specific epitope characteristics. Differences in the aa sequence had an effect on the affinity in ELISA, too. Of note, in the mapped area of the epitope complex #1 (aa residues 26-39) is the GAG binding site (aa residues 21-34), in which the KKPE segment (aa residues 26-29) is essential for binding heparan sulphate (Chung et al., 1998; Shih et al., 2009). So, the mAb 3F5/2D5 directing to epitope #1C (aa 26-**KKPE**AK-31) influences the attachment by binding the KKPE region. However, this antibody was not able to neutralize VACV without complement *in vitro*. As previously seen with the anti-D8 1.2.2.-H9 ab, this could be due to the fact, that there are four other proteins involved in the attachment (Chiu et al., 2007; Chung et al., 1998; Foo et al., 2009; Hsiao et al., 1999; Lin et al., 2000; Moss, 2016).

In contrast to epitope complex #1, the epitopes #4 and #5 could not be used for species-specific differentiations. Epitope #4 (9-DDDLAI-14) was conserved among all OPXVs with the exception of 3/25 BPXV sequences and 3/3 SkPXV. Epitope #5 (68-IEKC-71) was constant among 380/381 OPXV sequences. Epitope #5 is located in the CCD (aa residues 43-84) and contains a cysteine, which is essential for the binding of the A26 protein (Ching et al., 2009). But even this antibody has no influence on the VACV neutralization without complement, even though A26 is also an attachment protein (Chiu et al., 2007), and the binding of A26 is partially blocked by the binding of the antibody. However, as already mentioned, there are other proteins responsible for the attachment of the virus. These data suggest that, in future, the antibodies should be combined in one product.

Because of the high affinity of the mAb 5B4/2F2 directed to epitope #1A (even with aa exchanges in the epitope region), we decided to use its epitope sequence as tag for the antibody detection (data not shown). Similar to other tag-studies, our epitope tag has a defined aa sequence representing a linear epitope (Bastin et al., 1996). Another important

aspect of selecting this antibody as an epitope tag was, that the mAb was able to recognize its epitope in different immunological assays (Bastin et al., 1996) as well as with different epitope variations. Therefore, we decided to fuse the 5B4/2F2-tag to the N-terminal end of the 32 kDa D8 adsorption protein of VACV. The results indicated that the 5B4/2F2-tag did not interact with the tertiary structure of the tagged D8 protein. Moreover, the tagged recombinant construct could be detected by the mAb 5B4/2F2 in ELISA, which showed that the N-terminal fusion did not affect the functionality of the tag (figure 6).

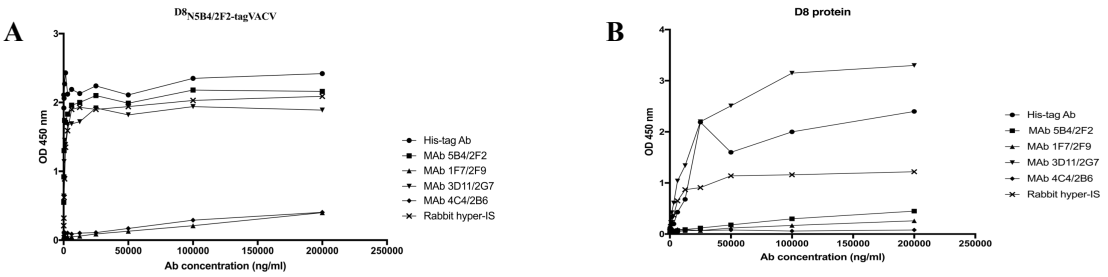


**FIG 6** The binding affinities were measured using an indirect ELISA on 5B4/2F2-tagged D8 (A) and recombinant D8 protein (B) with two-fold serial dilutions starting with 200 µg/ml. The mAb 4C4/2B6 served as negative control, while the rabbit serum, the his-tag ab and the mAb 3D11/2G7 served as positive D8 controls. The mAb 5B4/2F2 was the positive tag-control.

In future, it could also be a good purifying agent, since no imidazole is needed for the elution of the protein and thus it is also protected for precipitation (Hefti et al., 2001). In addition, the imidazole content, needed for the his-tagged purification, is not suitable for long time storage (Sharma et al., 1992). Thus, epitope tags are a useful tool for increasing the solubility of the proteins as well as the protein yield in general (Baneyx, 1999; Kapust and Waugh, 1999; LaVallie and McCoy, 1995).

Based on our different neutralization studies, we can conclude, that an effective virus neutralization is only possible, if many targets are bound. So, the goal in passive and therapeutic protection might be a combination of different specific monoclonal antibodies, which can block a poxvirus infection at different replication stages. Therefore, we took a closer look at the B-cell epitopes of the D8 protein. As mentioned above, the type 1 membrane protein is involved in the virus attachment by binding to GAG chondroitin

sulfate (Hsiao et al., 1999; Maa et al., 1990). First, mapping was performed by peptide microarrays (data not shown), whereby one linear epitope was discovered using the polyclonal anti-MVA-rabbit immune serum (aa residues: 139-DSIRSANTSAP-FDSVFYLDNL-159). This epitope was mapped in the carbonic anhydrase (CAH) domain (residues 1-234), which is important for chondroitin sulfate binding (Matho et al., 2012). So, anti-MVA-rabbit immune serum may possibly affect the adsorption of the virus to the host cell. In other studies, there were different anti-D8 mAbs targeting the CAH domain rather than the stalk region (aa residues 235-273) (Matho et al., 2014; Matho et al., 2012). However, in our investigations, the anti D8-mAbs did not show any reaction in microarray analysis. Because of this, we supposed that the epitopes are conformation dependent, and we decided to construct truncated recombinant D8 fragments. Therefore, the D8L-genes of VACV Elstree, CPXV KR2 Brighton, ECTV Munich 1 and CMLV CP1 were divided into five differently sized fragments, because of their 2D secondary structure prediction (figure 7A). By using western blotting analysis, one epitope region was recognized by three antibodies at the C-terminus (aa residues 222-289) of the D8 protein (figure 7B-D).

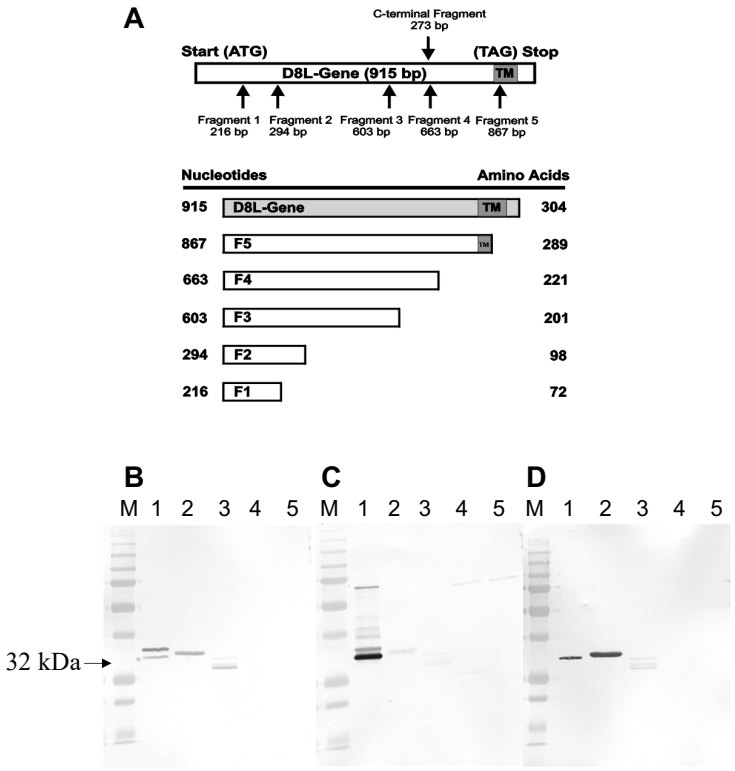


**FIG 7 (**A) Layout of the truncated D8 fragments. (B) Western blot on VACV Elstree gradient (1), recombinant D8 protein (2), recombinant fragment #5 (3) recombinant fragment #4 (4) and recombinant fragment #3 (5). B: 50 µg/ml of the mAb 3D11/2G7 were used for detection. C: 50 µg/ml of the mAb 1F7/2F9 were used for detection. D: 50 µg/ml of the IgG1 1.2.2.H9 were used for detection.

In addition to the last 12 aa of the CAH domain (residues 1-234), this target region also includes the disordered stalk region (residues 235-273) as well as a part of the transmembrane domain that anchors the D8 protein (aa residues 274-294) (Maa et al., 1990; Niles and Seto, 1988). By comparing all D8 protein homologs of OPXVs which are available online, sequence alignments revealed a significant homology. However, there was an increasingly occurring hypervariable region at the C-terminus, in which we mapped the conformational epitope area (aa residues 222-289). This confirmed the species-specific differences regarding the epitope phylogeny, leading to 35 different aa motifs. While BPXV, CMLV, HSPV, ECTV, MPXV, RPXV, TaPXV and VARV were largely conserved, and the New World Poxviruses (RCNV, SkPXV and VPXV) were completely conserved, CPXV and VACV showed many different motifs in this hypervariable region.

However, CPXV were known to be very polyphyletic (Carroll et al., 2011; Dabrowski et al., 2013; Franke et al., 2017). The different sequence motifs in this area enabled us to interpret species-specific epitope variations in order to get an impression of their phylogenic relationships.

In summary, we accurately mapped the antigenic sites on the A27, D8 and F13 proteins of OPXVs. Therefore, we were able to interpret species-specific epitope variations and conservations of various OPXVs. To interpret these relationships, crystallizations of the protein-antibody complexes might be helpful for future investigations. The neutralization results suggest the construction of a multi-epitope vaccine for active immunization against OPXVs, stemming from these immunogenic proteins which are involved in the attachment. For this development, further knowledge about OPXVs-morphology and the exact position of immunogenic epitopes is indispensable. Moreover, epitope evaluations are essential for a target directed screening of human immunoglobulin libraries to generate specific human recombinant antibodies, which might help to control any future outbreaks of zoonotic orthopoxviruses.

## 3.2 CONCLUSIONS

The following conclusions were drawn in these investigations:

1. Six sequential antigenic sites of 4-8 aa were identified and mapped on the A27 protein of OPXVs.
2. Epitope #4 (9-DDDLAI-14) was conserved among all OPXVs, except 3/25 BPXVs and SkPXVs.
3. Epitope #5 (68-IEKC-71) was constant among 380/381 sequences.
4. Epitope complex #1 (26-KKPEAKREAIVKAD-39) was hypervariable and therefore responsible for conserved species-specific epitope characteristics.
5. Nearly all of the 381 OPXV sequences offered a monophyletic genus-specific epitope conservation.
6. CPXV sequences were polyphyletic and could be subdivided into 7 different motifs.
7. The constructed anti-OPXV-immunoglobulin-library displayed a diversity of >4x108 independent colonies.
8. The epitope of the obtained scFv-1.2.2-H9 was mapped on the D8 protein to a similar region like the mAb 1F7/2F9.
9. Partial *in vivo* neutralization of VACV Munich1 by scFv and IgG1-1.2.2-H9.
10. High viral loads are found in spleen and kidney of mice after lethal challenge of VACV Munich1.
11. *In vitro* VACV-neutralization is complement-dependent.
12. The sequential antigenic-site of the obtained scFv 3E2 was mapped on the F13 protein of VACV.

## 3.3 FUTURE PROSPECTS

The further prospects of these investigations are:

1. Crystallization of the A27 protein together with the 6 mAbs.
2. Crystallization of the 1.2.2-H9-D8 complex and so fine mapping of the epitope.
3. Crystallization of the F13 protein together with the scFv 3E2.
4. Library screening against the further attachment proteins H3, A26 and L1 as well as against the immunogenic B5 protein to get target-specific antibodies.
5. Combination of neutralizing recombinant antibodies against different epitopes (D8, A27, H3, A26, L1, B5) to obtain a general *in vivo* protection without clinical symptoms.
6. Further development of the 5B4/2F2-epitope-tag for the purification of proteins.

## 4 SUMMARY

The genus *Orthopoxvirus* (OPXV) contains a group of large (130-380 kb) and closely related double-stranded DNA viruses within the *Poxviridae* family, which replicates in the cytoplasm of vertebrate or invertebrate cells. Vaccinia virus (VACV), the prototype of the OPXV genus, was applied as a vaccine against the closely related Variola virus (VARV). VARV, the causative agent of smallpox, elicited more fatalities than all other human diseases taken together. However, strict VACV-vaccination campaign led to the eradication of smallpox. Another advantage of the vaccination is the achievement of cross protection against all the other OPXVs. Unfortunately, due to the termination of the vaccination campaign, the majority of humans is not protected anymore. Therefore, there is considerable concern regarding the use of VARV and monkeypox virus (MPXV) as potential biological weapons, especially after recurrent outbreaks of MPXV in Africa, America and Europe. Moreover, reservoirs for other closely related OPXVs, e.g. cowpox viruses (CPXV), exist in the environment and may also endanger human health, especially in immuno-compromised humans. Therefore, it is crucial to join forces in the development of safer vaccines, antiviral agents, and protective human recombinant antibodies for passive immunization. Morphogenesis of VACV results mainly in two distinct virus particle forms. The majority (>90%) consists of the "intracellular mature virus" (IMV), which mediates host-to-host transmission. "Extracellular enveloped virus" (EEV) on the other hand is important for direct cell-to-cell transmission inside the host and is surrounded by an additional golgi-derived envelope. Another difference between IMVs and EEVs is the distribution of envelope proteins, as several structural proteins of immunological relevance were identified on the IMV (A10, A13, A14, A17, A25, A26, A27, A28, C3, D8, D13, H3 and L1), and on the EEV (A33, A56, B5 and F13).

In my PhD thesis, studies on antibody-viral interaction with focus on the VACV proteins A27, D8 and F13 were conducted, because of their important functions in the virus replication cycle. One of the best characterized envelope proteins is the A27, which is encoded by the open reading frame (ORF) A27L. This conserved protein is present in all members of OPXVs. A27 is important for virus attachment, by binding to the glycosaminoglycan (GAG) heparan sulfate on the surface of mammalian cells. In this study, the binding sites of six specific A27 monoclonal antibodies (mAbs) were identified by peptide SPOT synthesis and peptide microarray technology. In the region of amino acids (aa) 26 to 39, a complex of four antigenic sites was identified (epitope #1A: aa 32-39, #1B: aa 28-33, #1C: aa 26-31, #1D: 28-34), and another two at the N-terminus (epitope #4: aa 9-14) and C-terminus (epitope #5: aa 68-71). Binding affinities were determined using ELISAs with different purified OPXV reference strains. Interestingly, all mAbs directed to epitope complex #1 showed strong binding activities to VACV, CPXV and camelpox virus (CMLV) but either did not react or only bound weakly to ectromelia virus (ECTV) and MPXV. These differences are caused by amino acid exchanges of the epitope regions. To determine the sequence variability of the six antigenic sites, 391 published sequences of A27 protein homologs were compared. Epitope #4 was conserved among almost all OPXVs with the exception of three buffalopox viruses (BPXV), three skunkpox viruses (SkPXV), 12 truncated OPXV sequences and one VACV sequence, while epitope #5 was constant among 389 of the 391 sequences. The sequential epitope complex #1A-D was more variable and, therefore, responsible for species-specific epitope characteristics, which is in correspondence to the ELISA results. Moreover, the neutralization capabilities of A27 specific mAbs were tested, whereby the mAbs detecting epitopes #1A-D and #4 neutralized VACV Elstree in the presence of 1% complement (50% plaque-reduction: 12.5-200 µg/ml).

Another crucial IMV protein is the D8 type 1 membrane protein, which is highly conserved in poxviruses. It plays an important role in virus attachment to the host cell *via* binding to the GAG chondroitin sulfate (CS). For neutralization studies, specific human anti-D8 antibodies were generated. Therefore, the IgG repertoire from four donors vaccinated intracutaneously with live vaccinia virus vaccine was amplified, cloned and displayed onto M13K07ΔpIII phage. This library displayed a diversity of $\geq 4x10^8$ independent colonies. Different immuno-screening protocols against VACV Elstree revealed a predominant selection of scFv-clones specifically binding to the D8 protein, which is known to induce strong antibody responses. To improve the binding affinity and the immune response, the obtained scFv-1.2.2.H9 was also engineered into the larger human scFv-Fc-1.2.2.H9 and IgG1-1.2.2.H9 formats. Similar binding affinities were shown by scFv-1.2.2.H9 and scFv-Fc-1.2.2.H9 (1.61 nM and 7.68 nM, respectively), whereas, IgG1-1.2.2.H9 was much more efficient (43.82 pM). However, none of the purified recombinant 1.2.2.H9 antibodies were able to neutralize 100 pfu of VACV Elstree *in vitro.* Interestingly, after addition of 1% human complement, the neutralization abilities of the larger antibody formats scFv-Fc-1.2.2.H9 and IgG1-1.2.2.H9 could be improved (0.0776 µM and 0.01324 µM, respectively). In an *in vivo* passive immunization NMRI-mouse-model, 100 µg of scFv-1.2.2.H9 and the IgG1-1.2.2.H9 partially protected the mice against the challenge with $4LD_{50}$ VACV Munich 1 as 3/6 animals survived. In contrast, the mice inoculated with scFv-Fc-1.2.2.H9 showed no protection.

Moreover, the existing OPXV phage library was screened against the F13 protein of VACV, which is the major envelope protein of EEV particles. The nonglycosylated F13 membrane protein is encoded by the ORF F13L gene. The F13 protein has no transmembrane domain, instead, its N- and C-terminus are both directed towards the inner side within the EEV membrane. Because of its location in the TGN membrane, it plays an important role in the virion wrapping progress as well as the EEV production. After

applying immuno-screening protocols against F13, one anti-F13 scFv was isolated and characterized. Interestingly, two antigenic binding sites (139-GSIHTIKTLGVYSDY-153 and 169-AFNSAKNSWLNL-188) were mapped using a cellulose membrane encompassing 372 15-mere peptides with 12 overlaps, therefore covering the whole F13 protein. Because of the inner location of the protein, scFv 3E2 showed no capability of VACV neutralization.

In conclusion, more research on poxvirus replication is crucial. The epitope mapping on immuno-protective proteins such as the A27 and D8 proteins of VACV provides more insights into host-pathogen interaction. Moreover, data on virus species-specific epitope variations will enable the future development of safer vaccines or antivirals. The construction of recombinant scFv phage libraries is a promising strategy to produce target specific antibodies which are useful to investigate the replication cycle of poxviruses. Moreover, these libraries are of high interest because they enable generating specifically engineered human recombinant scFv antibodies, which might be a helpful tool for controlling any future eruption of zoonotic OPXV infections.

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## 5 ZUSAMMENFASSUNG

Die Gattung der *Orthopockenviren* (OPXV) lässt sich der Familie der *Poxviridae* zuordnen, die eine Gruppe großer, doppelsträngiger DNA Viren umfasst. Diese replizieren sich im Cytoplasma von Wirbel- und Wirbellosentieren, welches eine Besonderheit unter den DNA-Viren darstellt. Vaccinia Virus (VACV) wurde, aufgrund der Kreuzreaktivität zwischen den nah miteinander verwandten Pockenviren, als Impfstoff gegen Variola Virus (VARV) eingesetzt. Es wird angenommen, dass VARV mehr Todesfälle, als alle anderen menschlichen Infektionskrankheiten zusammen, auslöste. Der strikten VACV-Impfkampange ist es jedoch zu verdanken, dass VARV vollständig ausgerottet werden konnte. Aufgrund der Beendigung dieser Impfkampagne ist jedoch ein Großteil der Menschheit nicht mehr geschützt, weswegen VARV sowie auch die Affenpockenviren (MPXV) als potentielle biologische Waffen genutzt werden könnten. Auch andere zoonotische OPXV Arten, wie z.B. Kuhpockenviren (CPXV), können die menschliche Gesundheit, insbesondere die, immungeschwächter Menschen, gefährden. Daher ist die Entwicklung sicherer Impfstoffe, antiviraler Wirkstoffe und rekombinanter humaner Antikörper essentiell. Die Morphogenese von VACV führt zu zwei verschiedenen Haupt-Viruspartikelformen. Die Mehrheit (>90%) besteht aus dem "intracellular mature virus" (IMV), dass die Übertragung von Wirt zu Wirt vermittelt. Während das „extracellular enveloped virus“ (EEV) für die direkte Übertragung von Zelle zu Zelle innerhalb des Wirts wichtig ist. Hervorzuheben ist außerdem, dass das EEV von einer zusätzlichen Hülle umgeben ist. Ein weiterer Unterschied zwischen den IMVs und den EEVs sind die unterschiedlichen Hüllproteine. Unter anderem wurden am IMV A10, A13, A14, A17, A25, A27, A28, C3, D8, D13, H3 und L1 und A33, A56, B5 und F13 am EEV identifiziert.

Im Laufe meiner Dissertation habe ich mich auf die VACV Proteine A27, D8 und F13 konzentrieren, da diese wichtige Funktionen im Virusreplikationszyklus übernehmen.

Eines der am besten charakterisierten Hüllproteine ist das A27, dass vom offenen Leserahmen (ORF) A27L kodiert wird. Dieses konservierte Protein ist in allen OPXV-Arten vorhanden. Es ist ein wichtiger Bestandteil für die Virusanheftung, indem es an das Glycosaminoglycan (GAG) Heparansulfat auf der Oberfläche von Säugetierzellen bindet. In dieser Arbeit konnten die Bindungsstellen von sechs monoklonalen A27-spezifischen Antikörpern (mAks) mit Hilfe der Peptid-SPOT-Synthese und der Peptid-Microarray-Technologie identifiziert werden. Im Bereich der Aminosäuren (AS) 26 bis 39 wurde ein Komplex von vier antigenen Bereichen detektiert (Epitop #1A: AS 32-39, #1B: AS 28-33, #1C: AS 26-31, #1D: AS 28-34). Des Weiteren wurden ein N-terminales (Epitop #4: AS 9-14) und ein C-terminales Epitop (Epitop #5: AS 68-71) identifiziert. Durch ELISA-Tests mit verschiedenen OPXVs-Referenzstämmen konnten die Bindungsaffinitäten bestimmt werden. Interessanterweise zeigten alle, gegen den Epitopkomplex #1 gerichteten mAKs eine starke Bindung mit VACV, CPXV und Camelpox-Viren (CMLV), reagierten jedoch nicht oder nur schwach mit Ektromelie-Virus (ECTV) und MPXV. Diese Unterschiede sind auf die Austausche in der Aminosäuresequenz der Epitopregion zurück zu führen. Um diese Sequenzvariabilität der sechs antigenen Bereiche zu bestimmen, wurden 391 bisher veröffentlichte A27 OPXV Sequenzen miteinander verglichen. Das Epitop #4 zeigte sich bei fast allen OPXV Arten konserviert. Ausnahmen bildeten jedoch drei Büffelpocken Viren (BPXV), drei Stinktierpocken Viren (SkPXV), 12 N-terminal verkürzte OPXV-Sequenzen und eine VACV-Sequenz. Das Epitop #5 war in 389 der insgesamt 391 Sequenzen konstant. Der Epitopkomplex #1A-D zeigte sich variabler und ist daher für die spezies-spezifischen Eigenschaften des Epitops verantwortlich, welche auch durch die ELISA-Ergebnisse bestätigt werden konnten. Darüber hinaus wurden die Neutralisationsfähigkeiten der A27-spezifischen mAks getestet, wobei die mAks, die die Epitope #1A-D und #4 detektieren, VACV Elstree in Anwesenheit von einem Prozent humanem Komplement neutralisierten (50% Plaquereduktion: 12,5-200 µg/ml).

Ein weiteres IMV-Protein ist das D8 Protein, ein Membranprotein Typ 1, welches bei der Anheftung von Viren an die Wirtszelle durch das GAG-Chondroitinsulfat (CS) ebenfalls eine wichtige Rolle spielt. Um Neutralisationsstudien durchführen zu können, wurden spezifische humane anti-D8-Antikörper generiert. Daher wurde das IgG-Repertoire von vier Spendern, die zuvor mit dem Vaccinia-Lebendvirus geimpft wurden, amplifiziert, kloniert und von M13K07ΔpIII-Phagen präsentiert. Diese Bibliothek zeigte eine Vielfalt von $\geq 4\times10^8$ unabhängigen Kolonien. Verschiedene Screening-Protokolle gegen VACV Elstree zeigten eine vermehrte Selektion von spezifischen anti-D8 scFv-Klonen. D8 ist für die Induktion starker Antikörperreaktionen bekannt. Um die Bindungsaffinität und die Immunantwort verbessern zu können, wurde das scFv-1.2.2.H9 durch die größeren scFv-Fc-1.2.2.H9 und IgG1-1.2.2.H9 Formate erweitert. Die scFv-1.2.2.H9 und scFv-Fc-1.2.2.H9 Formate zeigten ähnliche Bindungsaffinitäten (1,61 nM bzw. 7,68 nM), wohingegen das IgG1-1.2.2.H9 eine viel effizientere Bindung aufwies (43,82 pM). Jedoch neutralisierte keiner der gereinigten rekombinanten 1.2.2.H9-Antikörperformate 100 pfu VACV Elstree *in vitro*. Interessanterweise konnten, nach der Zugabe von einem Prozent humanem Komplement, die Neutralisationsfähigkeiten der größeren Antikörperformate scFv-Fc-1.2.2.H9 und IgG1-1.2.2.H9 verbessert werden (0,0776 uM bzw. 0,01324 uM). *In vivo*, hingegen, konnten 100 µg des scFv-1.2.2.H9 sowie auch des IgG1-1.2.2.H9 teilweise (3/6 Mäusen) gegen die Infektion mit $4LD_{50}$ VACV München1 schützen. Unerwarteterweise zeigte scFv-Fc-1.2.2.H9 keine Schutzwirkung.

Zudem wurde die bestehende OPXV-Phagenbibliothek erneut gegen das VACV F13 Protein gescreent. Bei dem F13 Protein handelt es sich um ein nicht glykosyliertes Membranprotein, welches von dem ORF-F13L-Gen kodiert wird. Das F13 Protein wurde im Bereich des Trans-Golgi-Netzes und der Plasmamembran der Wirtszelle nachgewiesen. Es liegt jedoch nicht exponiert auf der Oberfläche der Viren vor, sondern befindet sich überwiegend an der Innenseite der Virushülle. Das F13 Protein ist, ebenso wie das A27

Protein, für die Umhüllung der IMV und damit für die Ausbildung der EEV essentiell. Nachdem die OPXV Phagenbibliothek gegen das F13 Protein gescreent wurde, wurde das scFv 3E2 isoliert und mit Hilfe der Peptid-SPOT-Synthese weiter charakterisiert. Interessanterweise ergaben sich für das scFv 3E2 zwei antigene Bereiche (139-GSIHTIKT-LGVYSDY-153 und 169-AFNSAKNSWLNL-188). Es konnte keine VACV-Neutralisation *in vitro* erzielt werden, da sich das F13 Protein (und somit das Epitop) auf der Innenseite der Virushülle befindet.

Zusammenfassend ist die weitere Forschung der Replikationszyklen von Pockenviren von großer Bedeutung. Besonders sollte die Kartierung funktioneller Epitope der A27 und D8 VACV Proteine im Vordergrund stehen, da Antikörper gegen diese Proteine bei der Immunantwort im Höchsten Maße verfügbar sind. Darüber hinaus ermöglicht das Wissen über mögliche spezies-spezifische Epitopvarianten die zukünftige Entwicklung sichererer Impfstoffe und Virostatika. Die Konstruktion rekombinanter scFv-Phagenbibliotheken ist darüber hinaus eine vielversprechende Strategie zur Hestellung spezifisch konstruierter humaner rekombinanter scFv-Antikörper, die dazu beitragen können, zukünftige Ausbrüche von zoonotischen OPXV-Infektionen zu kontrollieren.

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# 7 APPENDIX

## ABBREVIATIONS

| | |
|---|---|
| A | Adenine |
| AA | Amino acid |
| Ab | Antibody |
| ATI | A-type inclusion |
| BLAST | Basic Local Alignment Search Tool |
| bp | Base pair |
| BPXV | Buffalopox virus |
| C | Cytosine |
| CCD | Coiled-coil domain |
| CDR | Complementary determining region |
| CEV | Cell-associated enveloped virus |
| CMLV | Camelpox virus |
| CPXV | Cowpox virus |
| DNA | Deoxyribonucleic acid |
| dsDNA | Double-stranded deoxyribonucleic acid |
| ECTV | Ectromelia virus |
| EEV | Extracellular enveloped virus |
| EFC | Entry fusion complex |
| ELISA | Enzyme Linked Immunosorbent Assay |

| | |
|---|---|
| G | Guanine |
| GAG | Glycosaminoglycan |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| HBD | Heparin binding domain |
| HKD | His, Lys, Asp. |
| HSPV | Horsepox virus |
| IEV | Intracellular enveloped virus |
| Ig | Immunoglobulin |
| IMV | Intracellular mature virus |
| ITR | Inverted terminal repeat |
| Kb | Kilobase |
| kDa | Kilodalton |
| L | Left |
| LB | Lateral body |
| LZD | Leucine zipper motif |
| MAb | Monoclonal Antibody |
| MPXV | Monkeypox virus |
| NCBI | National Center for Biotechnology Information |
| Nt | Nucleotide |

| | |
|---|---|
| ORF | Open reading frame |
| OPXV | Orthopoxvirus |
| PBMC | Peripheral blood mononuclear cells |
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| PFU | Plaque-forming unit |
| PRT | Plaque reduction test |
| R | Right |
| RPXV | Rabbitpox virus |
| RCNV | Raccoonpox virus |
| ScFv | Single-chain variable fragment |
| SkPXV | Skunkpox virus |
| T | Thymine |
| TaPXV | Taterapox virus |
| TGN | *trans* Golgi network |
| VACV | Vaccinia virus |
| VARV | Variola virus |
| VPXV | Volepox virus |
| WHO | World Health Organisation |
| WR | Western Reserve |

## LIST OF PUBLICATIONS

This thesis is based on the work contained in the following publications:

I. Ahsendorf, H.P., Gan, L.L., Eltom, K.H., Abd El Wahed, A., Hotop, S.K., Roper, R.L., Beutling, U., Broenstrup, M., Stahl-Hennig, C., Hoelzle, L.E., Czerny, C.P., 2019. Species-Specific Conservation of Linear Antigenic Sites on Vaccinia Virus A27 Protein Homologs of Orthopoxviruses. Viruses 11.

II. Diesterbeck, U.S., Ahsendorf, H.P., Frenzel, A., Sharifi, A.R., Schirrmann, T., Czerny, C.P., **submitted to PLOS Pathogens 2019**. Characterization of an in vivo neutralizing anti-vaccinia virus D8 single chain Fragment variable (scFv) from a human anti-vaccinia virus-specific recombinant library.

III. Ahsendorf, H.P., Diesterbeck, U.S., Hotop, S.K., Winkler, M., Broenstrup, M., Abd El Wahed, A., Czerny, C.P. **to be submitted in 2019**. Characterization of an anti-vaccinia virus F13 single chain Fragment variable from a human anti-vaccinia virus-specific recombinant library.

## LIST OF PRESENTATIONS

I. Virology Network (14.01.2014 Göttingen):

H.P. Ahsendorf, U.S. Diesterbeck, A. Frenzel, T. Schirrmann, S. Dübel, C.P. Czerny

"Importance of complement in the neutralization mechanisms of poxviruses"

II. Virology Network (08.07.2015 Göttingen):

H.P. Ahsendorf and C.P. Czerny

"Mapping of antigenic sites on the VACV D8 protein and its heterologs in orthopox viruses"

III. Virology Network (08.06.2016 Göttingen):

H.P. Ahsendorf, A. Abd El Wahed, S.K. Hotop, M. Broenstrup, C.P. Czerny

"Construction of a human anti-F13 single chain antibody fragment"

IV. International Poxvirus, Asfarvirus & Iridovirus Conference (26.05 - 30.05.2018 Taipei, Taiwan)

H.P. Ahsendorf, L.L. Gan, K.H. Eltom, A. Abd El Wahed, S.K. Hotop, R.L. Roper, U. Beutling, M. Broenstrup, R. Frank, C. Stahl-Hennig, L.E. Hoelzle, C.P. Czerny

"Species-specific preservation of Antigenic Sites on vaccinia virus A27 protein homologues in the evolution of Orthopoxviruses"

V. Virology Network (13.06.2018 Göttingen):

H.P. Ahsendorf, L.L. Gan, K.H. Eltom, A. Abd El Wahed, S.K. Hotop, R.L. Roper, U. Beutling, M. Broenstrup, R. Frank, C. Stahl-Hennig, L.E. Hoelzle, C.P. Czerny

"Preservation of antigenic sites on VACV A27 protein homologues in the evolution of OPXV"

**LIST OF POSTERS**

I. International Poxvirus, Asfarvirus & Iridovirus Conference (26.09 30.09.2014 Victoria, Canada)

H.P. Ahsendorf, A. Siemon, C.P. Czerny

"An octapeptide-tag derived from the vaccinia virus A27 protein"

II. International Poxvirus, Asfarvirus & Iridovirus Conference (01.07 05.07.2016 Bischenberg, France)

H.P. Ahsendorf, A. Abd El Wahed, S.K. Hotop, M. Broenstrup, J. Krijnse Locker, C.P. Czerny

"Construction and characterization of an anti-vaccinia virus F13 single chain Fragment"

III. International Poxvirus, Asfarvirus & Iridovirus Conference (26.05 30.05.2018 Taipei, Taiwan)

H.P. Ahsendorf, S. Dede Davi, M. Faye, J. Kissenkötter, S. Boehlken-Fascher, C. Stahl-Hennig, O. Faye, A.A. Sall, M. Weidmann, F.T. Hufert, C.P. Czerny, A. Abd El Wahed

"Recombinase polymerase amplification assay for rapid detection of Monkey-pox virus at point of need"

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**DECLARATION**

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examination body either in its present or a similar form.

   Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

   Göttingen, ……

   ……

   (Signature)

   ……

   (Name in block capitals)

2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorized aid.

   Göttingen, ……

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   (Signature)

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   (Name in block capitals)