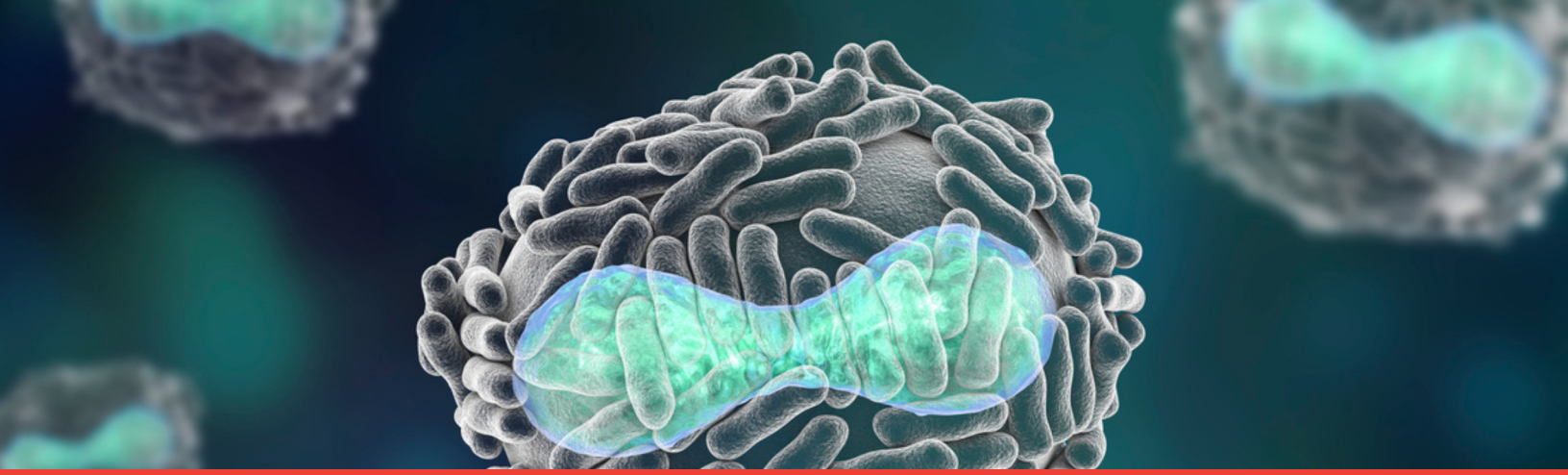


MONOCLONAL ANTIBODIES AGAINST MONKEYPOX VIRUS & OTHER EMERGING INFECTIOUS DISEASES: FAST-TRACK TO RESEARCH & ASSAY DEVELOPMENT

MONOGRAPH

M2206-A

Leinco Technologies, Inc.™

30 YEARS OF SUPPORTING LIFE SCIENCES

Leinco Technologies, Inc.
YEARS OF SUPPORTING LIFE SCIENCES

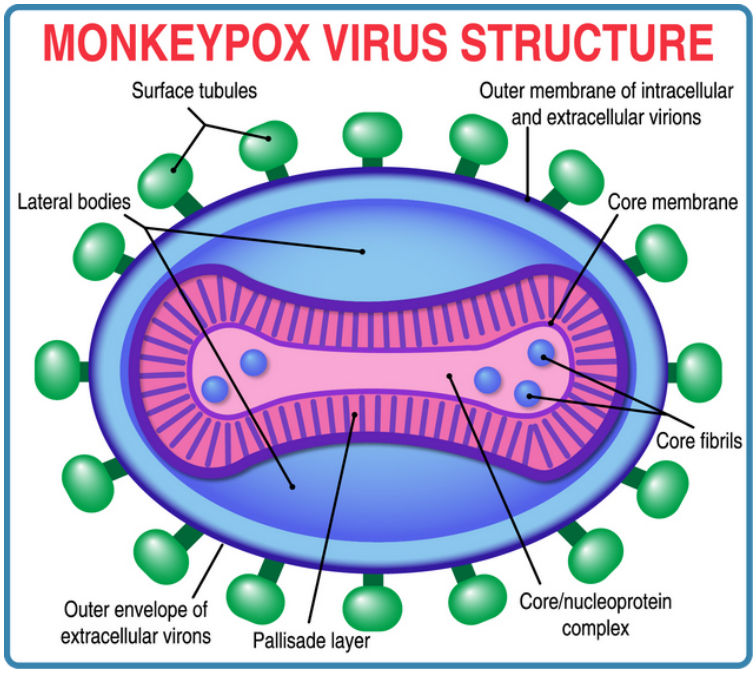
MONOCLONAL ANTIBODIES AGAINST MONKEYPOX VIRUS & OTHER EMERGING INFECTIOUS DISEASES: FAST-TRACK TO RESEARCH & ASSAY DEVELOPMENT

Table of Contents

info@imtec.be | www.imtec.be

Monkeypox Virus Background

Monkeypox virus (MPXV)is a zoonotic member of the *Orthopoxvirus* genus in the *Poxviridae* family. It is the next most pathogenic poxvirus after smallpox[1]. Two genetic clades, West African and Central African (Congo Basin), have been characterized; the latter is capable of human-to-human transmission[1,2]. Monkeypox has gained clinical relevance due to the eradication of smallpox, which has created opportunities for increased prevalence and viral mutations that may affect virulence[1,2]. Rodents are thought to be the natural reservoir, with transmission through contact with bodily fluids and feces. Case fatality rates are <1% and up to 11% for the West African and Central Basin clades, respectively[1,2]. An infection with one orthopoxvirus of any one species, or vaccinia virus vaccination, protects against infection by other orthopoxviruses[3,4,5].



MPXV is an enveloped virus with a linear, double-stranded DNA genome[2] and a large, complex proteome of over 200 proteins[6]. During infection, the virus exists in two antigenically distinct forms: mature virions (MV) or enveloped virions (EV)[6].

An FDA approved attenuated live virus vaccine, JYNNEO™, is also being evaluated for the protection of those at risk of occupational exposure to orthopoxviruses[7]. There are no proven, safe treatments for monkeypox virus infection.

Antibody Clone Descriptions

MPXV-26 was generated from peripheral blood mononuclear cells obtained from donors who had either recovered from naturally-occurring MPXV infection or been immunized against vaccinia[5]. Complementary screening approaches were used to identify orthopoxvirus-specific monoclonal antibodies (mAbs) to MPXV, cowpox virus (CPXV), variola virus (VARV), and vaccinia virus (VACV). MPXV-26 is reactive to VACV antigen, CPXV lysate, VARV antigen and lysate, but not MPXV lysate. MPXV-26 binds to VACV and VARV purified antigens and CPXV virus-infected cell lysate but not VACV and MPXV cell lysates. MPXV-26 neutralizes VACV MV and MV plus complement (MV+C'), CPXV MV and MV+C', and weakly neutralizes MPXV MV and MV+C'. MPXV-26 is reactive against L1, an MV surface protein.

When MPXV-26 is mixed with other mAbs, neutralization and cross-neutralization are more efficient than in individual assays[5]. These mAb mixes also provide protection against VACV in mice. While not completely protective on its own, MPXV-26 induces delayed morbidity and mortality in mice, and mixes that exclude MPXV-26 are less effective prophylactics.

Isotype: IgG1[5]
Applications: ELISA[5], neutralization assay[5], binding assay[5], *in vivo* protection study[5]

MPXV-56 was generated from peripheral blood mononuclear cells obtained from donors who had either recovered from naturally-occurring MPXV infection or been immunized against vaccinia[5]. Complementary screening approaches were used to identify orthopoxvirus-specific mAbs to MPXV, cowpox virus (CPXV), variola virus (VARV), and vaccinia virus (VACV). MPXV-56 is reactive against VACV antigen and lysate, CPXV lysate, MPXV lysate, and VARV antigen and lysate. MPXV-56 binds to VACV and VARV purified antigen and weakly binds virus-infected cell lysates of VACV, CPXV, and MPXV.

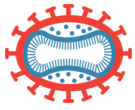
MPXV-56 neutralizes VACV EV plus complement (+C') but not MV, MV+C', or EV. MPXV-56 also neutralizes CPXV EV+C' but not EV and weakly neutralizes MPXV EV+C' but not MV, MV+C', or EV. MPXV-56 is reactive against A33, a surface antigen on the EV form of the virus.

Isotype: IgG2
Applications: ELISA[5], neutralization assay[5], binding assay[5]

MPXV-13 was generated from peripheral blood mononuclear cells obtained from donors who had either recovered from naturally-occurring MPXV infection or been immunized against vaccinia[5]. Complementary screening approaches were used to identify orthopoxvirus-specific mAbs to MPXV, cowpox virus (CPXV), variola virus (VARV), and vaccinia virus (VACV).

MPXV-13 reacts to VACV antigen and lysate, CPXV lysate, MPXV lysate, and VARV antigen and lysate. MPXV-13 weakly binds to VACV purified antigen, VACV and MPXV virus-infected cell lysates, and strongly binds VARV purified antigen and CPXV virus-infected cell lysate. MPXV-13 neutralizes VACV EV plus complement (+C') but not MV, MV+C', or EV. MPXV-13 also binds to CPXV EV+C' but not EV. MPXV-13 does not neutralize MPXV MV, MV+C', EV, or EV+C'. MPXV-13 reacts to B5, an EV surface antigen.

Isotype: IgG1
Applications: ELISA[5], neutralization assay[5], binding assay[5]



ANTIBODIES AGAINST OTHER EMERGING INFECTIOUS DISEASES

Pathogen	Clone	Specificity	Antigen Distribution	Applications
Chikungunya (CHIKV)	CHK152	CHK-152 activity is directed against the A domain of CHIKV E2	E2 is expressed on the surface of the virus.	Neutralization; competition ELISA
	CHKV-24	CHKV-24 targets the CHIKV E2 glycoprotein		Neutralization; competition ELISA; protective efficacy study; plaque reduction neutralization test
	CHKV-35	CHKV-35 binds to and neutralizes CHIKV vaccine strain 181/25		Neutralization; ELISA;
Dengue Virus (DENV)	DENV-1C19	DENV-1C19 activity is directed against the bc loop of domain II of the E glycoprotein adjacent to the fusion loop (FL), is quaternary structure dependent, and cross-reactive against DENV-1, 2, 3, 4.	E is expressed on the surface of DENV	Competitive binding assay, neutralization assay, focus reduction neutralization titer assay (FRNT), binding assay, yeast surface display, shotgun mutagenesis screening, capture ELISA, dot blot
	DENV-1F4	DENV-1F4 activity is DENV-1 specific and directed against one E protein within a homodimer at DI and the DI/DII hinge region in a quaternary structure dependent manner. The quaternary structure epitope is present only on intact E protein assembled on a viral particle.		Neutralization assay, capture ELISA, dot blot, binding assay, ELISA, flow cytometric neutralization assay, immunoassay, competition assay, immunoblotting, Ab-dependent enhancement (ADE) assay
	DENV- 2D22	DENV-2D22 activity is DENV-2 specific and directed against the E homodimer at the DIII+glycan loop with serotype specificity on one E protein and DII around the fusion loop on the other E protein.		Binding assay, neutralization assay, capture ELISA, dot blot, ELISA, immunoblotting, Ab-dependent enhancement (ADE) assay, flow cytometry-based neutralization assay, cryo-electron microscopy

Pathogen	Clone	Specificity	Antigen Distribution	Applications
Eastern Equine Encephalitis virus (EEEV)	EEEV-129	EEEV-129activity is directed against the B domain of the E2 glycoprotein.	E2 is expressed on the surface of EEEV	Neutralization, focus reduction neutralization test, ELISA, competition-binding studies, biolayer interferometry, alanine-scanning mutagenesis
Influenza (Flu)	Flu-5J8	Flu-5J8activity is directed against a conserved H1 epitope adjacent to the receptor binding site domain on the HA globular head.	HA is on the viral surface	Fast protein liquid chromatography (FPLC), hemagglutination inhibition assay, neutralization assay, microneutralization assay, binding affinity assay, biolayer interferometry, *in vivo* therapeutic assay, *in vitro* binding assay, *in vivo* protection assay, X-ray crystallography, electron microscopy, biolayer interferometry-based competition, flow cytometry, capture ELISA, competition assay using biolayer interferometry, competition ELISA
	FluA-20	FluA-20 activity is directed against a novel epitope at the trimer interface of the hemagglutinin (HA) head domain of most influenza A viruses.		ELISA, *in vivo* protection study, prophylaxis study, therapeutic study, bio-layer interferometry, crystallography, hydrogen deuterium exchange mass spectrometry, flow cytometry, hemagglutinin inhibition assay, microneutralization assay, plaque reduction neutralization test, egress inhibition assay, negative-stain electron microscopy, affinity chromatography, Ab-dependent cellular cytotoxicity (ADCC) assay, binding kinetic assay, site-directed mutagenesis, HA cleavage inhibition assay, pH dependent conformational change assay, NK cell activation assay

Pathogen	Clone	Specificity	Antigen Distribution	Applications
Andes virus (hantavirus) (ANDV)	ANDV-44	ANDV-44 specificity is directed against the surface glycoprotein spike	The glycoprotein spike is expressed on the ANDV envelope surface	Competition binding assay, neutralization assay, fusion inhibition assay, plaque reduction neutralization test, flow cytometric-based competition-binding analysis, ELISA, animal protection study
Sin Nombre virus (hantavirus) (SNV)	SNV-53	SNV-53 specificity is directed against the surface glycoprotein spike	The glycoprotein spike is expressed on the SNV envelope surface.	Competition binding assay, neutralization assay, fusion inhibition assay, plaque reduction neutralization test, flow cytometric-based competition-binding analysis, ELISA, animal protection study
Henipavirus (HENV)	HENV-103	HENV-103 activity is directed against an area spanning the β1 and β6 propeller blades of receptor binding protein (RBP)	RBP is an envelope glycoprotein	surface plasmon resonance competition binding, binding, neutralization, ephrin-B2 competition binding, negative stain electron microscopy, animal protection challenge, flow cytometry, neutralization synergy
	HENV-117	HENV-117 activity is directed against the receptor-binding domain of RBP		surface plasmon resonance competition binding, binding, neutralization, ephrin-B2 competition binding, negative stain electron microscopy, animal protection challenge, flow cytometry, neutralization synergy.

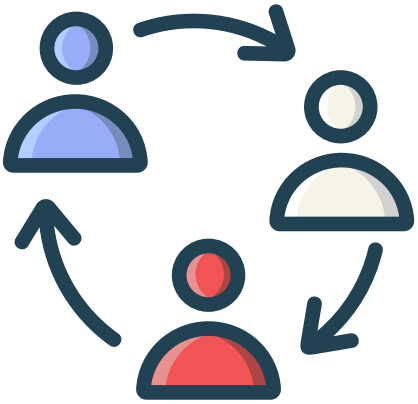
Pathogen	Clone	Specificity	Antigen Distribution	Applications
Japanese Encephalitis virus (JEV)	JEV-75	JEV-75activity is directed against the E ectodomain.	E is expressed on the surface of JEV.	neutralization, ELISA,focus reduction neutralization test, pre-attachment neutralization assay, post-attachment neutralization assay, fusion-from-without assay, alanine-scanning site-directed mutagenesis
	JEV-69	JEV-69activity is directed against DIII-LRof the E protein		neutralization, ELISA,focus reduction neutralization test, pre-attachment neutralization assay, post-attachment neutralization assay, fusion-from-without assay, alanine-scanning site-directed mutagenesis
Respiratory Syncytial virus (RSV)	RSV-3M3	RSV-3M3activity is directed against antigenic site IV of the RSV fusion (F)protein	F protein is a surface glycoprotein	ELISA,neutralization assay, competition binding assay, biolayer interferometry-based competition-binding assay, peptide binding assay, Fab production
Ross River Virus (RRV)	RRV-19	RRV-19activity is directed against the B domain of the E2 protein.	E2 is expressed on the surface of RRV.	ELISA,binding assay, neutralization, focus reduction neutralization test, alanine scanning mutagenesis, fusion from without (FFWO)assay, quantitative competition-binding assay using biolayer interferometry, competition ELISA
	RRV-86	RRV-86activity is directed against the arch region/B domain of the E2 protein		
	RRV-12	RRV-12activity is directed against the E2 protein		

Pathogen	Clone	Specificity	Antigen Distribution	Applications
Rotavirus (RV)	RV6-26	RV6-26 activity is directed against a quaternary epitope on VP6	VP6 is a viral protein in the intermediate capsid layer of RV.	Immunoblotting, ELISA, neutralization, binding assay, cryo-electron microscopy, fast protein liquid chromatography (FPLC), ELISAbinding assay, intracellular neutralization assay, surface plasmon resonance affinity, flow cytometry, antibody-capture ELISA, sandwich ELISA, enhanced amide hydrogen/deuterium exchange mass spectroscopy (DXMS)
	RV6-25	RV6-25 activity is directed against the apical surface of the VP6 head domain.		Fast protein liquid chromatography (FPLC), ELISA, ELISA binding assay, intracellular neutralization assay, surface plasmon resonance affinity, antibody-capture ELISA, sandwich ELISA, flow cytometry, antibody-capture ELISA, sandwich ELISA, cryo-electron microscopy, hydrogen-deuterium exchange mass spectrometry (DXMS)
West Nile virus (WNV)	WNV-86	WNV-86 targets E domain II, preferentially recognizing mature virions lacking prM	E protein is preferentially displayed on mature virions	ELISA, affinity chromatography, RT-PCR, neutralization assay, flow cytometry, binding assay, neutralization escape assay, inhibition assay, pre-attachment assay, post-attachment assay, antibody competition binding study, bio-layer interferometry
	WNV-96	WNV-96 activity is directed against the β-Ladder, spaghetti loop of NS1.	NS1 is expressed as a dimer on the cell surface and as a soluble hexamer in the extracellular space and in circulation.	ELISA, flow cytometry, biolayer interferometry-based antibody competition, ELISA-basedantibody competition assay, mean fluorescence intensity, virus challenge
	WNV-99	WNV-99 activity is directed against the wing, flexible loop of NS1.		

Pathogen	Clone	Specificity	Antigen Distribution	Applications
Vaccinia Virus (VACV)	VACV-301	VACV-301 activity is directed against the A27 antigen	A27 is expressed on the mature virion particle surface	ELISA, binding assay, neutralization assay, plaque reduction neutralization (PRNT) assay, *in vivo* protection study
Zika Virus (ZIKV)	ZV67	Anti-Zika clone ZV-67 specifically targets the lateral ridge of the DIII domain of envelope protein (E)	ZIKV envelope	ELISA, neutralization, Western Blotting

Antibody Screening & Assay Development Services
ELISA or Lateral Flow Tests: Expertise in Assay Sensitivity & Specificity

With over 30 years of know how, proprietary methods and project management competencies, Leinco Technologies is your partner to bring assays and diagnostic tests to market more quickly. We are certified to ISO 9001 and ISO 13485.



Collaborative Approach

- Highly experienced scientific and project management staff
- Stringent quality and cGMP standards
- Focused on transparent communication at every project stage and on recommending data-driven decisions
- Achieve your goals more quickly using our biomarker immunoassay development and manufacturing experience for drug discovery and diagnostic applications

Assay Services

Delivering reproducibility and accuracy when an off-the-shelf assay option is not available.

Planning

- Collaboration
- Antibody selection & pairing (if relevant)
- Proof of concept
- Design inputs
- **Report:** Standard curve

Development

- Assay optimization
- Integration of cassettes, sample handler and reader systems
- Performance verification
- **Report:** sensitivity, cross-reactivity, shelf life (stability)

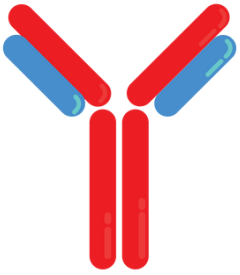
Validation

- Sample testing
- QC assay performance qualification
- Validate that product meets customer's requirement specifications
- **Report:** sensitivity, precision, longer range stability testing

cGMP Manufacturing

- Scale-up manufacturing
- Bulk lot production
- Lot release testing
- Delivery of final kits
- **Report:** SOPs and batch records

Partnership + Process + Proficiency



Assay Substrates & Reagents

Validated components for lot-to-lot consistency, higher signal to noise ratios, increased sensitivity and longer shelf life:

- Substrates and secondary antibody conjugates
- Blocking, coating, and washing buffers and stop solutions
- Stabilization diluents

info@imtec.be | www.imtec.be

Antibody Screening & Assay Development Services
ELISA or Lateral Flow Tests

Looking to improve an existing assay?
Trying to develop a new assay?

Our collaborative and flexible approach provides you with a dedicated team of experienced scientists who are equally adept at development and troubleshooting.

Qualifying Matched Antibody Pairs in Lateral Flow Tests

			Nanoparticles			
			1	2	3	4
Nitrocellulose	Clone 1	Virus		0	3	3
		No Virus		0	0	2
	Clone 2	Virus	0		0	0
		No Virus	0		0	0
	Clone 3	Virus	2	0		3
		No Virus	1	0		2
	Clone 4	Virus	2	0	2	
		No Virus	1	0	0	

Strong	3
Medium	2
Weak	1
No detection	0
None done	

All the data that are part of the verification and validation phases are provided. The design history file includes all the necessary details (SOPs, batch records, etc.) covering all the regulatory requirements.

info@imtec.be | www.imtec.be

References

1. Sklenovská N, Van Ranst M. Front Public Health. 6:241. 2018.
2. Moore M, Zahra F. 2021 Oct 19. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–.
3. McConnell S, Herman YF, Mattson DE, et al. Am JVet Res. 25:192-195. 1964.
4. Hammarlund E, Lewis MW, Carter SV, et al. Nat Med. 11(9):1005-1011. 2005.
5. Gilchuk I, Gilchuk P, Sapparapu G, et al. Cell. 167(3):684-694.e9. 2016.
6. Moss B. Immunol Rev. 239:8–26. 2011.
7. National Foundation for Infectious Diseases- monkeypox. https://www.nfid.org/infectious-diseases/monkeypox/