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Development of the First WHO International Standard for antibodies to vaccinia virus

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** Details provided in Appendix 1*

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposal(s) contained therein. Written comments on the proposal(s) MUST be received in English by **25 September 2026** and should be addressed to:

Biologicals Norms and Standards and Transplantation
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Comments should be submitted electronically to **Dr Ivana Knezevic** at email: knezevici@who.int

The distribution of this document is intended to provide information to a broad audience of potential stakeholders and to improve the transparency of the consultation process. Following consideration of all comments received, the proposal(s) will then be considered by the WHO Expert Committee on Biological Standardization (ECBS) prior to a final decision being made and published in the WHO Technical Report Series.

Summary

Following endorsement by the WHO Expert Committee on Biological Standardization (ECBS) in 2024, a candidate reference material for the standardisation of assays detecting antibodies to vaccinia virus (VACV) (NIBSC code 05/124) was evaluated in a multi-centre collaborative study. Establishment of an International Standard (IS) for antibodies to VACV will support the harmonisation of serological assays for the assessment of immune responses induced by current and novel orthopoxvirus vaccines. It will also facilitate seroepidemiological and Orthopoxviruses antibody cross-reactivity studies. The standard will complement the WHO IS for antibodies to monkeypox virus (MPXV).

The collaborative study for the evaluation of the candidate WHO IS for antibodies to VACV was conducted in parallel with the evaluation of the candidate WHO IS for antibodies to MPXV (NIBSC code 22/218), which was reported in WHO/BS/2026.2514. The choice of one collaborative study was driven by the current serological landscape in which most laboratories routinely test for both viruses; furthermore, the well-recognised cross-reactivity among orthopoxviruses makes the development and validation of truly MPXV-specific serological assays challenging.

Eighteen laboratories from eleven countries participated in the international collaborative study, testing a panel of vaccinee, mpox convalescent, smallpox convalescent and negative-control samples using a wide range of neutralisation (22 datasets) and binding antibody assays (56 datasets). The study assessed the ability of the candidate standards to harmonise assay results, characterise antibody specificity and cross-reactivity, and demonstrate commutability across different sample types and assay formats.

The candidate IS for antibodies to VACV (NIBSC 05/124) was shown to be stable when stored at the recommended temperature of –20°C and robust for shipment at ambient temperature. The candidate standard demonstrated consistent performance across a wide range of VACV neutralisation and binding antibody assays and substantially improved comparability of results, particularly for high-titre samples derived from vaccinated individuals and smallpox convalescent donors. Improved harmonisation was also observed for assays measuring antibodies against individual VACV antigens.

An important outcome of the study was the demonstration of extensive cross-reactivity between antibodies induced by mpox infection and vaccinia-based vaccination. The anti-VACV antibody standard showed strong reactivity in MPXV assays, while mpox convalescent samples reacted in VACV assays. No clear serological distinction between infection- and vaccination-derived responses could be consistently demonstrated across the assay platforms evaluated. These findings reflect the close immunological relationship among orthopoxviruses and highlight the challenges of developing highly specific serological assays.

Despite this cross-reactivity, the candidate preparation demonstrated a clear ability to harmonise biological potency estimates across laboratories and methods. The proposal is that NIBSC 05/124 (MX-02) be established as the First WHO IS for anti-Vaccinia virus antibodies with assigned potencies of 250 IU/ampoule for neutralising antibodies (05/124_NT) and 250 IU/ampoule for binding antibodies (IgG, 05/124_BA).

Overall, the study concluded that the two candidate standards provide valuable tools for standardising antibody measurements, supporting vaccine development, sero-epidemiological investigations and future efforts to define correlates of protection against mpox and other orthopoxvirus infections.

1. Introduction

Vaccinia virus (VACV) is an enveloped, double-stranded DNA virus of the *Orthopoxvirus* genus within the family *Poxviridae* and has played a critical role in human public health as the basis of the vaccines that led to the global eradication of smallpox [1]. This was possible due to VACV sharing a substantial genetic and antigenic similarity with other orthopoxviruses, including variola virus, the causative agent of smallpox, and monkeypox virus (MPXV), the aetiological cause of mpox disease. Indeed, evidence indicates that VACV-based vaccines can induce protective immunity and lessen disease severity of mpox [2,3].

Recent mpox outbreaks were declared by the WHO a Public Health Emergency of International Concern (PHEIC): on 23 July 2022 [4] and lifted in May 2023 [5], and on August 2024 after the emergence and spread of a new MPXV lineage in the Democratic Republic of Congo and neighbouring countries, which remained in place until September 2025 [6].

The two licensed vaccines available on the market and approved by WHO are IMVANEX (also known as JYNNEOS or IMVAMUNE), a highly attenuated modified vaccinia Ankara strain (MVA) developed by Bavarian Nordic and propagated in chicken embryo fibroblast cells; and LC16 KMB, used in Japan, which is derived from the Lister strain and grown in rabbit kidney cells. Additionally, in the USA a third vaccine is approved for smallpox and mpox, ACAM2000, a second-generation vaccine based on the New York City Board of Health (NYCBH) strain and produced in eukaryotic cell culture. Several mpox vaccines are under development at preclinical or early clinical stage [7,8].

The development and availability of serological assays to assess immune responses following vaccination are essential. These tools will facilitate the evaluation of both existing and emerging vaccines, support sero-epidemiological investigations, and improve understanding of antibody cross-reactivity among orthopoxviruses. To increase comparability between assays/laboratories and enable parameters, such as analytical sensitivity of tests, or clinical measures, including protective levels of antibody, to be better defined, an International Standard is required to act as a primary calibrant and define a common unitage to express the biological potency of immune responses in International Units (IU).

An International Standard for antibodies to monkeypox virus has been proposed and was endorsed by the WHO Expert Committee on Biological Standardization (ECBS) in March 2023 [9]. One year later, the International Standard for anti-VACV antibodies [10] was also endorsed. Two different standards are required to support immunoassays that distinguish between mpox and VACV responses. This distinction is important for understanding differences in immune responses between vaccination and infection, in sero-prevalence studies and in clinical trials for new mpox specific vaccines.

A multi-centre international collaborative study was organized, recruiting participants with established serological assays for MPXV or VACV antibodies. These methods included qualitative tests and quantitative assays measuring functional, i.e. neutralising, antibodies and binding antibodies. Few assays were reported to be able to distinguish between mpox infection and vaccination and were included in the study to evaluate the two candidate preparations as

the First WHO International Standard for antibodies to monkeypox virus and the First WHO International Standard for antibodies to vaccinia virus. The study was facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI).

The aims were to:

- Assess the suitability of the candidates to serve as WHO International Standards and harmonise data across a range of typical assays performed in different laboratories.
- Characterise the candidates' reactivity/specificity, including cross-poxvirus reactivities, in different assay systems.
- Assess commutability, i.e. establish the extent to which the candidates are suitable to serve as standards for a variety of different samples.
- Recommend to the WHO ECBS the suitability of the candidates to serve as WHO International Standards and propose an assigned IU.

The results of this collaborative study are also included in the report describing the establishment of the First WHO International Standard for antibodies to monkeypox virus (WHO/BS/2026.2514).

2. Bulk material

2.1 Ethical statement

Convalescent plasma or serum from individuals with an history of mpox infection from the Democratic Republic of Congo (DRC) were kindly provided by Infiuss Health, Inc and by the National Institute for Biomedical Research (INRB). Collections were approved by Ministry of Public Health, Democratic Republic of Congo, Zone Sankuru (N/Ref: 83/DPS/SANK/MS/765/AAO/2022) and National Health Ethics Committee (No. 452/CNES/BN/PMMF/2023 of 27/06/2024). Serum samples from UK MVA-vaccinated individuals were kindly provided by the University of Oxford and University College London. Studies were approved by the UK NHS Ethics committee (London–Surrey Borders Research REC ref 22/PR/1425) and the local Research Ethics Committee South Central Hampshire B (REC 19/SC/0423), respectively. All donors provided written informed consent.

2.2 Candidate WHO International Standard for anti-vaccinia virus antibodies

The candidate WHO International Standard for anti-VACV antibodies and its formulation has been already described in a previous study [11]. Briefly, the source material is a pool of defibrinated plasma collected from volunteers that have been immunised with the NYCBH strain of vaccinia virus. Three litres of plasma were donated by a Vaccinia Immunoglobulins manufacturer. This plasma was tested at NIBSC by plaque reduction neutralisation test (PRNT) against vaccinia virus Lister and NYCBH strains and considered suitable for the production of a candidate IS [11]. The plasma pool was tested at South Mimms laboratories for known blood borne viruses markers and found to be negative for HCV RNA, HIV1/2 antibodies, and HBsAg.

3. Processing of the candidate WHO IBRS

The pool of plasma for the production of the candidate IS for anti-VACV antibodies was filled and lyophilised by the Production/Manufacturing team at South Mimms laboratory (NIBSC) starting 23 June 2005 under product code 05/124. The mean weight of the fill was 1.0063gms (taken from a mean of 42 with check weights being taken at approximately two-minute intervals) with a coefficient of variation of 0.31, which is acceptable for a plasma fill [12]. Freeze drying commenced 23/06/05 and was completed 28/06/05. Results for testing for vacuum, cracks or pinholes showed nil failures. The mean dry weight of the fill measured by coulometric Karl Fischer was 0.0722g (taken from a mean of 6) and the residual moisture content 0.56%. The product 05/124 was prepared in 2005 when testing for residual oxygen content was not routinely carried out at NIBSC. Stability studies (see below) demonstrate that the material is stable at the recommended storage temperature -20°C. Summary of the product characteristics are shown in Table 1.

4. Stability studies

The stability of the candidate International Standard for anti-VACV antibodies (05/124) was evaluated through an accelerated degradation study described in the previous WHO report [11]. Briefly, ampoules of 05/124 were stored at −70°C, −20°C, 4°C, 20°C, 37°C, and 56°C for varying periods. The overall study was conducted from December 2006 to March 2010 (approximately 40 months).

Three ampoules for each temperature and time point were tested by PRNT, and median effective doses (ED50) for each ampoule were calculated, along with the geometric mean of these values. Relative potencies were expressed as percentages relative to the −20°C baseline.

The data presented in Table 2 demonstrate good stability of the candidate IS, with no detectable loss in potency at 4°C and only a 5% loss at 20°C after 1.4 years. Long-term stability estimated using the Arrhenius model [13] predicted a loss of 0.002% per year when stored at the recommended temperature of −20°C. No significant loss of potency was observed after storage at 37°C for 49 days, supporting the conclusion that the preparation is sufficiently stable for shipment at ambient temperature (Table 2).

5. Collaborative study

The collaborative study under NIBSC reference CS732 was launched at the end of September 2025. However, due to delays in obtaining import permits, some participants received the samples as late as April 2026. The first set of results was received on 14 January 2026, and the final data were submitted on 5 June 2026.

The study protocol is provided in Appendix 2. Participants were asked to test the study samples using their established method(s) for detecting antibodies against MPXV and/or VACV. They were requested to perform three independent assays using a fresh set of samples for each assay, preparing serial dilutions of the samples to be tested, with at least duplicate measurements. A results reporting sheet was provided for participants to record all essential information, including the raw data from each assay.

5.1 Collaborative study participants

Twenty laboratories accepted to participate in the study; however, two participants withdrew due to re-prioritisation of activities in their lab and delays in obtaining the import permit. Results were returned from 18 laboratories from 11 countries, representing 4 WHO regions: Canada (1), China (2), France (1), Germany (2), India (1), Italy (1), Japan (1), South Korea (1), Uganda (1), United Kingdom (3) and United States of America (4). All laboratories are referred to by a code number randomly allocated and not reflected in the order presented in Appendix 1. Participating organisations included vaccine developers, national control/reference laboratories, other government institutions, diagnostics laboratories, kit manufacturers, and academic laboratories

5.2 Study sample panel

In this collaborative study we have included seven samples for evaluation alongside the candidate ISs, provided coded and blinded to the participants (Table 3). These additional samples were:

- Candidate WHO International Standard for monkeypox virus, a pool of plasma from 100 mpox recovered individuals described in WHO/BS/2026.2514 (MX-01).
- Two plasma pools from six MVA-BN vaccinated individuals each from UK (MX-03 and MX-04).
  In house testing conducted at NIBSC/MHRA showed a significant difference in the potency of the two pools with MX-03 having higher neutralising antibodies titres. Each pool contained plasma from one donor born before 1971 who might have received a smallpox vaccination in the past. The donors received either 1 or 2 doses of Imvanex.
- One plasma pool from 30 MVA-BN vaccinated individuals from UK (MX-05). Samples were collected either 1 month post first dose, or 1-3 months post second dose. None of the donors had a prior smallpox vaccination.
- Two pools of sera from 27 (MX-07) or 30 (MX-09) mpox-recovered individuals from DRC.
  Samples were collected in 2024. Individual donations were tested and found negative for Mpox DNA using S3352E MPXV – Monkeypox virus Nucleic Acid Diagnostic Kit (Sansure). Pooling was designed based on results provided by collaborators at VisMederi, using a neutralisation assay against MPXV clade 2b and 1b [15] with MX-07 having higher neutralising antibodies titre than MX-09. Both pools were solvent/detergent treated to minimise the risk of the presence of enveloped viruses [12].
- Pool of smallpox convalescent serum from recovered patients, majority of whom had also been vaccinated (MX-08).
  The liquid preparation was generated from the same source material for the production of the first International Standard for anti-smallpox serum [16]. Before adding the sample to the study, the preparation was solvent/detergent treated at NIBSC/MHRA.
- Plasma from a healthy donor as negative control (MX-06).

All samples have been tested for blood borne viruses markers and found to be negative for HCV RNA, HIV1/2 antibodies and HBsAg, except sample MX-08 which tested positive for HBsAg, and samples MX-07 and MX-09 which tested positive for HBsAg as well as HIV-1/2 antibodies, but negative for HIV antigen. Since the solvent/detergent treatment is effective against Hepatitis B virus and HIV-1/2, the pool is considered not infectious.

5.3. Assay Methods

Assays used by the participants included methods to detect neutralising and binding antibodies and are summarised in Tables 4 and 5, respectively. Where laboratories performed multiple assay methods, laboratory codes are followed by a letter indicating the different methods/targets (e.g., laboratory 1a, 1b).

Twelve laboratories returned twenty-two datasets measuring the neutralising activity of the collaborative study samples against MPXV (n = 12) or VACV (n = 10). Most of the laboratories performed a plaque reduction neutralisation test (PRNT, n = 9), while the remaining participants tested the samples using a foci reduction neutralisation test (FRNT, n = 3) or a microneutralisation test through detection of cytopathic effects (CPE, MN=1) or foci (FRMN, n=1). Participants 2 and 6 tested the samples using two methods against MPXV (lab 2: PRNT and MN) or against both viruses (lab 6: PRNT and FRNT). Lab 3 provided two sets of results from the same method (PRNT), expressed as NT_{50} and NT_{80}.

Ten laboratories returned results on binding antibody potencies from 13 methods (Table 5). All methods were ELISA-based; seven targeted either a single antigen (labs 1a and 4), multiple individual antigens (lab 10b) pan-poxvirus antigen pools (lab 1b), or whole irradiated MPXV (lab 10b) and vaccinia virus (labs 9c, 15a, and 15b). The remaining methods were multiplex ELISAs targeting several antigens from either MPXV or VACV. Overall, 56 datasets for binding antibodies were returned. Only lab 15b detected IgM; all other laboratories measured IgG responses.

5.4 Statistical methods

All test details and raw data were returned to MHRA using the provided summary form to permit independent analysis.

Potency estimates for each of the study samples were calculated as the geometric mean (GM) from the participant reported data for the three independent experiments, with data reported and included in the statistical analysis if the sample was determined as positive in at least two out of three measurements by the participants.

Relative potency estimates were calculated by a sigmoidal curve model or parallel-line analysis comparing log transformed assay responses to log dose for results with sufficient dose-response data. Calculations were performed using CombiStats software [17]. For all assays, data for each test preparation was analysed separately against both candidate IS. Model fit was

assessed visually, and non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference sample.

For neutralisation assays reporting end-point titres, potency estimates were calculated as the ratio of endpoint titres of the test and reference samples within an assay run.

Valid relative potency estimates were combined to generate an unweighted geometric mean potency per laboratory. The laboratory GM values were then also used to calculate overall unweighted geometric mean potencies and median potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^{s}\text{-}1\} \times 100\%$) where s is the standard deviation of the $\log_{10}$ transformed potencies.

5.5 Results

5.5.1 Neutralising activity

The geometric means of the neutralising antibody titres reported by the participants were grouped based on the virus used in the method and are presented in Tables 6 (MPXV) and 7 (VACV).

All methods using MPXV detected antibodies in the candidate WHO International Standard (IS) for mpox antibodies (MX-01), albeit at low titres (Table 6). The same methods also detected neutralising activity against MPXV in the candidate anti-VACV antibody standard (MX-02), in the higher-titre pool of MVA vaccinees (MX-03), and in the high-titre pool of mpox convalescent individuals (MX-07). Lab 17 was the only laboratory that did not detect neutralising antibodies in the smallpox antiserum sample MX-08, which was otherwise ranked as having the highest antibody titres by all other laboratories, except lab 18a, which ranked it second after MX-02. As neither MX-02 nor MX-08 contains donations from individuals recovered from mpox, these responses are likely due to known orthopoxvirus cross-reactivity.

The other pool of mpox convalescent sera (MX-09) was detected by only 9 out of 12 tests, and the pools from MVA vaccinees were scored as positive in 7 (MX-04) or 8 (MX-05) out of 12 methods. All neutralisation tests against MPXV correctly identified sample MX-06 as negative.

Interestingly, overall, the neutralisation assays against VACV returned higher titre values for most samples than the methods using MPXV, but also produced more false-negative results (Table 7). The candidate WHO IS for anti-VACV antibodies (MX-02) was detected by all laboratories with one exception (lab 10a); however, in this method— the only one using MVA as the virus—only the smallpox antiserum MX-08 was scored as positive, possibly indicating lower assay sensitivity. The higher-titre pool of MVA vaccinees (MX-03) and the pool of mpox convalescent individuals (MX-07) were detected by almost all methods except lab10a. The candidate mpox antibody standard was detected in 7 out of 10 methods, while the pools from MVA vaccinees (MX-05) and mpox convalescent individuals (MX-09) tested positive in 6 out of 10 assays. Neutralising antibodies in the lower-titre MVA vaccinee pool (MX-04) were

reported by only 4 laboratories. Two laboratories also detected neutralising antibodies in the negative sample MX-06 (3 datasets).

In Table 8, MPXV neutralisation titres for each sample are expressed relative to sample MX-01, the candidate IS for mpox antibodies. For each sample, the spread of results between participants was wider when reported as absolute titres than when expressed relative to the candidate IS (Figure 1A–B). To facilitate comparison, the candidate International Standard was arbitrarily assigned a unitage of 60 International Units per millilitre (IU/mL), allowing the data to be plotted on the same y-axis scale. For all samples except MX-04, expressing MPXV neutralising titres relative to the candidate IS reduced the coefficient of variation (%GCV). As MX-04 is a pool of plasma from MVA vaccinees, it was not expected that the candidate mpox antibody standard would harmonise these data. For the two pools of mpox convalescent sera (MX-07 and MX-09), the variability between participants' values was reduced from 61- and 37-fold, respectively, to 8-fold when expressed relative to MX-01.

A reduction in inter-laboratory variation was also observed for VACV neutralisation assay results when expressed relative to the candidate WHO IS for anti-VACV antibodies, MX-02 (Table 9 and Figure 1C–D). For comparison purposes, this candidate IS was arbitrarily assigned a unitage of 1000 IU/mL to enable plotting on the same y-axis scale. Again, the low-titre MVA vaccinee pools MX-04 and MX-05 did not show harmonisation when expressed relative to a common reference reagent; these samples were challenging to detect, with 6/10 and 4/10 assays, respectively, scoring them as negative. Similarly, the low-titre mpox convalescent pool MX-09 exhibited a better coefficient of variation when reported as absolute titres than when expressed relative to MX-02. In contrast, the other mpox preparations—candidate WHO IS MX-01 and the high-titre mpox convalescent pool MX-07—demonstrated improved harmonisation. The variability between reported potencies for the higher-titre MVA vaccinee pool (MX-03) and the smallpox antiserum (MX-08), where some donors had also been vaccinated, was reduced from 43- and 221-fold to 13- and 8-fold, respectively.

5.5.2 Binding antibody potency

Fifty-six datasets were reported for methods detecting binding antibodies (Table 5). Statistical analysis was feasible only when at least three assays targeted the same analyte, i.e. the same viral antigen and immunoglobulin class. For all other methods, participant-reported data are presented in Table 10 and expressed relative to the corresponding candidate International Standards (IS) in Table 11. This includes the only IgM-specific assay (lab 15b). As both candidate ISs were negative for IgM, no relative measurements could be calculated.

For all other antigens, binding potencies as reported by the participants and expressed relative to the respective standards are summarised as geometric mean, median, and coefficient of variation (GCV) in Tables 12–18 (MPXV antigens), Tables 19–21 (VACV antigens), and Table 22 (antigen pools and whole virus).

For MPXV antigens A35R, B6R, and H3L, expressing potency relative to candidate IS MX-01 reduced inter-laboratory variability, with GCV values below 100%. A similar reduction was observed for E8L and M1R in mpox convalescent samples, although vaccine-derived samples

showed higher variability. For A29L and B2R, GCV was reduced across all samples, but the effect was minimal for the low-titre convalescent pool MX-09.

For VACV antigens, similar improvements in harmonisation were observed for A33R and B5R when data were expressed relative to candidate IS MX-02, with GCV values below 100% at least for vaccine-derived samples. For A27L, expressing results relative to MX-02 reduced inter-laboratory variation overall, but not for low-titre samples (MX-04, MX-05, and MX-09), consistent with observations for neutralising antibody titres.

For assays targeting antigen pools or whole virus, where cross-reactivity is expected, binding antibody titres were expressed relative to both candidate ISs (Table 22). Comparable GCV values were observed, demonstrating that the use of a standard improves inter-laboratory harmonisation.

5.5.3 Commutability

In addition to the reduction in GCV values noted above, the commutability of both candidate WHO International Standards was further evaluated using individual laboratory GM estimates. For neutralisation assays and for binding assays with at least five participating laboratories, geometric mean results were summarised and displayed using a colour-coded scheme, with shading intensity ranging from none to dark red according to the fold deviation from the study median. A decrease in fold deviation following calibration against the respective standards was interpreted as evidence of improved harmonisation. The proportion of laboratories reporting potency estimates within 50–200% or 25–400% of the study median was calculated and tabulated. Cases in which calibration against the relevant standard improved agreement between laboratories are highlighted in green (Tables 6 and 7 *versus* 8 and 9, and Tables 12-14, 22).

6. Discussion, Conclusions and Proposal

6.1 Discussion and Conclusions

The Poxviridae family has been identified by the WHO R&D Blueprint for Epidemics as posing a significant public health risk, with monkeypox virus (MPXV) and vaccinia virus (VACV) highlighted as prototype pathogens [18]. In line with this, the WHO research roadmap for mpox (September 2024) emphasised the urgent need to define correlates of protection and to support the development and standardisation of immunoassays [19]. A key component of this effort is the implementation of higher-order reference materials, such as WHO International Standards (IS), to enable comparability of immunogenicity data across laboratories and studies by providing a common unitage for expressing the biological potency of the immune responses to infection and vaccination, as discussed at a WHO Informal consultation on the Standardization of Biological products for Mpox and other Emerging Pathogens [20].

This study demonstrates the value of candidate WHO IS preparations in harmonising antibody assay outputs. The MPXV candidate IS, MX-01 (NIBSC 22/218), was detectable across all neutralisation assays targeting MPXV, albeit at relatively low titres. If MPXV-specific

responses were substantially distinct from VACV responses, the MPXV IS would be expected to exhibit greater relative reactivity than the VACV IS, MX-02 (NIBSC 05/124), when tested against mpox convalescent samples in MPXV-specific assays. However, this was not the case. The VACV IS (MX-02) consistently yielded titres approximately 5–10-fold higher in the same assays and against the same sample sets, regardless of whether the samples originated from mpox convalescent individuals or vaccine recipients. This observation is consistent with the well-established cross-reactive immunological relationship between orthopoxviruses, reflecting the historical use of VACV-based vaccines for both smallpox and mpox prevention.

The candidate IS for antibodies to VACV, MX-02 was effective in harmonising results for high-titre samples, particularly from vaccine recipients, although it was less successful for low-titre samples. This limitation was also evident in assays involving modified vaccinia Ankara (MVA)-based vaccines. MVA vaccines are known to elicit lower antibody responses than second-generation VACV vaccines, making it difficult to determine whether the reduced harmonisation observed is due to intrinsic assay variability, sample characteristics, or limitations of the standard itself. Notably, good harmonisation was observed for the anti-smallpox serum pool MX-08, suggesting that standard performance may be context-dependent and influenced by antibody titre levels.

For binding antibody assays, the use of candidate IS improved inter-laboratory agreement (generally reducing geometric coefficients of variation to below 100%) for several MPXV and VACV antigens, particularly in higher-titre samples. However, the degree of harmonisation was inconsistent across analytes and sample types. Lower-titre samples often showed poorer agreement, underscoring the challenges of assay sensitivity and precision near detection limits.

An additional limitation relates to assay specificity. Despite some of the assays being designed to target distinct MPXV or VACV antigens, cross-reactivity was observed in both mpox convalescent and vaccine-derived samples (Table 23). While such cross-reactivity is expected within the orthopoxvirus genus, it complicates the interpretation of antigen-specific responses and may mask true differences in immune specificity. Of note, the samples evaluated here were derived from pooled plasma or sera rather than individual clinical samples, which may have influenced cross-reactivity; unfortunately, large volumes from individual patients were not available.

In conclusion, the study did not demonstrate differential responses between samples from mpox-infected and vaccinated individuals. This may reflect limitations of the assays, mainly due to the challenge of developing antigens with exclusive specificity to MPXV or VACV, complexity of the immune responses to poxviruses and the nature of the collaborative study samples. However, both candidate WHO International Standards demonstrated a clear benefit in improving the comparability of antibody measurements for mpox and VACV, albeit with some limitations. The candidate IS for anti-VACV antibodies, MX-02, effectively reduced inter-laboratory variation in high-titre samples but had limited or no impact on lower-titre samples.

6.2 Proposal

We propose that sample MX-02, NIBSC code 05/124, be established as the First WHO International Standard for antibodies to vaccinia virus for neutralisation assays with an assigned potency of 250 IU/ampoule (05/124_NT) and 250 IU/ampoule for binding antibodies (IgG, 05/124_BA). There are approximately 2600 ampoules (0.25 mL/ampoule) available for distribution.

In the proposed Instruction for use (IFU, Appendices 3-4) it will be specified that for binding antibodies the potency will need to be specified per antigen and no comparison should be done between antigens.

Based on the stability study results, we propose that both International Standards can be shipped at ambient temperature. MHRA is the custodian laboratory, and the standard will be kept at -20˚C.

7. Comments Received from Collaborative Study Participants

This collaborative study report was shared with the study participants, and two proposals were put forward for consideration, with participants asked to indicate, based on their expert opinion, which proposal they supported. The alternative proposal, informed by the extensive cross-reactivity observed in the study, was to establish sample MX-02 (NIBSC code 05/124) as the First International Standard for antibodies to Orthopoxviruses. The study demonstrated that 05/124 could harmonise biological potency estimates across samples from recipients of vaccinia virus-based vaccines and convalescent individuals from mpox or smallpox infection.

Seven out of twelve participants, who provided an opinion, favoured the establishment of two distinct standards, as per proposal in 6.2. Also, two of the authors indicated a preference for two separate standards. A summary of the supporting statements is the following:

- Two separate standards option is always better accepted from developers, company and regulatory authorities.
- The data from the study, especially Table 8-9 support two distinct standards.
- VACV standard might not correctly report mpox specific responses due to the cross-reactivity observed within Orthopoxviruses.
- concern would be using anti-vaccinia sera as a reference for anti-MPXV sera (either from infection or post-immunisation with the new generation of MPXV-specific vaccines being worked on). Whilst antibody responses to VACV and MPXV look very similar and are cross-reactive, there appears to be some subtle differences to homologous antigen binding (e.g. VACV A33 versus MPXV A35) regarding epitopes and likely avidity/affinity.

Five of twelve participants preferred the alternative proposal of one standard for Orthopoxviruses for the following reasons:

- It more accurately represents the reality of what we see in the lab side when doing serological testing that samples are effectively being classified as orthopoxvirus

positive or negative as opposed to just mpox-positive due to the limitations of both the assays and our current understanding of cross-reactivity within orthopoxviruses.

- MX-02 (NIBSC code 05/124) yields the highest and most stable neutralising antibody titres, with robust neutralising and binding antibody levels against mpox and other orthopoxviruses.

Other comments on the collaborative study:

Lab 11 observed a matrix effect for these samples MX-01, MX-02, MX-04, MX-07 and MX-09 in the PRNT as follows: "The matrix effect consisted of dark spots observed in the cell monolayer. The effect was sample-specific, being observed throughout different aliquots of affected samples for both methods. Under visual inspection of the images, these spots or artefacts were plaque-unrelated but caused the Cytation reader protocol to erroneously count them as virus-induced plaques – presumably due to the higher than usual contrast of the dark spots. Additionally, this matrix effect displayed a concentration-dependent behaviour, i.e., being more pronounced at lower dilutions and disappearing along dilution steps".

Several participants reported that MX-02 after reconstitution became difficult to pipette (Lab 5, 6, 12, 17 and 18) with lab 12 adding "but mixing a bit more thoroughly seemed to solve it". Lab 17 suggested to increase the reconstitution volume to 1 mL.

In response to the last point, the IFUs (appendices 3 and 4) contain clear instructions on how to reconstitute the standard 05/124.

8. Acknowledgements

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10. Tables and Figures

10.1 Tables

Table 1. Summary characteristics of the candidate WHO IS for antibodies to VACV

General material characteristics		
Product code	05/124	
Presentation	lyophilised	
Appearance	white cake	
Container type	5 mL ampoule	
Number of containers available for distribution	2590*	
Date of fill	23 June 2005	
Storage temperature	-20°C	
Microbiological findings	All negative	
	Target value	Result
CV of fill mass (%)	< 1	0.31 (n=42)
Mean dry weight (CV%)	n/a	0.07
Mean residual moisture (%)	<1	0.56 (n=6)
Mean oxygen headspace (%)		Not tested

n = number of samples tested; CV= coefficient of variation; * as of 05 June 2026.

Table 2. Thermal degradation assessment of the candidate IS for antibodies to VACV

Relative potency of 05/124 after storage at the indicated temperatures and time, expressed relative to -20°C baseline (as percentage). These data have been extracted from previous study [11].

	-70°C	**4°C**	**20°C**	**37°C**	**56°C**
26 days			109	102	71
49 days			101	89	36
113 days	93	103			30
243 days				83	20
525 days	96	99	95		
1187 days	96	96	61	35	

Table 3. Collaborative study sample panel
Samples were shipped under study reference CS732

CS sample code	Abbreviation	Description	Volume (mL)	Appearance
MX-01	IS mpox	22/218- candidate WHO IS mpox Ab, plasma	0.25	freeze dried
MX-02	IS VACV	05/124- WHO IS VACV Ab, plasma-derived serum	0.25	freeze dried
MX-03	MVA#1	MVA-vaccinated, plasma	0.2	liquid
MX-04	MVA#2	MVA-vaccinated, plasma	0.2	liquid
MX-05	MVA#3	MVA-vaccinated, plasma	0.2	liquid
MX-06	Neg	Negative, plasma	0.2	liquid
MX-07	mpox-1	mpox convalescent plasma pool#1	0.2	liquid
MX-08	SMX	smallpox antiserum	0.2	liquid
MX-09	mpox-2	mpox convalescent plasma pool#2	0.2	liquid

Table 4. List of the neutralisation assay methods used in the study.

Lab	Methods	Virus/Strain	Readout	Output
2a	MN	MPXV clade IIb (2225/22 Sloveni ex Gran Canaria)	CPE	Endpoint titre
2b	MN	VACV Western reserve strain	CPE	Endpoint titre
2c	FRNT	MPXV clade IIb (2225/22 Sloveni ex Gran Canaria)	FFU	ND50
3a	PRNT	MPXV clade IIb (hMPXV/USA/MN001/2022)	PFU	NT50
3b	PRNT	MPXV clade IIb (hMPXV/USA/MN001/2022)	PFU	NT80
5a	PRNT	MPXV clade I	PFU	ND50
5b	PRNT	VACV Western reserve strain	PFU	ND50
6a	PRNT	MPX Clade IIa (Liberia strain)	PFU	Endpoint titre
6b	PRNT	VACV LC16m8	PFU	Endpoint titre
6c	FRNT	MPX Clade IIa (Liberia strain)	FFU	Endpoint titre
6d	FRNT	VACV LC16m8	FFU	Endpoint titre
9a	PRNT	VACV	PFU	NT50
9b	FRNT	MPXV	FFU	NT50
10a	FRMN	VACV (MVA)	PFU	ND50
11	PRNT	MPXV clade IIb (hMPXV/USA/MN001/2022)	PFU	NT50
13a	PRNT	MPXV clade IIb (hMPXV/USA/MN001/2022)	PFU	NT50
13b	PRNT	VACV Western reserve strain	PFU	NT50
14	PRNT	VACV	PFU	ND50
15c	PRNT	VACV Western reserve strain	PFU	NT50
17	PRNT	MPXV clade IIb	PFU	NT50
18a	PRNT	MPXV Clade IIb (hMPXV/Beijing.CHN/CMC-V-0800/2024)	PFU	NT50
18b	PRNT	VACV (Tiantan strain)	FFU	NT50

Grey highlighting, monkeypox virus MPXV; no highlighting: Vaccinia virus, VACV. MN: microneutralisation test; FRNT: foci reduction neutralisation test; PRNT: plaque reduction neutralisation test; FRMN: foci reduction microneutralisation test; CPE: cytopathic effect; FFU: foci forming units; PFU: plaque forming units; ND50: 50% neutralisation dose; NT50: 50% neutralisation titre; NT80: 80% neutralisation titre.

Table 5. List of the methods detecting binding antibodies used in the study.

Lab	Ig Class	MPXV antigen	VACV antigen	Readout	Output
1a	IgG	A27L, antigen pool		OD	endpoint titre
1b	IgG	Pan poxvirus antigen pool*		OD	endpoint titre
1c	IgG	E8L, B6R,B2R,M1R, A35R, H3L, A29L, A5L	B5R, A33R,A27L	MFI	endpoint titre
2d	IgG	A29L,A35R,B6R,E8L,M1R	A27L, A33R,B5R,D8L, L1R	AU/ml	AU/ml
4	IgG	E8L		OD	endpoint titre
7	IgG	H3L, A35R	A27L	OD	endpoint titre
8	IgG	A35R, A29L, E8L		OD	endpoint titre
9c	IgG		VACV	OD	endpoint titre
10b	IgG	M1R, B6R, B2R, E8L, A35, H3L, MPXV Clade IIb		OD	Relative to in-house serum
12	IgG	H3, B2R, B6R, A35, A5L E8L	B5R, A33R	MFI	pos/neg
15a	IgG		VACV	OD	pos/neg
15b	IgM		VACV	OD	pos/neg
16	IgG	A35R, A29L, B6R, E8L	A33R, A27L, B5R, D8L	MFI	endpoint titre

Ig: Immunoglobulin; OD: optical density; MFI: mean fluorescence intensity; AU: arbitrary units. * antigen pool included VACV B5 and MPXV A35, B2, B6 and E8.

Table 6. Geometric means of the neutralising antibody titres against MPXV as reported by the participants**.**

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
2a	71	1810	20	-	-	-	570	5747	71
2c	94	1862	57	-	-	-	1466	3101	221
3a	47	349	56	29	48	-	279	1626	21
3b	18	70	14	-	-	-	56	269	-
5a	209	1188	276	111	183	-	796	4245	80
6a	81	724	575	144	144	-	287	2299	29
6c	256	1625	697	69	512	-	1393	19112	83
9b	23	114	89	-	30	-	135	496	-
11	91	1040	706	826	1002	-	364	8177	n/a
13a	43	416	1844	1949	1516	-	328	2196	38
17	11	149	14	-	-	-	24	-	6
18a	41	423	12	11	9	-	79	174	69
GM	56	516	106	128	150	-	266	2019	46
Median	58	553	71	111	162	-	307	2299	69
% GCV	155.6	207.7	504.9	510.1	502.3	n/a	256.6	325.2	183.9
% 50-200	58%	42%	25%	43%	25%		42%	45%	44%
% 25-400	83%	83%	42%	57%	50%		67%	64%	89%
N	12	12	12	7	8	0	12	11	9

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories.
Shading represents laboratory geometric means as fold change from study median values (X).
No shading, X < 2; shading from lightest to darkest, 2 < X < 4, 4 < X < 8, X > 8, respectively.

Table 7. Geometric means of the neutralising antibody titres against VACV as reported by the participants.

Lab	MX-01 IS mpox	MX-02 IS VACV	MX-03 MVA#1	MX-04 MVA#2	MX-05 MVA#3	MX-06 Neg	MX-07 mpox#1	MX-08 SMX	MX-09 mpox#2
2b	25	905	25	-	-	-	202	2281	40
5b	-	299	62	-	57	-	69	1304	-
6b	645	10321	323	64	64	9	912	26008	128
6d	2048	20643	753	110	439	44	4778	65536	174
9a	32	386	38	-	-	-	97	>640	-
10a	-	-	-	-	-	-	-	1562	-
13b	-	552	206	-	75	-	42	2203	-
14	30	404	27	-	-	-	81	297	17
15c	575	18352	1076	72	396	-	1391	38553	92
18b	121	986	32	36	17	21	127	547	95
GM	156	1649	112	65	97	20	248	3629	71
Median	121	905	62	68	69	21	127	2203	94
% GCV	490.8	472.9	341.6	57.4	248.3	120.5	401.8	591.3	138.0
% 50-200	14%	33%	33%	100%	50%		56%	50%	67%
% 25-400	29%	67%	67%	100%	50%		67%	50%	83%
N	7	9	9	4	6	3	9	8	6

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories.
Shading represents laboratory geometric means as fold change from study median values (X).
No shading, X < 2; shading from lightest to darkest, 2 < X < 4, 4 < X < 8, X > 8, respectively

Table 8. Geometric means of the neutralising antibody titres against MPXV when expressed relative to the candidate IS for mpox antibodies (MX-01)

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
2a	60	1524	17	-	-	-	480	4838	60
2c	60	1188	36	-	-	-	936	1979	141
3a	60	450	72	37	62	-	359	2095	21
3b	60	258	44	-	-	-	194	946	-
5a	60	342	79	32	53	-	229	1221	23
6a	60	539	428	107	107	-	214	1711	21
6c	60	381	163	16	120	-	327	4479	17
9b	60	300	234	-	79	-	354	1301	-
11	60	713	550	645	601	-	142	6372	n/a
13a	60	580	1525	2376	1263	-	406	2885	37
17	60	836	78	-	-	-	134	-	32
18a	60	619	18	14	13	-	115	255	101
GM	60	560	109	89	115	-	271	1888	38
Median	60	559	79	37	92	-	273	1979	32
% GCV		71.9	303.1	606.7	322.2		83.6	146.3	110.8
% 50-200		75%	33%	29%	63%		75%	55%	78%
% 25-400		100%	58%	71%	63%		100%	91%	89%
N	12	12	12	7	8	0	12	11	9

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories. Shading represents laboratory geometric means as fold change from study median values (X). No shading, X < 2; shading from lightest to darkest, 2 < X < 4, 4 < X < 8, X > 8, respectively. Green highlighting, increased in the percentage of laboratories within the stated range of the study median.

Table 9. Geometric means of the neutralising antibody titres against VACV when expressed relative to the candidate IS for anti-VACV antibodies (MX-02)

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
2b	28	1000	28	-	-	-	223	2520	44
5b	-	1000	206	-	203	-	230	4357	-
6b	63	1000	31	6	6	1	88	2520	12
6d	99	1000	36	5	21	2	231	3175	8
9a	84	1000	98	-	-	-	250	n/a	-
10a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
13b	-	1000	374	-	121	-	77	3988	-
14	74	1000	66	-	-	-	201	735	41
15c	31	1000	59	4	22	-	76	2101	3
18b	123	1000	32	37	17	22	129	555	97
GM	63	1000	68	8	33	3	150	2040	19
Median	74	1000	59	6	21	2	201	2520	23
% GCV	75.9		148.7	175.6	271.1		66.9	113.9	263.2
% 50-200	71%		67%	75%	50%		67%	75%	50%
% 25-400	100%		89%	75%	67%		100%	88%	67%
N	7	9	9	4	6	3	9	8	6

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories. Shading represents laboratory geometric means as fold change from study median values (X). No shading, X < 2; shading from lightest to darkest, 2 < X < 4, 4 < X < 8, X > 8, respectively. Green highlighting, increased in the percentage of laboratories within the stated range of the study median.

Table 10. Geometric means of the binding antibody titres for methods as reported by the participants.

Lab	Antigen	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
		IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
2d	VACV D8L	40359	160456	165965	17428	84894	164	175372	1716609	5987
16		200	800	566	50	400	-	800	6400	28
2d	VACV L1R	35710	584979	84620	5894	11508	120	118597	8863804	11081
1c	MPXV A5L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
12		3283	2786	-	-	-	-	5534	11420	-
15b	VACV IgM	-	-	-	-	-	-	0.432	0.564	-

n/a: participant provided raw data, not analysed value.

Table 11. Geometric means of the binding antibody titres expressed relative to the corresponding candidate International Standard.

Lab	Antigen	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
		IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
2d	VACV D8L	0.25	1.00	1.03	0.11	0.53	0.001	1.09	10.70	0.04
16		0.23	1.00	0.60	0.06	0.33	-	0.88	8.02	0.05
2d	VACV L1R	0.06	1.00	0.14	0.01	0.02	0.0002	0.20	15.15	0.02
1c	MPXV A5L	1.00	0.12	0.07	0.04	0.05	0.01	1.72	0.55	0.36
12		1.00	1.06	-	-	-	-	2.25	10.37	-

Value for VACV D8L and VACV L1R reported relative to MX-02; values for MPXV A5L relative to MX-01.

Table 12. Geometric means of the binding antibody titres against **MPXV A35R**.

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	400	800	200	-	50	-	>3200	>3200	100
2d	32151	139514	23753	234	4057	151	256768	1677742	9880
7	100	173	-	-	Neg	-	900	2700	-
8	1008	12800	3200	504	504	504	12800	81275	504
10b	469	1795	247	25	77	-	4495	27555	298
12	5394	15779	2532	-	Neg	-	21482	41629	2502
16	356	1600	141	-	35	-	2540	14368	126
GM	1023	3789	1051	144	195	276	9479	42707	600
Median	469	1795	791	234	77	276	7585	33869	387
% GCV	598.2	827.3	661.5	374.3	626.7	n/a	619.7	749.7	500.1
%50-200	43%	29%	0%	33%	40%	n/a	33%	33%	33%
%25-400	57%	43%	50%	67%	60%	n/a	67%	67%	67%
N	7	7	6	3	5	2	6	6	6

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	1000	1854	441	Neg	102	-	15538	46871	291
2d	1000	4339	739	7	126	5	7986	52183	307
7	1000	2536	-	-	Neg	-	8390	49567	-
8	1000	4545	773	NP	440	312	9377	56872	404
10b	1000	3799	527	53	166	-	10329	65211	693
12	1000	4575	415	-	Neg	-	7721	70323	400
16	1000	4842	388	-	74	-	7401	43877	382
GM	1000	3598	527	20	147	38	9243	54275	396
Median	1000	4339	482	20	126	38	8390	52183	391
% GCV	0.0	44.1	34.7	n/a	97.3	n/a	29.2	18.9	36.0
%50-200		86%	100%	0%	80%	n/a	100%	100%	100%
%25-400		100%	100%	100%	100%	n/a	100%	100%	100%
N	7	7	6	2	5	2	7	7	6

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories. Shading represents laboratory geometric means as fold change from study median values (X). No shading, X < 2; shading from lightest to darkest, 2 < X < 4, 4 < X < 8, X > 8, respectively. Green highlighting, increased in the percentage of laboratories within the stated range of the study median.

Table 13 Geometric means of the binding activity titres against **MPXV B6R.**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	400	>3200	1008	50	159	-	>3200	>3200	126
2d	35503	584979	84620	5952	11075	124	118597	8863804	11228
10b	265	2598	529	47	112	-	1519	51505	126
12	4641	25515	7161	-	-	-	13217	45047	-
16	224	3200	112	-	45	-	1270	20319	112
GM	1314	18768	2051	241	306	124	7416	142974	376
Median	400	9036	1008	50	133	124	4481	48168	126
% GCV	812.7	1135.7	1191.6	1508.8	1061.4	n/a	743.8	1514.9	863.1
%50-200	60%	0%	40%	67%	50%	n/a	0%	50%	75%
%25-400	60%	75%	40%	67%	75%	n/a	75%	75%	75%
N	5	4	5	3	4	1	4	4	4

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	1000	10771	2795	100	371	-	8551	238370	324
2d	1000	16477	2383	168	312	3	3341	249665	316
10b	1000	10219	2080	166	414	-	6600	252072	520
12	1000	13517	1791	-	-	-	4267	209417	-
16	1000	12760	460	-	198	-	4849	95569	724
GM	1000	12562	1627	141	312	3	5227	197466	443
Median	1000	12760	2080	166	340	3	4849	238370	411
% GCV	0.0	21.0	106.6	34.4	38.4	n/a	44.6	51.0	49.1
%50-200		100%	80%	100%	100%	n/a	100%	80%	100%
%25-400		100%	80%	100%	100%	n/a	100%	100%	100%
N	5	5	5	3	4	1	5	5	4

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories. Shading represents laboratory geometric means as fold change from study median values (X). No shading, X < 2; shading from lightest to darkest, 2 < X < 4, 4 < X < 8, X > 8, respectively. Green highlighting, increased in the percentage of laboratories within the stated range of the study median.

Table 14. Geometric means of the binding activity titres against **MPXV E8L.**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	400	1008	635	25	317	-	>3200	>3200	50
2d	53365	103598	85862	2223	36633	352	516340	1267196	9073
4	25803	72982	57926	34443	40960	24355	91952	655360	20480
8	12800	25600	51200	504	504	317	51200	204800	2016
10b	420	1183	1390	41	408	-	2473	13810	132
12	3761	9147	6952	-	3492	-	13825	31630	1257
16	356	1008	566	-	252	-	2540	7184	100
GM	3267	8017	7071	524	1890	1395	24400	90063	846
Median	3761	9147	6952	504	504	352	26605	80484	1257
% GCV	750.2	671.6	815.3	1854.7	834.5	1089.9.6	726.7	738.6	932.6
%50-200	14%	14%	14%	20%	43%	n/a	33%	0%	29%
%25-400	29%	29%	14%	20%	57%	n/a	50%	33%	29%
N	7	7	7	5	7	3	6	6	7

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	1000	2336	1644	55	765	-	13037	26789	129
2d	1000	1941	1609	42	686	7	9676	23746	170
4	1000	3374	2179	862	1328	511	4105	20355	547
8	1000	2704	2531	108	69	22	7453	32802	179
10b	1000	2816	3066	91	887	-	6731	37842	325
12	1000	3292	2116	-	893	-	6212	36359	294
16	1000	2861	1323	-	560	-	7432	19087	355
GM	1000	2719	1994	114	577	42	7386	27249	256
Median	1000	2816	2116	91	765	22	7432	26789	294
% GCV	0.0	21.3	33.7	229.2	164.9	842.8	43.4	31.8	65.7
%50-200		100%	100%	60%	86%	n/a	100%	100%	86%
%25-400		100%	100%	80%	86%	n/a	100%	100%	100%
N	7	7	7	5	7	3	7	7	7

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories. Shading represents laboratory geometric means as fold change from study median values (X). No shading, X < 2; shading from lightest to darkest, 2 < X < 4, 4 < X < 8, X > 8, respectively. Green highlighting, increased in the percentage of laboratories within the stated range of the study median.

Table 15. Geometric means of the binding activity titres against **MPXV H3L.**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	317	1008	400	79	63	-	1600	>3200	40
7	200	2844	200	-	-	-	300	8100	100
10b	431	24705	707	105	194	67	875	54123	188
12	10645	25827	15892	3320	2504	-	23996	42130	3106
GM	735	6540	974	302	313	67	1782	26433	220
Median	370	8383	532	105	194	67	1183	42130	137
% GCV	511.0	403.7	590.3	700.5	560.0	n/a	546.6	180.6	554.4
N	4	4	4	3	3	1	4	3	4

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	1000	3031	1317	269	217	-	8961	50505	152
7	1000	2735	1128	-	-	-	3019	59640	507
10b	1000	7865	1795	170	553	144	1774	125880	406
12	1000	5931	1877	227	150	-	4354	115274	183
GM	1000	4435	1496	218	262	144	3802	81310	275
Median	1000	4240	1538	227	217	144	3626	82916	272
% GCV	0.0	67.1	27.8	26.1	96.1	n/a	97.4	58.5	80.4
N	4	4	4	3	3	1	4	4	4

Table 16. Geometric means of the binding activity titres against **MPXV M1R.**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	283	1600	1600	35	400	-	800	>3200	25
2d	3017	58631	30368	491	5522	320	10470	192666	1307
10b	96	499	423	15	99	9	148	2236	21
GM	434	3605	2740	65	603	54	1073	20756	88
Median	283	1600	1600	35	400	54	800	20756	25
% GCV	484.3	1099.5	790.5	508.6	669.1	n/a	754.8	n/a	934.1
N	3	3	3	3	3	2	3	2	3

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	1000	5975	7215	172	1832	-	3002	36390	NP
2d	1000	19431	10064	163	1830	106	3470	63852	433
10b	1000	5143	4321	179	1295	107	1940	30069	274
GM	1000	8421	6795	171	1631	107	2724	41187	345
Median	1000	5975	7215	172	1830	107	3002	36390	345
% GCV	0.0	107.1	53.1	4.8	22.2	n/a	35.3	47.9	n/a
N	3	3	3	3	3	2	3	3	2

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories

Table 17. Geometric means of the binding activity titres against **MPXV A29L.**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2d	9308	70725	954	789	1327	239	11648	213369	1812
8	800	12800	2016	504	504	252	3200	20319	200
16	89	1270	-	-	-	-	356	1796	200
GM	872	10476	1387	631	818	245	2368	19820	417
Median	800	12800	1387	631	818	245	3200	20319	200
% GCV	923.4	651.9	n/a	n/a	n/a	n/a	482.9	990.1	257.0
N	3	3	2	2	2	2	3	3	3

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	1000	2715	-	-	153	-	2524	12684	255
2d	1000	7599	102	85	143	26	1251	22924	195
8	1000	8305	459	176	257	-	2582	19770	240
16	1000	14633	-	-	-	-	3396	-	2201
GM	1000	7076	217	122	178	26	2294	17914	403
Median	1000	7944	217	122	153	26	2553	19770	248
% GCV	0.0	101.7	n/a	n/a	38.0	n/a	53.1	36.1	212.2
N	4	4	2	2	3	1	4	3	4

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories;
n/a: participant provided raw data, not analysed value.

Table 18. Geometric means of the binding activity titres against **MPXV B2R.**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	317	2016	504	50	79	-	2016	3200	63
10b	265	1280	169	63	37	3	1232	3943	54
12	4382	17404	5519	-	-	-	17836	39402	-
GM	717	1904	778	56	55	3	3538	7922	58
Median	317	2016	504	56	55	3	2016	3943	58
% GCV	380.8	45.0	494.2	n/a	n/a	n/a	314.7	302.8	n/a
N	3	3	3	2	2	1	3	3	2

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	1000	7661	1863	NP	167	-	6855	52796	152
10b	1000	2497	679	260	154	16	5231	16660	230
12	1000	7771	1347	-	-	-	7706	56658	-
GM	1000	5297	1194	260	160	16	6514	36800	187
Median	1000	4405	956	260	154	16	6349	30724	230
% GCV	0.0	123.2	62.3	n/a	n/a	n/a	31.5	137.6	n/a
N	3	3	3	1	2	1	3	3	2

Table 19. Geometric means of the binding activity titres against **VACV A33R**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	200	800	200	-	50	-	1600	>3200	63
2d	15698	184396	33812	383	11833	161	81022	2379899	5269
12	6416	28301	6814	-	2550	-	21771	48220	5145
16	283	3200	200	-	56	-	1600	28735	100
GM	1545	10751	1742	383	539	161	8197	148844	643
Median	1347	9516	1167	383	378	161	5902	48220	717
% GCV	798.8	998.7	1224.6	n/a	1468.1	n/a	610.9	1018.4	1027.7
N	4	4	4	1	4	1	4	3	4

Relative to MX-02

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	243	1000	220	-	64	-	2639	30298	87
2d	85	1000	183	2	64	1	439	12906	29
7	98	1000	109	-	35	-	630	15229	75
16	103	1000	89	-	26	-	585	9204	53
GM	120	1000	141	2	44	1	809	15301	56
Median	101	1000	141	2	47	1	607	14020	63
% GCV	60.8	0.0	53.2	n/a	58.6	n/a	123.4	65.1	63.8
N	4	4	4	1	4	1	4	4	4

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories

Table 20. Geometric means of the binding activity titres against **VACV B5R**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	200	2016	504	79	126	-	1008	>3200	50
2d	28578	528305	99044	6605	14555	199	80979	10666550	8916
12	2844	24705	10017	1280	2674	-	8886	46113	803
16	89	1425	126	-	45	-	356	18102	25
GM	1097	10777	2817	875	684	199	4010	207263	308
Median	754	4232	2247	1280	580	199	2993	46113	200
% GCV	1286.1	1408.2	1897.3	834.7	1357.1	n/a	1014.0	3033.6	1386.8
N	4	4	4	3	4	1	4	3	4

Relative to MX-02

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1c	74	1000	243	27	50	-	438	28865	20
2d	54	1000	187	13	28	0	153	20190	17
12	46	1000	229	18	43	-	191	24888	12
16	65	1000	73	-	26	-	252	12377	46
GM	59	1000	166	18	35	0	238	20584	21
Median	59	1000	207	18	34	0	219	22416	19
% GCV	22.8	0.0	75.0	46.4	37.1	n/a	57.4	44.7	78.1
N	4	4	4	3	4	1	4	4	4

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories

Table 21. Geometric means of the binding activity titres against **VACV A27L**

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1a	800	32254	50	50	50	71	8063	51200	200
1c	63	800	-	-	-	-	800	1600	-
2d	6534	76258	1080	718	1784	242	9354	222550	1243
7	635	900	159	141	100	-	900	2700	200
16	283	2851	-	-	25	-	1270	3592	356
GM	568	5503	205	172	122	131	2332	11207	365
Median	635	2851	159	141	71	131	1270	3592	267
% GCV	441.7	708.3	372.2	282.9	552.0	n/a	236.5	751.5	136.6
%50-200	40%	20%	33%	33%	50%	n/a	60%	40%	75%
%25-400	60%	60%	67%	67%	75%	n/a	60%	60%	75%
N	5	5	3	3	4	2	5	5	4

Relative to MX-02

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1a	32	1000	6	5	10	10	228	2002	10
1c	65	1000	-	-	-	-	909	2359	-
2d	86	1000	14	9	23	3	123	2918	16
7	594	1000	194	134	NP	-	1060	3228	195
16	101	1000	-	-	9	-	431	1338	154
GM	102	1000	26	19	13	6	410	2264	47
Median	86	1000	14	9	10	6	431	2359	50
% GCV	194.2	0.0	503.6	454.3	71.7	n/a	149.4	41.6	361.5
%50-200	60%		33%	67%	67%	n/a	40%	100%	0%
%25-400	80%		67%	67%	100%	n/a	100%	100%	75%
N	5	5	3	3	3	2	5	5	4

GM: geometric mean; %GCV: geometric coefficient of variation; N: number of laboratories. Shading represents laboratory geometric means as fold change from study median values (X). No shading, $X < 2$; shading from lightest to darkest, $2 < X < 4$, $4 < X < 8$, $X > 8$, respectively. Green highlighting, increased in the percentage of laboratories within the stated range of the study median.

Table 22. Geometric means of binding activity titres against pool of antigens or whole virus.

As reported by the participants

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1a	8063	81275	12800	800	3200	126	51200	819200	3200
1b	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
9c	200	1796	449	100	238	-	400	5702	119
10b	46	630	23	4	4	1	81	2763	7
15a*	0.65	1.69	0.66	Neg	0.20	-	0.76	1.85	Neg
GM	421	4513	507	68	143	13	1184	23456	139
Median	200	1796	449	100	238	13	400	5702	119
% GCV	1330.0	1190.6	2280.3	1361.4	2869.4	n/a	2773.9	2116.3	2038.6
N	3	3	3	3	3	2	3	3	3

Relative to MX-01

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1a	1000	11582	1679	120	379	NP	4524	148753	326
1b	1000	13653	1913	133	422	59	5446	190383	249
9c	1000	10638	2030	378	804	-	1724	NP	492
10b	1000	13937	NP	99	81	34	2190	64156	249
GM	1000	12374	1868	157	320	45	3106	122024	316
Median	1000	12575	1913	127	400	45	3147	148753	285
% GCV	0.0	13.9	10.2	82.2	164.2	n/a	74.3	76.9	38.0
N	4	4	3	4	4	2	4	3	4

Relative to MX-02

Lab	MX-01	MX-02	MX-03	MX-04	MX-05	MX-06	MX-07	MX-08	MX-09
	IS mpox	IS VACV	MVA#1	MVA#2	MVA#3	Neg	mpox#1	SMX	mpox#2
1a	86	1000	145	10	33	NP	391	12844	28
1b	73	1000	140	10	31	3	475	14614	28
9c	94	1000	231	38	NP	-	214	6839	59
10b	72	1000	NP	7	6	3	157	4603	18
GM	81	1000	167	13	18	3	281	8768	30
Median	80	1000	145	10	31	3	289	9372	28
% GCV	13.9	0.0	32.4	109.2	166.3	n/a	67.3	72.1	64.6
N	4	4	3	4	3	2	4	4	4

Lab 15a was excluded from the statistical calculation, due to lack of dilution series;
NP: not parallel; n/a: : participant provided raw data, not analysed value.
Highlighted grey MPXV virus (lab 10b) or pool of antigen (lab 1a); no highlighting VACV(lab 9c and 15a) or pan orthopoxvirus (Lab 1b)

Table 23. Participants' qualitative evaluation on the collaborative study sample testing

Lab	MX-01 IS mpox	MX-02 IS VACV	MX-03 MVA# 1	MX-04 MVA# 2	MX-05 MVA# 3	MX-06 Neg	MX-07 mpox-1	MX-08 SMX	MX-09 mpox-2	Target
1a	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	A27L MPXV+ 2nd gen vacci
1c	P/P/P	P/P/P	P/P/P	P/N/P	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	MPOX + 2nd gen vaccine
4	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	E8L MPXV
7	P/P/P	P/P/P	P/P/P	N/N/N	N/N/N	N/N/N	P/P/P	P/P/P	P/P/P	H3L MPXV
	P/P/P	P/P/P	N/N/N	N/N/N	N/N/N	N/N/N	P/P/P	P/P/P	N/N/N	A35R MPXV
	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	A27L VACV
8	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N	N/N/N	P/P/P	P/P/P	N/N/N	A35R MPXV
	N/N/N	P/P/P	P/P/P	N/N/N	N/N/N	N/N/N	P/P/P	P/P/P	N/N/N	A29L MPXV
	P/P/P	P/P/P	P/P/P	N/N/N	N/N/N	N/N/N	P/P/P	P/P/P	P/P/P	E8L MPXV
16	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	A35R MPXV
	P/P/P	P/P/P	N/N/N	N/N/N	N/N/N	N/N/N	P/P/P	P/P/P	P/P/P	A29L MPXV
	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	B6R MPXV
	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	E8L MPXV
	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	A33R VACV
	P/P/P	P/P/P	N/N/N	N/N/N	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	A27L VACV
	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	B5R VACV
	P/P/P	P/P/P	P/P/P	P/P/P	P/P/P	N/N/N	P/P/P	P/P/P	P/P/P	D8L VACV

Red: false positive or negative; blue: cross- reactivity

10.2 Figures

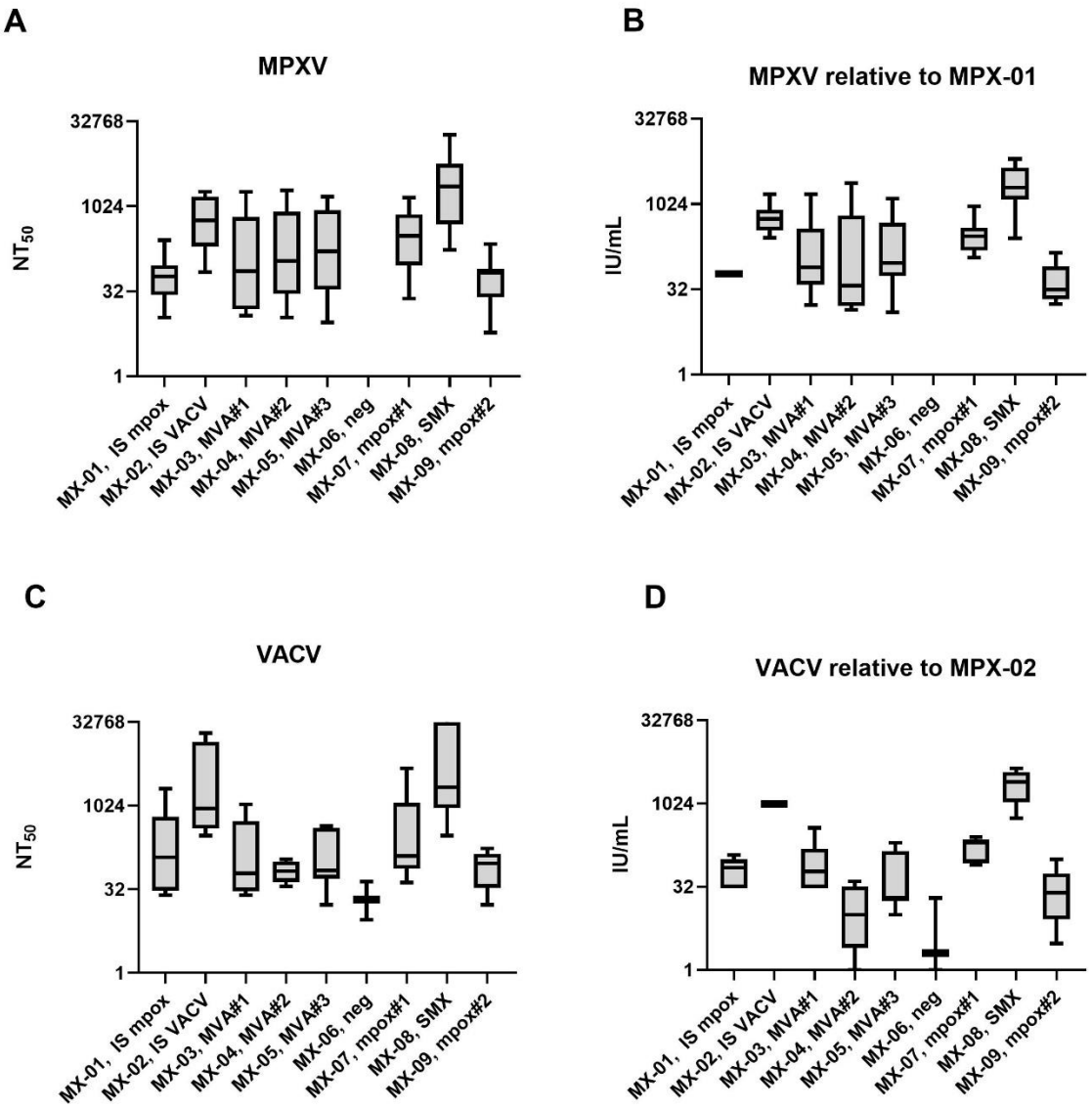

Figure 1. Harmonisation of neutralising antibody titres when reported relative to the candidate WHO International Standards.
The laboratory reported geometric mean neutralising antibody titres for each sample in NT_{50} against monkeypox virus, MPXV (**A**) and Vaccinia virus, VACV (**C**); potencies reported relative to the candidate WHO IS for mpox antibodies MX-01 in IU/mL with an assigned value of 60 IU/mL (B) or the candidate WHO IS for anti-VACV antibodies MX-02 in IU/mL with an arbitrarily assigned value of 1000 IU/mL (**D**). Data are shown as box-and-whisker plots, with the median indicated by a horizontal line and boxes representing the interquartile range (IQR). Whiskers denote the full range of values. Each box represents the distribution of results across datasets/laboratories for each sample.

11. Appendices

Appendix 1: List of participants

in alphabetic order by country, and by Institution within the same country

Participant	Organisation	Country
Sean Li, Caroline Gravel	Health Canada	Canada
Youchun Wang, Qiangming Sun	Institute of Medical Biology, Chinese Academy of Medical Science & Peking Union Medical College (IMBCAMS)	China
Cao Shouchun, Mao Qunying	National Institute for Food and Drug Control (NIFDC)	China
Olivier Ferraris, Audrey Ferrier-Rembert, H Timera, E Jospin, G Frenois-Veyrat	Institute of Biomedical Research of the Armed Forces (IRBA)	France
Filipe Colaco Mariz, Jacqueline Möbius, Darja Schmidt	IQVIA Laboratories Vaccines/ Nexelis Marburg	Germany
Alex Dulovic, Patrick Marsall, Daniel Junker, Johanna Griesbaum	NMI Natural and Medical Sciences Institute at the University of Tübingen	Germany
Gayathri Kudupudi, Raju Dugyala, Brunda Ganneru	Bharat Biotech International Limited	India
Alessandro Manenti, Giulia Roscia, Giulia Lapini, Eva Puccioni	Vismederi	Italy
Akira Ainai, Shiori Ueno, Akiko Sataka, Koji Ishii, Tadaki Suzuki	Japanese Institute of Health Security (JIHS)	Japan
You-Jin Kim, Hye-Sook Jeong, Hyeonguk Kim, June-Woo Lee, Yun-Ho Hwang, WonSeok Gwak, Min-Chul Jung, SunKyung Yoon	National Institute of Health, Korea Disease Control and Prevention Agency (KDCA)	South Korea
Jackson Sembera, Deborah Mukisa, Opio Solomon, Nambi Ruth Priscilla, Victoria Mirembe, Pontiano Kaleebu, Joseph Ssbwana Katende, Jennifer Serwanga	Uganda Virus Research Institute (UVRI)	Uganda
Mary Matheson, Holly Humphries, Lorraine Stapley, Kelly Thomas, Sue Charlton, Kerry Godwin, Kirsty Bulpitt, Katherine Raynor	Clinical Evaluation Team, Countermeasures, Development, Evaluation and Preparedness, UK Health Security Agency (UKHSA)	UK
Scott Jones, Bethany Hicks	Emerging Pathogen Serology, Countermeasures, Development, Evaluation and Preparedness UK Health Security Agency (UKHSA)	UK
Emma Summersgill	Medicines and Healthcare products Regulatory Agency	UK
Jennifer Garver, Thomas Rudge, Chris Cirimotich, Erin Altonen	Battelle	USA
Sathesh Panayampalli, Michael Townsend	Centers for Disease Control and Prevention	USA
Sanjay Sarkar, Jennifer Pickens	Southern Research	USA
McKenna Roe, Kevin Mcgill, Lucille Washington, Eric Laing	Uniformed Services University	USA

Appendix 2: Collaborative study protocol

Collaborative Study CS732
Development of WHO International Standards for Mpox antibodies and anti-Vaccinia virus antibodies

Study Background and Aims

Mpox disease was first described in humans in 1970 and is now endemic in West and Central Africa. Outbreaks have occurred in the past linked to international travel or the export of animals from endemic regions. The largest outbreak started in May 2022 leading to the WHO declaring Mpox a Public Health Emergency of International Concern (PHEIC) on 23rd July 2022. In May 2023, this first PHEIC was declared over, but a second PHEIC was declared in August 2024, associated with the rapid spread of a new virus strain, clade Ib, in the Democratic Republic of Congo and neighboring countries. Monkeypox virus is a member of the Poxviridae family, Orthopoxvirus genus which encompasses 12 species, including variola, cowpox and vaccinia virus. There are two distinct clades of the virus: clade I (with subclades Ia and Ib) mainly affecting central Africa, and clade II (with subclades IIa and IIb) predominant in West Africa with clade IIb responsible for the outbreak outside Africa in 2022. Vaccinia virus (VACV) based vaccines have been used since the late 18th century, to control and eventually eradicate smallpox, a highly contagious and often deadly disease caused by variola virus. Smallpox vaccines have been deployed to contain Mpox outbreaks as preclinical studies have shown that VACV vaccines provide a level of protective immunity and are efficacious in reducing severity of illness. There are three licensed vaccines which are based on VACV. ACAM2000 vaccine is second generation smallpox vaccine, approved in the USA and is derived from the first generation Dryvax, following selection and propagation in eukaryotic cells; IMVANEX (also licensed as JYNNEOS and IMVAMUNE) vaccine, is a highly modified Vaccinia Ankara strain produced by Bavarian Nordic which has been extensively passaged in chicken embryo fibroblast cells; in Japan, LC16 KMB vaccine is derived from the Lister strain and passaged in rabbit kidney cells. New vaccines are in preclinical phase trials including those based on a horsepox virus and mRNA technology. Availability of serological assays to compare immune responses elicited by vaccination will help evaluate current and new vaccines, support sero-epidemiology studies and understand the antibodies cross-reactivity between Orthopoxviruses.

This multi-centre International collaborative study will evaluate two candidate preparations to serve as the First WHO International Standard for Mpox antibodies and the First WHO International Standard for anti-Vaccinia virus antibodies. The study is organised by the MHRA on behalf of the World Health Organization (WHO) and is facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI).

International Standards (IS) are recognised as the highest order of reference material for biological substances and are assigned a potency in International Units (IU). They are intended to be used to calibrate assays to report the relative biological activity of samples in terms of IU. This allows for increased comparability between assays/laboratories and enables

parameters, such as analytical sensitivity of tests, or clinical measures, such as protective levels of antibody, to be better defined.

The family Poxviridae have been listed as priority family in the recently published WHO pathogens prioritisation framework with variola, vaccinia and monkeypox indicated as a priority and/or prototype pathogens [1]. The availability of International Standards for Mpox antibodies and for anti-VACV antibodies will support the development and harmonisation of methods for the quantification of **binding and neutralising** antibodies **and assay technology** as outlined in the Mpox virus Research Roadmap [2]. The study will follow published WHO guidelines [3] and be submitted for formal establishment by the WHO.

The aims of this study are to:

- Assess the suitability of the candidate to serve as a WHO International Standard and harmonise data in a range of typical assays performed in different laboratories.
- Characterise the candidate's reactivity/specificity, including cross-poxvirus reactivities, in different assay systems.
- Assess commutability, i.e. establish the extent to which the candidate is suitable to serve as a standard for a variety of different samples.
- Recommend to the WHO ECBS the suitability of the candidate to serve as a WHO International Standard and propose an assigned IU .

Study Samples

All study samples are provided coded and blinded to participants (Table 1). Each participant is provided with four sets of study samples MX-01 to MX-09 per method, which allows for three independent assays and includes a spare sample set. Participants requiring higher volumes, and therefore more sample sets will only receive one spare set.

The samples are comprised of plasma or sera of human origin which have been collected from healthy donors who were either vaccinated with a VACV-based vaccine or have recovered from mpox infection. The samples were collected with the approval of local ethics committees.

All mpox convalescent samples have tested negative for the presence of Monkeypox virus DNA using molecular PCR-based methods. Samples with indeterminate results have also been confirmatory tested via culture on permissive cells, examined for cytopathic effect (CPE).

All of the samples tested negative for HCV RNA, but some of the samples tested positive for HBsAg and HIV-1 antibodies. These samples have undergone a inactivation treatment using a validated Solvent-Detergent method to inactivate enveloped viruses and the samples are provided as non-infectious [4].

Table 1. CS732 Study Sample Panel.

All samples should be stored at -20°C or below

Sample Code	Formulation	Volume (mL)	Vial
MX-01	Freeze-dried	0.25	DIN Ampoule
MX-02	Freeze-dried	0.25	DIN Ampoule

MX-03	Liquid frozen	0.2	Screw Cap Vial
MX-04			
MX-05			
MX-06			
MX-07			
MX-08			
MX-09			

CAUTION: As with all materials of biological origin, the material should be regarded as potentially hazardous to health.

Study Protocol

As per the Instructions for Use (IFU), freeze-dried samples MPX-01 and MPX-02 require reconstitution in 0.25mL of sterile ultra-pure H_2O. Allow 10 minutes for complete dissolution and mix gently. All other samples should be thawed at room temperature prior to assay.

Participants are requested to:

- Perform three independent tests, i.e. different days or different operators.
- Use a fresh set of samples for each independent test. However, due to limited volume of each collaborative study sample, **IF a laboratory has multiple methods which require less than 100 µl of sample, the same ampoule/vial should be shared between methods**. After reconstitution or thawing the samples can be stored at 4°C for up to five days IF kept sterile.
- Prepare dilutions of the samples in the sample matrix routinely used in the test method (e.g. plasma/media/buffer). The optimal dilution range should cover at least five points and include at least one point beyond the endpoint dilution. If needed, dilutions can be adjusted for subsequent assays and recorded clearly in the results reporting sheet.
- Test samples at least in duplicate, ideally performing an independent dilution series for each replicate
- If feasible, test all samples within the same assay so that sample potencies relative to each other can be calculated. Please indicate in the results reporting sheet if samples have not been tested concurrently.

Results Reporting

An Excel reporting sheet is provided so that all essential information can be recorded, including details of test method and the raw data obtained from each assay. The use of the reporting sheet facilitates the analysis and interpretation of results.

- Within the reporting sheet, under the 'Result Summary' record the qualitative (+/-) and where applicable quantitative (endpoint titre/IC50 etc.) result for each sample as per analysis in your laboratory. It is important to know whether the samples are considered positive, as per analysis in your laboratory.
- Under the 'Raw data' section of the reporting sheet record the raw assay readout for each dilution tested (e.g. absorbance OD, plaques, foci, etc.). Ensure that the dilutions tested are correctly recorded.

- Include the assay cut-off value indicating sero-reactivity for each assay. Our statistician will use the raw data readouts to perform statistical analysis.
- Where multiple methods have been used, complete one reporting sheet per method or add a new tab to the template excel file.
- Record in the Excel reporting sheet any deviations from the study protocol and complete all fields requesting additional method details.

Deadline for the return of results is 28th February 2026

All completed results spreadsheets should be returned electronically to:
Collaborative study organiser: Giada Mattiuzzo Giada.Mattiuzzo@mhra.gov.uk

Data Analysis

Analysis of the study data will assess the potencies of each sample relative to each other, and their performance within the different assay methods. The anonymity of each laboratory is maintained by reporting datasets with a blind code, details of the assay performed will be included.

A draft study report will be sent to participants for comment. The report will include data analysis, proposed conclusions and recommendations on the selection, use and unitage of the candidate WHO IS for mpox antibodies and WHO IS for anti-VACV antibodies. Participants' comments will be included in the report prior to submission to the WHO in July 2026. Study participants will be notified of the outcome of the study after the WHO ECBS meeting due to be held in October 2026.

References

[1] Pathogens prioritization: a scientific framework for epidemic and pandemic research preparedness

[2] a-coordinated-research-roadmap-monkeypox-virus-immediate-research-next-steps-to-contribute-to-control-the-outbreak.pdf

[3] Recommendations for the preparation, characterization and establishment of international and other biological reference standards, Annex 2, TRS No 932

[4] Dichtelmüller HO, *et al.*. Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion. 2009 Sep;49(9):1931-43. doi: 10.1111/j.1537-2995.2009.02222.x. PMID: 19497061.

As outlined in the study questionnaire, participation in the collaborative study is conducted under the following WHO Collaborative Study Terms and Conditions:

- The study samples have been prepared from Materials provided by donors and therefore must be treated as proprietary;
- The Materials provided must not be shared with anyone outside of the study;
- The Materials must not be used for application in human subjects or animals in the human food chain in any manner or form;
- There must be no attempt to reverse engineer, ascertain the chemical structure of, modify, or make derivatives of, any of the Materials;

- Participants accept responsibility for safe handling and disposal of the materials provided in according to the local regulations in their organisation/country.
- Data obtained through testing of the Materials must not be published or cited before the formal establishment of the standard by World Health Organization, and without the express permission of the MHRA study organiser.

The MHRA, as the Collaborative Study coordinator, notes that:

- It is normal practice to acknowledge all participants as contributors of data rather than co-authors in publications;
- Data published from participating labs will be anonymised;
- Participation in this study is at your discretion and does not include remuneration costs;
- Prior to the establishment of the standard, the MHRA reserves the right to disclose specific information about the use of the Material(s), without acknowledgement of the study participants;
- Participants will receive a copy of the report of the study with proposed conclusions and recommendations for comment before it is further distributed.

Appendix 3: Proposed Instruction for Use

WHO International Standard
First International Standard for antibodies to vaccinia virus
for neutralisation assay
NIBSC code: 05/124_NT
Instructions for use

1. INTENDED USE

The First WHO International Standard for antibodies to vaccinia virus is a freeze-dried preparation of pooled plasma defibrinated plasma collected from volunteers that have been immunised with the NYCBH strain of vaccinia virus (1). The preparation has been evaluated in a WHO International collaborative study (2). The intended use of the International Standard is for the calibration and harmonisation of neutralisation assays against vaccinia virus (VACV).

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg , anti-HIV1/2 and HCV RNA.

This preparation is not for administration to humans or animals. As for all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for neutralising antibodies against VACV is 250 IU/ampoule. These values have been arbitrarily chosen and do not reflect the proportion of the antibody in the preparation. After reconstitution of the lyophilised cake in 0.25 mL of distilled water or other matrix, the final concentration will be 1000 IU/mL.

International Units are arbitrary assigned to a WHO International Standard to express the biological activity of the standard, and as such there is no conversion factor between IU and mass (e.g. ng of antibody).

4. CONTENTS

Country of origin of biological material: Canada.

Each ampoule contains the freeze-dried equivalent of a mL of pooled human plasma.

5. STORAGE

Ampoules should be stored at -20°C or below until use.

Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an ‘easy-open’ coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

The contents of each ampoule should be reconstituted in **0.25mL distilled water**. Following addition of the distilled water, the material must be allowed to become fully reconstituted before use. End-users have found that it may take > 30 mins with agitation for the material to become fully reconstituted. Gentle pipetting is recommended.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

It is the policy of WHO not to assign an expiry date to International Standards. They remain valid with the assigned potency and status until withdrawn or amended. Please note that the stability of International Standard when reconstituted has not been specifically determined. Therefore, it is recommended that the reconstituted material is for single use only. Should users wish to store reconstituted material, they should determine the stability of reconstituted material according to their own method of preparation, storage and use

9. REFERENCES

(1) Bentley M, Christian P, Hamill M and Heath A. Report on a Collaborative Study to Assess the Suitability of a First International Standard for Anti-Vaccinia plasma, Human. 2010. WHO/BS/10.2134

(2) Summersgill et al Development of WHO International Standards for antibodies to vaccinia virus. WHO/BS/2026.2515

10. ACKNOWLEDGEMENTS

We would like to wholeheartedly thank the anonymous donors of the serum and plasma samples for their consent which has allowed this study to be undertaken. We gratefully acknowledge the important contributions of the collaborative study participants and the clinical study teams who collected the samples.

The project has been funded by the Coalition for Epidemic Preparedness Innovations

11. FURTHER INFORMATION

Further information can be obtained as follows:

This material: enquiries@nibsc.org

WHO Biological Standards: http://www.who.int/biologicals/en/

JCTLM Higher order reference materials: http://www.bipm.org/en/committees/jc/jctlm/

Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx

Ordering standards from NIBSC: http://www.nibsc.org/products/ordering.aspx

NIBSC Terms & Conditions: http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze dried		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling:	See caution, Section 2
Other (specify):	human origin		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation:	Seek medical advice		
Ingestion:Seek medical advice			
Contact with eyes: Wash with copious amounts of water. Seek medical advice			
Contact with skin: Wash thoroughly with water.			
Action on Spillage and Method of Disposal			
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.			

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 1 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached: Not Applicable**

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

Appendix 4: Proposed Instruction for Use

WHO International Standard
First International Standard for antibodies to vaccinia virus
for binding antibodies assay
NIBSC code: 05/124_BA
Instructions for use

1. INTENDED USE

The First WHO International Standard for antibodies to vaccinia virus is a freeze-dried preparation of pooled plasma defibrinated plasma collected from volunteers that have been immunised with the NYCBH strain of vaccinia virus (1). The preparation has been evaluated in a WHO International collaborative study (2). The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting binding antibodies (IgG) against vaccinia virus (VACV) antigens.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg , anti-HIV1/2 and HCV RNA.
This preparation is not for administration to humans or animals. As for all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for binding antibodies against VACV is 250 IU/ampoule. These values have been arbitrarily chosen and do not reflect the proportion of the antibody in the preparation. After reconstitution of the lyophilised cake in 0.25 mL of distilled water or other matrix, the final concentration will be 1000 IU/mL.

International Units are arbitrary assigned to a WHO International Standard to express the biological activity of the standard, and as such there is no conversion factor between IU and mass (e.g. ng of antibody). Harmonisation of the binding antibody titres against several antigens was demonstrated in the collaborative study (2). The same numeric value should use to report results for each antigen. The standard is not to be used to compare responses to different antigens, but to compare results against the same VACV antigen.

4. CONTENTS

Country of origin of biological material: Canada.

Each ampoule contains the freeze-dried equivalent of a mL of pooled human plasma.

5. STORAGE

Ampoules should be stored at -20°C or below until use.

Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

11. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

12. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

The contents of each ampoule should be reconstituted in **0.25mL distilled water**. Following addition of the distilled water, the material must be allowed to become fully reconstituted before use. End-users have found that it may take > 30 mins with agitation for the material to become fully reconstitued. Gentle pipetting is recommended.

13. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

It is the policy of WHO not to assign an expiry date to International Standards. They remain valid with the assigned potency and status until withdrawn or amended. Please note that the stability of International Standard when reconstituted has not been specifically determined. Therefore, it is recommended that the reconstituted material is for single use only. Should users wish to store reconstituted material, they should determine the stability of reconstituted material according to their own method of preparation, storage and use

14. REFERENCES

(1) Bentley M, Christian P, Hamill M and Heath A. Report on a Collaborative Study to Assess the Suitability of a First International Standard for Anti-Vaccinia plasma, Human. 2010. WHO/BS/10.2134

(2) Summersgill et al Development of WHO International Standards for antibodies to vaccinia virus. WHO/BS/2026.2515

15. ACKNOWLEDGEMENTS

We would like to wholeheartedly thank the anonymous donors of the serum and plasma samples for their consent which has allowed this study to be undertaken. We gratefully acknowledge the important contributions of the collaborative study participants and the clinical study teams who collected the samples.
The project has been funded by the Coalition for Epidemic Preparedness Innovations

11. FURTHER INFORMATION

Further information can be obtained as follows:

This material: enquiries@nibsc.org
WHO Biological Standards: http://www.who.int/biologicals/en/
JCTLM Higher order reference materials: http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC: http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions: http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze dried		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling:	See caution, Section 2
Other (specify): human origin			
Toxicological properties			
Effects of inhalation: Not established, avoid inhalation			
Effects of ingestion: Not established, avoid ingestion			
Effects of skin absorption: Not established, avoid contact with skin			
Suggested First Aid			
Inhalation: Seek medical advice			
Ingestion:Seek medical advice			
Contact with eyes: Wash with copious amounts of water. Seek medical advice			
Contact with skin: Wash thoroughly with water.			
Action on Spillage and Method of Disposal			
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.			

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

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16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 1 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached: Not Applicable**

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev200

4.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.