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Collaborative Study to Evaluate the Proposed First WHO International Standard for Sudan Virus Antibodies (Human Serum).

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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 **19 September 2025** and should be addressed to:

Department of Health Products Policy and Standards
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Comments may also 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

Sudan virus disease (SVD) is a severe, often fatal, zoonotic illness caused by Sudan virus (SUDV), a filovirus discovered in South Sudan in 1976. A total of nine outbreaks of SVD have been reported in South Sudan and Uganda, the most recent one occurring in 2025 in Uganda. There is currently no approved antiviral treatment to protect against SVD, but promising combinations of monoclonal antibodies have been shown to protect against a lethal challenge in animal models, and were used therapeutically during the 2022 outbreak in Uganda. Similarly, there are currently no licenced vaccines available against SVD, but many candidates have been in development and four vector based vaccines have completed phase 1 clinical trials. To evaluate the immune response elicited by vaccinations and determine still elusive correlates of protection against SVD, a variety of immunological assays have been developed, including serological methods measuring binding and neutralising antibodies. In order to facilitate the comparison of results obtained from these assays, an International Standard (IS) for anti-SUDV antibodies is required. The present report describes a multicentre collaborative study to evaluate the suitability of a candidate first WHO IS for anti-SUDV antibodies (24/124). The candidate WHO IS, a pool of 14 sera from survivors of SUDV infection during the 2000 and 2012 outbreaks in Uganda, was evaluated alongside convalescent sera by 23 laboratories including vaccine manufacturers, academic institutions and public organisations. Statistical analysis of 36 data sets generated by binding and neutralising assays showed that the WHO IS candidate could harmonise, to varying degrees, the data generated by both binding and neutralisation assays, across and within laboratories. It is therefore proposed that 24/124 be established as the first WHO IS for anti-SUDV antibodies for binding assays and the first WHO IS for anti-SUDV antibodies for neutralisation assays, with an assigned unitage of 250 International Units/ampoule for anti-glycoprotein IgG and 250 IU/ampoule for neutralising activity, respectively. In addition, we showed that harmonisation of anti-SUDV antibody quantifications could also be achieved when results are reported relative to samples composed of a combination of binding and neutralising monoclonal antibodies against SUDV glycoprotein. With the challenges associated with sourcing large volumes of high titre human sera from convalescent individuals in order to produce reference reagent for emerging pathogens, a carefully designed cocktail of monoclonal antibodies might prove a suitable alternative as a primary calibrant.

Introduction

Sudan Virus (SUDV) is a negative sense single stranded RNA virus which belongs to the *Filoviridae* family, species *Orthoebolavirus sudanense* [1]. SUDV is the causative agent of Sudan Virus Disease (SVD), a severe disease with a high case fatality rate (CFR) ranging from 29% to 100% (median 50%) [2][3], and clinical manifestations similar to illnesses caused by other filoviruses, characterised by multiple gastrointestinal, respiratory, vascular and neurological disorders, and haemorrhagic fever [4].

SVD is a zoonotic disease which circulation in animals is poorly understood. The natural reservoir has not been identified despite suggestions that SUDV may have originated in the fruit bat of the *Pteropodidae* family [5]. Spill over to humans is thought to occur through direct contact with infected sick or deceased wild or potentially domestic animals [6], while secondary human-to-human transmission occurs through direct contact with infected blood or bodily fluids or indirectly through contaminated surfaces or fabrics [4].

The first outbreak of SUDV occurred in 1976 in Sudan (now South Sudan), where a total of 284 cases and 151 deaths (53% CFR) were reported between June and November [7]. Since 1976, SUDV has reemerged sporadically in East Africa, causing two additional outbreaks in South Sudan and six outbreaks in Uganda, including the most recent one in 2025, where 14 confirmed cases and four deaths were reported across six districts [8].

There are currently no approved antiviral therapies to prevent or treat SVD in humans, and patient management mainly relies on supportive care. Nevertheless, several monoclonal antibody (mAb) cocktails have shown effective protection against lethal challenge in guinea pigs, ferrets, and non-human primates (NHP) [9][10][11][12], and one antibody cocktail, $MBP134^{AF}$, has been used in combination with Remdesivir in clinical trials during the 2022 SUDV outbreak in Uganda [13]. Similarly, there are currently no licensed vaccines against SVD, but multiple candidates have been evaluated at preclinical stages [14] and four vaccines based on adenovirus or vesicular stomatitis virus (VSV) vectors have progressed to early clinical trials [15][16][17][18][19], among which three were used in ring vaccination trials in Uganda to combat the 2022 outbreak [20].

To support the development of SUDV vaccine candidates, a number of serological immune assays have been developed. An International Standard (IS) for anti-SUDV antibodies is therefore required to harmonise results generated by these assays in order to facilitate comparison across different test facilities and vaccine candidates. The WHO IS are the highest order of reference reagent for biological substances, with assigned potencies in International Units (IU). Quantifications of biological activity from assays calibrated against the IS are reported in IU, allowing better comparability of data obtained by different laboratories using different assays. In November 2018, the WHO Expert Committee on Biological Standardization (ECBS) endorsed the preparation of an IS for anti-SUDV antibodies.

The candidate IS is a pool of sera obtained from survivors of the 2000 and 2012 SUDV outbreaks in Uganda. Samples were collected through a collaboration between the Uganda

Virus Research Institute (UVRI) and Integrum Scientific and was supported by the Coalition for Epidemic Preparedness Innovations (CEPI). The formulation and evaluation of the candidate IS through a multi-centre collaborative study was conducted on behalf of the WHO in partnership with Battelle and sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) Division of Microbiology and Infectious Diseases (DMID).

The main objectives of this WHO International collaborative study were to:

- Assess the suitability of the candidate WHO IS for anti-SUDV antibodies in serological assays.
- Recommend to the WHO ECBS the antibody preparation found to be suitable to serve as the IS and demonstrate it is able to reduce quantification variability across assays and laboratories.
- Propose an assigned unit for the proposed WHO IS for anti-SUDV antibodies, for both binding and neutralising activities.
- Evaluate four preparations of monoclonal antibodies for their suitability to act as a primary calibrant.

Materials and Methods

Ethics Statement

The sera from 28 healthy individuals who survived SUDV infection in 2000 or 2012 in Uganda were collected under informed consent by UVRI in partnership with Integrum Scientific. This collection was approved by the UVRI Research Ethics Committee (reference GC/127/351).

Candidate First WHO IS

The candidate first WHO IS for anti-SUDV antibodies (NIBSC code 24/124) is a freeze-dried preparation of a pool of sera from individuals who survived SUDV infection during the 2000 or 2012 outbreaks in Uganda [21][22]. All 28 serum samples collected by UVRI were tested and confirmed negative for SUDV RNA by RT-qPCR using an in-house assay based on a protocol developed by the US Centers for Disease Control and Prevention [23]. All samples were subsequently tested at UVRI for HBsAg, HCV Ab, HIV Ag and HIV Ab, using the Elecsys HBsAg II, Elecsys Anti-HCV II, and Elecsys HIV Combi PT kits, respectively (all assays performed using the Roche Cobas 6000 platform). Eighteen samples were found negative for all viral markers tested and 10 samples were found positive for HIV Ag/Ab and/or HBsAg. All samples were found negative for HCV Ab. All sera were subsequently treated with solvent and detergent at the Science Campus of the MHRA to minimise the risk of the presence of infectious enveloped viruses and filtered through a 0.2 µm cellulose acetate filter. This solvent/detergent treatment has been shown to effectively inactivate various enveloped viruses in plasma, including HIV, HBV and HCV [24][25], and has been validated at the MHRA for HIV (Appendix 2). In addition, no cytotoxicity has been observed for samples treated by solvent and detergent [25]. The 18 sera found negative for blood borne virus markers were tested in parallel at the Science Campus of the MHRA and Battelle for anti-SUDV glycoprotein (GP) Immunoglobulin G (IgG) and neutralising activity using enzyme-linked immunosorbent

assay (ELISA), and VSV-based pseudotyped virus (PV) neutralisation assay, respectively. Among the sera found negative for blood borne viruses, the 14 sera with the highest binding and neutralising activities were pooled for a total of 524 mL and the bulk preparation was confirmed negative for blood borne viruses markers (HBsAg, HCV RNA and HIV Ag/Ab).

Filling and Lyophilization of the Candidate WHO IS (24/124)

The material was aliquoted by the manufacturing team at the MHRA using the AVF5090 filling line (Bausch & Stroebel, Ilshofen, Germany). The homogeneity of the fill was maintained by on-line check-weighing of a proportion of the filled ampoules. Filled ampoules were partially stoppered with halobutyl 13 mm diameter igloo closures and lyophilised in a CS100 freezer drier. Ampoules were loaded onto the shelves at 4°C and primary freezing was performed to -50°C over 4 hours. Primary drying was performed at -35°C for 30 hours at 100 µbar vacuum followed by a ramp to 25°C over 10 hours, then 30 hours secondary drying at 25°C and 30 µbar vacuum. Vials were back filled with dry nitrogen to atmospheric pressure and flame sealed. The sealed vials were stored at -20°C under continuous temperature monitoring. Assessments of residual moisture and oxygen content, as indicators of freeze-drying completion and vial integrity after sealing, were determined for 12 vials of freeze-dried product. Residual moisture was measured destructively using colorimetric Karl Fischer (CA-200, Mitsubishi Instruments obtained through A1-Environsciences Ltd, Blyth, UK) operated within a dry box and checking performance before analysis using an Aquamicron Check P water standard (A1 Environsciences) to give % w/w moisture readings. Oxygen content was measured non-invasively by frequency modulated infra-red spectroscopy using an FMS-760 Oxygen Headspace Analyzer (Lighthouse Instruments, Charlottesville, VA, USA).

Study Sample Panel

The study sample panel to be evaluated during the collaborative study was composed of 14 samples:

- The candidate WHO IS (24/124), lyophilised
- The bulk candidate WHO IS, liquid form
- Seven sera, each from an individual who survived the 2000 outbreak of SUDV in Uganda, pre-selected to cover a range of titres.
- A serum negative for anti-SUDV antibodies, produced by defibrination of a plasma sample obtained from a healthy blood donor from the UK.
- Four samples composed of one or multiple mAbs spiked in negative serum for anti-SUDV antibodies.

Table 1 lists the collaborative study samples provided coded and blinded to the participants and Table 2 summarises the composition of the four mAb preparations. A minimum of three panels were shipped on dry ice to each participant by our logistics team, starting in February 2024. Participant 2 did not receive the four mAb samples because of restrictions with the Material Transfer Agreement.

Participants

Twenty-seven laboratories agreed to participate in the study and were provided with the study sample panels except two laboratories which withdrew before the samples were shipped. Twenty-three laboratories provided results and were located in seven countries: China (1), Germany (4), Italy (2), Japan (1), United Kingdom (6), Uganda (2) and USA (7) in four WHO regions (Appendix 1). 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, public institutions, and academic laboratories.

Study Design

The study protocol is provided in Appendix 2. Participants were requested to test the study samples using their method(s) for the detection of antibodies against SUDV. It was requested that three independent assays to be performed, using a fresh set of samples for each assay, and preparing serial dilutions of the samples to be tested, at least in duplicate. A results-reporting sheet was provided for participants to record all essential information including the raw data from each assay. Participants were asked to return results within 10 weeks of receipt of the materials.

Assay Methods

The participants were recruited based on their capacity to perform assays detecting anti-SUDV binding and/or neutralising antibodies. The assays used by the participants are summarized in Table 3. Where laboratories performed multiple assays, had multiple targets, performed independent testing by several operators, or reported the data in different units, the laboratory code is followed by a letter indicating the different data set (*e.g.*, laboratory 5a, 5b, 5c). A total of 36 assays were used to evaluate the samples, detecting anti-SUDV binding or neutralising antibodies by 29 quantitative methods and seven qualitative or semi-quantitative methods.

Statistical Methods

For the neutralisation assays, the potency of each sample relative to the candidate standard (SU-10) was calculated as the ratio of the neutralisation titres of the sample to the candidate IS, based on the results provided by the participants. Quantitative ELISA data were analysed using a sigmoidal curve model or parallel line analysis with Log transformed responses. Calculations were performed using Combistats™ software [26]. Model fit was assessed visually, and non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration. The samples were concluded to be non-parallel when the slope ratio was outside of the range 0.70-1.43. It should be noted that this range was intended for use in the analysis of data from this study only, in order to apply consistent criteria to all laboratories and asses their relative performance. It should not be interpreted as suitable for routine use in the assessment of assay validity and may be overly stringent or lenient in the case of some laboratories. Relative potency estimates from all valid assays were combined to generate an unweighted geometric mean (GM) for each laboratory and assay type, with these laboratory means being used to calculate overall unweighted GM and median values. Variability between laboratories has been expressed using the geometric coefficient of

variation (GCV = [10^s-1] x 100% where s is the standard deviation of the Log_{10} transformed estimates). Grubbs' test was applied to the log transformed laboratory GM estimates in order to identify any outliers in the results obtained for each of the study samples [27].

Stability Testing of the Lyophilized Candidate WHO IS (24/124)

Stability of the lyophilised candidate WHO IS was evaluated in an accelerated thermal degradation study where samples were stored at elevated temperatures. Fifteen ampoules of 24/124 were stored at each of the following temperatures -20°C, +4°C, +20°C, +37°C, +45°C. Three ampoules for each temperature were retrieved at the following time points: two weeks, one month, three months, six months and 12 months. The activity of 24/124 was assessed at the Science Campus of the MHRA by an in-house ELISA and PV neutralisation assay. For the ELISA, recombinant SUDV GP (Boniface) obtained from the Native Antigen Company (REC32011-100) was first coated in sodium carbonate-bicarbonate buffer (pH 9.6) onto flat-bottom 96-well MaxiSorp plates overnight at 4°C, then blocked in PBS, 5% skimmed milk (w/v), 0.05% Tween20 at 37°C for 1 hour, to minimize non-specific binding of the samples. Serially diluted serum samples, assay calibrant, and negative control were then added to the plates in duplicate and allowed to bind to the coated antigen for 2 hours at 37°C on a shaking incubator. Plates were washed and binding antibodies were detected by a HorseRadish Peroxidase (HRP)-conjugated goat anti-human antibody, followed by the addition of TetraMethylBenzidine (TMB) substrate. After the addition of Stop Solution (2N sulfuric acid), plates were read at 450 nm on a BMG LabTech Omega plate reader. ODs were then plotted using Combistats™ and the potency of the samples against the -20°C baseline was calculated. For the neutralisation assay, the method used a recombinant VSV in which the GP gene had been replaced with a firefly luciferase gene (ΔG-luciferase(luc) rVSV). The ΔG-luciferase(luc) rVSV system was purchased from Kerafast and used together with a pCAGGS plasmid expressing the SUDV Boniface GP. Briefly, 200 TCID50/well of previously titrated PV was added to serially diluted test samples, incubated for 1 hour at 37°C, and the mixture was added to a confluent monolayer of Vero ATCC cells (ATCC CCL-81) previously seeded in a 96-well plate. Luminescence from luciferase activity was measured 24 hours post-inoculation using the Glomax Navigator Microplate Luminometer Promega using the BrightGlo™ Luciferase Assay System (Promega #E2620). To determine the 50% neutralisation titre (NT50), infectivity normalized data were plotted against Log10 transformed sample dilutions factors, using GraphPad Prism (v10.4.2), and a 4-parameter non-linear regression was performed. The NT50 was calculated by GraphPad Prism. The prediction of long-term stability of the candidate WHO IS was assessed using the Arrhenius model for accelerated degradation studies [28].

Results and Data Analysis

Production of the Candidate WHO IS (24/124)

Serum from 28 SUDV survivors were received from UVRI, solvent/detergent treated [24][25], and sterilised by filtration. The 18 sera found negative for blood borne virus markers (see M&M section) were assessed in parallel at the MHRA and Battelle for binding and neutralising antibodies using an anti-SUDV GP human IgG ELISA (Boniface or Gulu antigen, respectively) and VSV-based PV neutralisation assays (Boniface GP). Detection of anti-SUDV NP

antibodies was not performed. Fourteen sera with the highest titres were selected and pooled, resulting in 524mL of the candidate IS which was aliquoted on 31st May 2024 and freeze-dried over the following 4 days. A total of 1739 glass DIN ampoules (2.5 mL in size) were filled with approximately 0.25 mL (mean mass 0.27g) of the candidate IS. The % CV of the mass per ampoule was 1.02, the mean % residual moisture was 0.35 and the mean % oxygen head space was 0.36; all aligned with target values recommended by the WHO [29]. The product summary for the candidate IS (24/124) is shown in Table 4.

The appearance of the lyophilized material was as expected, with the formation of characteristic "cake". Reconstitution of the product at room temperature in 250 µL of water was quick with no aggregates forming, indicative of adequate solubility. No microbiological contamination was detected in 24/124.

Stability Study of the Candidate WHO IS (24/124)

Stability of the candidate IS was evaluated at the Science Campus of the MHRA by an accelerated thermal degradation study (see M&M section). Ampoules of the candidate WHO IS, NIBSC code 24/124 were stored at different temperatures, -20°C (baseline), +4°C, +20°C, +37°C and +45°C for two weeks, one month, three months, six months and 12 months. The freeze-dried preparations retrieved were reconstituted as per instructions for use (Appendix 2) and tested concurrently by anti-SUDV GP IgG ELISA and VSV-PV neutralisation assay. All assays were performed two independent times as described in the Materials and Methods section. Real time data on the degradation samples are reported as titration curves and showed no evidence of binding potency loss up to 12 months at all temperatures (Figure 1A). For neutralising activity, a slight increase in potency was generally observed at +4°C, +20°C, +37°C and a reduction of potency after six months at +45°C and 12 months at +37°C and +45°C (Figure 1B). However, these results suggest that shipment of the candidate IS at ambient temperature would be suitable as the increase of activity after two weeks and one month at +20°C is only a modest 17% and 25%, respectively. The long-term stability of the candidate WHO IS could not be estimated as the data did not fit the Arrhenius model. Nevertheless, the results obtained suggested that the preparation 24/124 is adequately stable to serve as WHO IS for anti-SUDV antibodies.

Collaborative Study Data Received

The collaborative study under the MHRA reference CS742 started Monday 10th February 2025 with the shipment of the first samples to the participants by a dedicated logistics team at the MHRA. The first set of results were received on 8th April 2025. Twenty-three participants returned 36 data sets using 29 quantitative methods and seven qualitative or semi-quantitative methods (Table 3).

Evaluation of the panel for binding antibodies was performed by 20 assays, including 14 ELISAs and six immunofluorescence assays (IFA). ELISAs detected human IgG against either GP (7a, 7b, 7c, 11b, 12a, 12b, 13a, 14, 16, 20a, 21a), NP (27a, 27b) or whole virus (WV) (4). Assays 27a and 27b are commercially available ELISA kits intended for research use only and detecting human IgG binding to two different regions of NP. Assay 7 is commercially available and designed to quantify human IgG against SUDV GP. Participant 16 and 27 highlighted that

their assay was at a very early stage of development and additional optimisation and validation were being arranged. Quantification from ELISAs was reported in ELISA unit (EU), endpoint dilution or antibody activity unit (AAU). Five IFAs assessed human IgG against either GP (5b, 24), WV (25, 26) and NP recombinantly expressed in HeLa cells (10b). IFA 5a was the only assay in our study detecting human IgM; the samples were treated with sorbent to deplete IgG and incubate on a confluent layer of VeroE6 cell line infected with replicative VSV expressing the Gulu GP. Six binding assays used the Boniface strain, and 13 assays used the Gulu strain, with one assay detecting NP of the Yambuku Ebola Virus (EBOV) strain.

Evaluation of the panel for neutralising antibodies was performed by 16 assays, using either lentivirus (LV)-based (3, 9, 20b, 21b) or VSV-based (5c, 7d, 11a, 13b, 13c, 22) PV, viral like particles (VLP) (8), or authentic live SUDV performed in a containment level 4 facility (1, 2, 6, 10a, 18). Ten assays used the Boniface strains, five assays the Gulu strain and one assay the EboSud-602 strain. Neutralising activities were reported in 50% (NT_{50}), 80% (NT_{80}), or 100% (NT_{100}) inhibition of infectivity.

Binding Antibody Assays

The GM of the binding antibodies titres as reported by the participants are summarised in Table 5 and Figure 2A. The results are divided based on the Immunoglobulin (Ig) class detected (IgG or IgM), and the type of antigen (GP, NP or whole virus). The only assay in our study detecting IgM (5a), was qualitative and could only detect activity at the assay limit of detection (LOD) in four samples, including the candidate IS (SU-10). For IgG, the candidate IS (SU-10) was detected by all assays measuring anti-GP antibodies, whether the target antigen was purified recombinant protein (ELISAs), or expressed at the surface of the MDCK SIAT-1 cell line (24). All three assays using WV in infected cells as target antigen could also measure binding activity in SU-10. Among assays detecting anti-NP IgG, only one, 27b, which specifically detects IgG against the very C-terminal section of NP (aa641-738) did not detect binding activity in SU-10.

The GM binding titre for SU-10 was 215 (arbitrary unit, a.u.) with a median of 273. The variability of quantification across assays for SU-10, as expressed in % Geometric Coefficient of Variation (%GCV), was 364. The negative control (SU-01), a serum sourced from a blood donor in the United Kingdom, was found positive in three assays (7c, 12a and 12b). The three samples with the highest activity were SU-11, composed of a single mAb spiked in negative serum, and SU-12 and SU-14, composed of a combination of three or five mAbs, respectively. Interestingly, the four samples containing mAbs have shown an atypical staining in assay 26, restricted to the plasma membrane, where GP is likely located. This differed from the usual pattern observed with sera from convalescent patient, characterised by granule-like structures within the cytoplasm, probably reflecting binding of antibodies to additional SUDV antigens, such as NP. Among samples from individual SUDV survivors, the highest activity was found in SU-08 (GM=353) and the lowest in SU-03 (GM=99), consistent with preliminary testing of the sera at the MHRA (data not shown). The %GCV of binding activity quantification was high and varied from 179 (SU-01) to 784 (SU-07) with a median of 465.

Inter-assay variability within laboratories was evaluated by calculating the ratio of the highest and lowest reported binding antibody quantification for each sample (Table 9), across the three independent repeats performed. Overall low variability was observed with 80% of ratios below or equal to 2.0.

Binding Antibody Titres Expressed as Relative to the Candidate WHO IS (24/124)

To determine whether the expression of the binding assays results relative to the candidate IS could decrease the inter-laboratory variability of the quantification of each study sample, SU-10 was assigned an arbitrary unit of 1000 IU/mL and the quantification of binding antibody titres expressed relative to SU-10, for applicable assays (Table 6, Figure 2B). Four assays were excluded for this analysis. First, assays 10b and 25, two qualitative assays, did not reach an endpoint after dilution of the samples and consequently the reported titres of several sera are only a minimum value. Second, assays 27a and 27b are diagnostic ELISAs and report an output, based on the OD for a sample dilution of 1/100, as positive, negative, or equivocal.

Harmonisation of the results was demonstrated by a reduction of inter assay %GCV when results were reported relative to SU-10, with the exception of SU-04, for which a modest 1.4-fold increase of quantification variability was observed. Fold reduction of %GCV varied depending on the samples, with strong decrease (>=3.0-fold reduction) observed for 11 samples and a smaller 2.5 and 1.4-fold reduction observed for SU-03 and SU-08, respectively, with a median fold reduction of 3.6. After exclusion of only four outlier data points, as identified by Grubbs' test, a further increase in harmonisation was observed for SU-05, SU-06, SU-12 and SU-13, reaching 11.5, 21.4, 6.7 and 8.0-fold reduction of the %GCV, respectively. Similarly, when selecting assays specifically detecting anti-GP antibodies (exclusion of assays 4 and 26), the inter-assay variability further diminishes (median fold reduction of %GCV of 4.5) (Table 8).

In total, 79% of laboratory GM estimate of the sera from convalescent patients (SU-02 to SU-10) were within a two-fold range of the study median values calculated for each sample, further supporting the harmonisation achieved by expressing potencies relative to the candidate IS.

Assay-to-assay variability within laboratories as measured by the max/min ratio across the three independent repeats, was also reduced or remained unchanged for 11 assays out of 16, when quantifications were reported relative to SU-10 (Table 9B). For applicable quantitative binding assays, slope ratios from the parallel-line analysis are shown in Figure 4. Acceptable parallelism was observed with most slope ratios (90%) in the range 0.70-1.43.

Binding Antibody Titres Expressed as Relative to Samples Composed of One or Multiple Monoclonal Antibodies

The binding potency of the panel members was also calculated relative to each of the four samples composed of a single or multiple anti-SUDV mAbs spiked in negative human serum (SU-11, SU-12, SU-13 and SU-14) (Table 7, Figure 2C-F). Similarly to the candidate IS, harmonisation of quantification obtained for the human sera (SU-01 to SU-10) was demonstrated (Table 7B, Table 8, Figure 2B-F), and varies depending on the samples, with reduction of %GCV better achieved for samples SU-05, SU-06 and SU-09. Overall, when all

applicable binding assays were considered, SU-12 and SU-13, composed of the same three mAbs but at different concentrations, were the most efficient at harmonising data (identical median fold reduction of %GCV of 4.5, after exclusion of outliers). SU-13 and SU-14 were the best candidates to reduce variability when anti-GP assays only were selected (median fold reduction of %GCV of 4.4 and 4.9, respectively).

Neutralising Antibody Assays

The GM of the neutralising antibody titres as reported by the participants are summarised in Table 10 and Figure 3A. The results are divided based on the type of assay performed, using authentic live virus, PV, or VLP.

All assays detected neutralising activity in the candidate IS (SU-10) except assay 8, using VLP. For this assay, the positivity threshold is set by the manufacturer at NT_{50}>170, and while SU-10 did not reach this potency and was found negative, laboratory 8 reported a value between the positivity threshold and the reciprocal of the lowest dilution tested (1/20), suggesting that a low neutralising activity was detected. The GM neutralising potency of SU-10 was 98 (a.u.) with the five assays using authentic virus detecting low activity, ranging from 10 to 46 NT_{50} and the assays using PV reporting higher potency with a median of 263 (a.u.). The %GCV of quantified neutralising activity across all assays was 400 for SU-10 (Table 12). The samples with the highest neutralising activity were SU-11, SU-12 and SU-14 with GM titres of 237, 223 and 245, respectively. Amongst the samples obtained from convalescent individuals, SU-05 and SU-06 were the most potent with a GM of 143 and 149 (a.u.), respectively. The variability of neutralising activity quantification across all assays was high, and depended on the samples, ranging from a %GCV of 142 (SU-02) to 719 (SU-05). Two assays (20b, 21b) detected low level of neutralising activity in the negative control (SU-01).

Inter-assay variability of results within laboratories was evaluated by calculating the ratio of the highest and lowest reported neutralising antibody quantification for each sample, across the three independent repeats performed (Table 13A). Fifty-two percent of calculated ratio were above 2.0, with a median ratio of 2.5 for assays using authentic virus, and 2.1 for assays using PV.

Neutralising Antibody Titres Expressed as Relative to the Candidate WHO IS (24/124)

Similarly to the binding assays, quantification of neutralising activity was calculated relative to SU-10 with an arbitrary assigned value of 1000 IU/mL to determine if the candidate IS could harmonise quantification across neutralisation assays and laboratories. Compared to binding assays, the relative potency for neutralisation assays is calculated as the ratio of reported value, which allows the inclusion of qualitative and semi-quantitative assays in this analysis.

Strong harmonisation of the data (>3.0-fold reduction of %GCV) was observed for 3 out of 4 samples containing mAb(s) and minor reduction of variability was found for SU-02 and SU-06 (Table 11, Figure 3B). A slight increase in variability was seen for SU-08 and SU-09 and a greater increase for SU-03 and SU-04, which were found negative by 10 and 12 assays, respectively. However, when only five outlier data points, as identified by Grubbs' test, were excluded, much stronger harmonisation was observed for SU-05, SU-06, and SU-08, the three

samples with the highest potency from convalescent patients. In total, 58% of laboratory GM estimate of the sera from convalescent patients (SU-02 to SU-10) were within a 2-fold range of the study median values calculated for each sample.

Improved assay-to-assay harmonisation was also observed as measured by the max/min ratio across the three independent repeats, where the calculated ratio decreased or remained unchanged for 12 assays out of 16 methods (Table 13B).

Neutralising Antibody Titres Expressed as Relative to Samples Composed of One or Multiple Monoclonal Antibodies.

The neutralising activity of the panel members was also calculated against each of the four samples composed of a single or multiple anti-SUDV mAbs spiked in negative human serum (SU-11, SU-12, SU-13 and SU-14) (Table 12, Figure 3C-F). Harmonisation of the quantification of neutralising activity was consistently observed for SU-05, SU-06, SU-08, SU-10 and the samples composed of the mAbs. Minor increases or decreases in variability were found for samples SU-02, SU-03, SU-04 and SU-09. Overall, results relative SU-14 achieved the highest reduction of %GCV with a median fold reduction of %GCV for SU-01 to SU-10, of 2.7.

Discussion and Conclusion

The unprecedented scale of the EBOV outbreak in West Africa in 2013-2016, highlighted the urgent need to develop vaccines for viruses in the *Filoviridae* family. Consequently, several vaccine candidates against SUDV have been in development with four of them progressing to phase 1 clinical trials, all aiming to elicit an immune response against GP. To increase harmonisation of the quantification of serological response elicited by these vaccines, and facilitate comparison between candidates, a WHO IS is required. The key analyte(s) to measure for evaluating vaccine efficacy is not known, given that the correlate of protection from vaccination against lethal challenge is not currently established for SUDV. Similarly to other filoviruses like Marburg virus, the level of anti-GP IgG seems to be an effective predictor of protection in NHP for several vaccine candidates [30][31][32][33], even though this may be vector dependent, as the strong humoral response induced by the ChAdOx1 biEBOV vaccine failed to protect cynomolgus macaques against lethal challenge [34]. The impact of vaccine induced neutralising antibodies on protection is not fully determined and results from two phase 1 clinical trials showed very low levels elicited by both the rVSV-SEBOV-GP [19] and the ChAdOx1 biEBOV vaccines [16]. A protective role conferred by neutralising antibodies has however been demonstrated by passive transfer studies where the administration of MBP134^{AF}, which combines two potent broadly neutralising antibodies, ADI-15878^{AF} and ADI-23774^{AF}, achieved therapeutic protection of ferrets and NHP against lethal challenge [10].

In this context, the objective of this study was to evaluate whether a pool of sera collected from individuals who survived SVD, could serve as a suitable WHO IS for anti-SUDV antibodies, particularly those detecting SUDV GP and neutralising antibodies. WHO ISs are the highest order of biological standards and are used as primary assay calibrants. Reporting the biological

activity in IU allows a better comparability of data obtained by different laboratories using different assays quantifying the same analyte. In the present study, we therefore assessed whether the variability of quantification of analytes from multiple serological assays could be reduced when values are reported relative to the candidate IS. A panel of 14 samples was evaluated by 23 participants in 7 countries, performing 20 binding assays (ELISA, IFA) and 16 neutralisation assays, using authentic or PV-based viruses (Table 3).

First, for the binding assays, the quantified potency of the 14 panel samples was highly variable across participating laboratories (Table 5, Figure 2A). This was likely due to a variety of reagents, type of antigens, SUDV strains, protocols, data analysis procedure, readouts, and output units being used, but also because most assays are performed in-house, still under development and not fully validated. This variability further highlights the need to establish a common calibrant in order to increase results harmonisation. Despite a long period of time between exposure to SUDV and collection of the samples, binding activity was detected in most individual sera from convalescent patients, consistent with previous studies demonstrating stability of humoral response after infection [35][36][37][38]. With only one exception, antibody binding activity was detected in the candidate IS (SU-10) by all assays, regardless of whether using a Gulu or Boniface antigen, which are the two main strains employed in SUDV studies and vaccine development (Table 5). SU-10 was only found negative in assay 27b, which specifically detects IgG against the very C-terminal domain (CTD) of NP. This observation aligns with preliminary data previously generated by participant 27 in a small set of sera from SUDV survivors where reactivity with NP CTD was limited to serum samples with a SUDV (WV) IgG IFA titre above or equal to 1280. Similarly, detection of anti-EBOV antibodies in EBOV survivor samples by an EBOV NP CTD IgG ELISA significantly improved with higher serum concentration (dilution 1:10 instead of standard protocol 1:100), and a MARV NP N-terminal domain (NTD) IgG ELISA showed a higher sensitivity than a MARV NP CTD IgG ELISA when testing MARV survivor sera. Therefore, the lack of detection by assay 27b could be explained by a limited immunogenicity of NP CTD during infection and/or a decline of a subset of antibodies after infection, resulting in the absence of anti-(aa641-738)NP IgG in SU-10. In addition, the conformation of NP CTD, expressed for assay 27b in *E. coli*, might differ from the one on the native virus. Nevertheless, anti-SUDV NP antibodies were detected in SU-10 by assays 10b and 27a, consistent with the convalescent nature of the sera used to prepare the candidate IS. Very low levels of anti-GP IgM were detected by assay 5a at the assay LOD, which would reflect the extended period between exposure to SUDV and collection of the blood from the patients. The GM of binding potency for SU-10 was 215 (a.u.), allowing this reagent, for most assays, to be serially diluted and generate a calibration curve supporting its use as a primary calibrant. Importantly, expression of the study samples potencies relative to SU-10 achieved a reduction of the inter-laboratory variability (Table 7A, Table 9B, Figure 2). This decrease is further enhanced when selecting assays detecting only anti-GP antibodies, which are the most relevant in the context of assessing serological response from GP based vaccine candidates (Table 8). SU-04 was the only sample for which a slight increase in variability was observed (0.7-fold reduction of %GCV). However, SU-04 was only found positive by four assays (12a, 12b, 26 and 5a) out

of 20. Notably, assays 12a and 12b also detected binding activity in the negative control (SU-01) whereas assay 5a detected activity in SU-04 at the assay LOD (Table 5). The very high potency of SU-04 measured by assay 26, an IFA against WV, could be due to the detection of antibodies not targeting GP while the poor harmonisation of assay 26 for SU-04 when relative to SU-10 (Table 6) could be due to a unique composition of antibodies targeting non-GP antigens in this serum, which may differ from the candidate IS. To support this, harmonisation of results is demonstrated for SU-04 for assays detecting anti-GP antibodies only (Table 8). Overall, potential issues with assay specificity and detection of different analytes might explain the lack of harmonisation for SU-04, but also SU-06 and SU-09 for assay 26, as well as SU-07 for assay 12a and 12b (Table 6, Figure 2B). Similarly, the relative potency of SU-08 was very high for two IFAs (assays 5b and 24), compared to all other binding assays (Table 6, Figure 2B), explaining only a modest 1.4-fold decrease in %GCV for SU-08. This could be caused by a subpopulation of anti-GP antibodies in SU-08 which were well detected by IFA, due potentially to an increased accessibility of their epitopes when the GP is expressed in the cells, but present at lower levels, or absent, in SU-10. Suboptimal harmonisation was also observed for assay 7c for SU-05, SU-07 and SU-08. Assay 7c is a commercially available ELISA and results obtained from a similar kit during a previous study has also been proven challenging [41]. Reduction of assay-to-assay variability was also observed, particularly for assays 12a, 12b and 24, demonstrating the capacity of a reference reagent to harmonise inter-assay results within a laboratory, between assays (Table 9).

The slope ratio analysis indicates good parallelism between the fitted curves obtained from SU-10 and test sera, for most assays, which further supports commutability of the candidate IS (Figure 4). A comprehensive assessment of the commutability of the candidate IS was unfortunately not conducted due to our inability to source sera and samples from vaccinees. Similar studies assessing antibody WHO IS against viruses have also primarily relied on samples from convalescent patients during the collaborative study, and the supply of these established ISs for vaccine development has not raised concerns by the scientific community [39][40][41]. Additionally, the test samples used in our study were all solvent/detergent treated and therefore would differ from samples tested during clinical trials. It would therefore be beneficial to evaluate the parallelism between the fitted curve obtained from non-solvent/detergent treated samples and from the candidate WHO IS.

Neutralising activity was detected in the candidate IS by all assays expect one (Table 5). The reported potencies were lower for assays using authentic virus than for assay using PV, which is in agreement with the higher sensitivity usually observed for PV-based assays [42]. Harmonisation of neutralising potency was also achieved when results were reported relative to the candidate IS (SU-10), but to a lesser extent than for the binding assays, likely due to the higher intrinsic variability of cell-based assays, as exemplified by higher max/min ratios across the three repeats performed by the participants (Table 13A). Similarly to the binding assays, most of the neutralisation assays used by different laboratories, are also not fully validated and used for research purposes only, which might lead to increased variability. The %GCV was diminished for four of the nine samples composed of convalescent sera (SU-02, SU-05, SU-06 and SU-08), and three of the four samples composed of mAbs (SU-11, SU-12 and SU-14),

when five outliers results were excluded, as identified by Grubbs' test (Table 12). This suggests that harmonisation is more effective on samples with the highest titres. Three outlier results were reported by participant 18 (Table 11), which used a neutralisation assay with authentic virus at containment level 4, and generally reported very high titres. Ineffective harmonisation of data from assay 18 could be partly attributed to a very low quantification of SU-10 during one of the three repeats, as shown by the max/min ratio (Table 13A), potentially due to inefficient resuspension of one of the three freeze-dried SU-10 sample. This resulted to reduce the overall GM for SU-10 and therefore led to very high relative potency for all the other samples. Another contributing factor to explain the outlier behaviour of assay 18 could be the high sample dilution factor (25-fold) used in this assay, which could have affected the accuracy of the quantifications. Increase of %GCV was observed for SU-03 and SU-04 (Table 12). These two samples were found positive by only six and four assays, respectively, out of 16, including two assays (20b and 21b) which also detected neutralising activity in the negative control. We therefore hypothesised that a potential issue with assay specificity could have contributed to increased %GCV for SU-03 and SU-04.

The identification, sourcing and characterisation of human sera to produce reference reagents to support the development of vaccines against emerging viruses such as filoviruses, is challenging, mainly due to the limited number of survivors of infection, logistics associated with the transfer of materials, the inherent biological risk associated with handling the samples, and the difficulty to find high titre samples. In addition, access to human sera collected through vaccine clinical trials has also proven difficult and only limited volume is available, often incompatible with the production of a reference material to be shared globally for several years. In order to consider an alternative type of reagent, we therefore had the secondary objective to investigate the suitability of human sera spiked with one or multiple mAbs to act as a primary calibrant (Table 2). To this aim, a selection of anti-GP mAbs isolated from either individuals vaccinated with a recombinant adenovirus expressing EBOV GP [43] or rabbits immunised with a combination of cells expressing the GP from EBOV, SUDV, Bundibugyo ebolavirus, and Tai Forest ebolavirus [44], were kindly provided by Dr Simon Draper at Oxford University. The rabbit mAbs were further humanised to allow detection in binding assays. Four samples were prepared at the MHRA, as described in Table 2, using antibodies with characterised binding and neutralising activity. The design strategy was to include multiple binding and neutralising mAbs to better represent the diverse epitope coverage typically observed in sera from convalescent individuals while also selecting mAbs with non-overlapping epitopes to minimise competition. SU-11, which contains only one mAb with binding and neutralising activity was used as control to assess whether a cocktail of mAbs would be a more appropriate calibrant. For assays only detecting anti-GP binding antibodies, we found that the variability of the quantification of SU-01 to SU-10 across assays was similar when reported relative to SU-13, SU-14 or the candidate IS with a median %GCV fold reduction of 4.4, 4.9 and 4.5, respectively (Table 8, Figure 2C-F). For neutralisation assays, harmonisation of the quantification of SU-01 to SU-10 was further improved when reported relative to SU-11, SU-12 and SU-14, compared to the candidate IS (Table 12, Figure 3C-F). Results reported relative to SU-11, containing a single mAb, were also harmonised, particularly for neutralisation

assays. However, SU-14, a human serum spiked with a combination of five mAbs was the most efficient at harmonising results from assays detecting anti-SUDV GP binding activity and anti-SUDV neutralising activity, to a degree similar to the candidate IS; therefore, strongly supporting the suitability of this type of material as a primary calibrant (Table 7B, Table 8, Figure 2F and Figure 3F). This has important implications in the design of reference reagents composed of mAbs, suggesting that more complex combinations of mAbs, better representing a polyclonal human serum, might achieve better reduction of inter-laboratory variability of analyte quantification. Such a reagent with defined antibody composition, would have the added advantage to be easily produced multiple times (*e.g.*, when the IS needs to be replenished) with very limited batch-to-batch variability. This would ensure better continuity of the IU and avoid potential drift that could arise if the first and subsequent IS are not prepared from the same human sera.

In conclusion, calibration of serological assays with the candidate IS leads to improved harmonisation of the quantification of anti-SUDV GP IgG and neutralising activity against SUDV, of test samples, across multiple assays. Satisfactory stability of binding and neutralising activities was observed for the candidate IS. In addition, a proof of concept was presented, suggesting that a carefully designed combination of monoclonal antibodies spiked in negative human serum could potentially offer a suitable alternative as primary calibrant for binding and neutralisation assays.

Proposal

It is proposed that the pool of sera collected from survivors of SVD, sample SU-10, MHRA code 24/124, be established as the first WHO IS for anti-SUDV antibodies for binding assays (human serum) (24/124_BA), and the first WHO IS for anti-SUDV antibodies for neutralisation assays (human serum) (24/124_NT), similar to that established for anti-Nipah virus antibodies [45]. It is proposed to assign a unitage of 250 IU/ampoule anti-glycoprotein IgG to for 24/124_BA, and 250 IU/ampoule for neutralising antibodies to 24/124_NT. Approximately 1500 ampoules (0.25mL/ ampoule) are available for distribution at the MHRA and the batch of 24/124 will be divided into 24/124_BA and 24/124_NT based on demand. The instructions for use of the proposed WHO IS are presented in Appendix 3 and 4. Based on the stability study results, we proposed that the IS is to be kept at -20˚C for long term storage but can be shipped at ambient temperature.

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Comments from participants

Participants 1, 3, 4, 8, 9, 11 and 22 reviewed the manuscript and did not have any comments.
Participants 27 provided useful input in the discussion regarding assays 27a and 27b.
Participants 6 and 20 requested a change in the participant list (Appendix 1).
Participant 16 updated the strain used in their ELISA to Gulu (Table 3).
Participant 7, 13, 16 provided useful additions, minor corrections and suggestions.

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Table 1. Collaborative study samples. ELISA performed at the MHRA and Battelle during preliminary screening of the individual samples were used to select samples with a range of potencies, which are abbreviated as shown below.

CS code	MHRA code	Description	Abbreviation	Formulation	Volume (mL)
SU-01		Negative serum	neg	Liquid	0.1
SU-02		Individual serum for SUDV survivor (mid)	mid1	Liquid	0.1
SU-03		Individual serum for SUDV survivor (low)	low1	Liquid	0.1
SU-04		Individual serum for SUDV survivor (unkn)	unkn	Liquid	0.1
SU-05		Individual serum for SUDV survivor (high)	hig1	Liquid	0.1
SU-06		Liquid bulk of candidate WHO IS	liq ISc	Liquid	0.1
SU-07		Individual serum for SUDV survivor (low)	low2	Liquid	0.1
SU-08		Individual serum for SUDV survivor (high)	hig2	Liquid	0.1
SU-09		Individual serum for SUDV survivor (mid)	mid2	Liquid	0.1
SU-10	24/124	Candidate WHO International Standard	F/D ISc	F/D	0.25
SU-11		Monoclonal antibody	mAb	Liquid	0.1
SU-12		Monoclonal antibody cocktail 1	cmAb1	Liquid	0.1
SU-13		Monoclonal antibody cocktail 2	cmAb2	Liquid	0.1
SU-14		Monoclonal antibody cocktail 3	cmAb3	Liquid	0.1

ISc: candidate International Standard; **F/D**: Freeze-Dried; **Hig**: High; **Liq**: Liquid; **Unkn**: Unknown; **Mid**: medium potency.

Table 2. Composition of sample SU-11, SU-12, SU-13 and SU-14.

Sample	mAbs	Concentration	Epitope	Activity
SU-11	11883[1]	40µg/mL	Receptor binding region	BA[2], NA[2]
SU-12	11883[1]	40µg/mL	Receptor binding region	BA[2], NA[2]
	11886[1]	40µg/mL	bridging the glycan cap, 3_{10} pocket and GP2 N-terminus	BA[2], NA[2]
	66-3-9C	8µg/mL	286 loop	BA[3]
SU-13	11883[1]	4µg/mL	Receptor binding region	BA[2], NA[2]
	11886[1]	4µg/mL	bridging the glycan cap, 3_{10} pocket and GP2 N-terminus	BA[2], NA[2]
	66-3-9C	0.8µg/mL	286 loop	BA[3]
SU-14	11883[1]	40µg/mL	Receptor binding region	BA[2], NA[2]
	11886[1]	40µg/mL	bridging the glycan cap, 3_{10} pocket and GP2 N-terminus	BA[2], NA[2]
	66-3-9C	8µg/mL	286 loop	BA[3]
	66-4-C12	2µg/mL	GP1-GP2 base	BA[3]
	66-3-4A	40µg/mL	Glycan cap 2	BA[3]

BA: Binding activity against SUDV; **NA**: Neutralising activity against SUDV.

[1]These mAbs were isolated from rabbits and subsequently humanised.

[2]See reference [44]

[3]See reference [43]

Table 3. Assay methods

Lab	Assay	Target	Isolate	Readout	Output
1	Auth. neut.	WV	Boniface	CPE (Microscopy)	VNT100
2	Auth. neut. (FRNA)	WV	Gulu	Fluorescence	NT50
3	LV-PV neut.	GP	EboSud-602	RLU	NT50
4	ELISA (IgG)	WV	Gulu	OD	Endpoint dilution
5a	IFA (IgM)	GP	Gulu	Fluorescence	Endpoint dilution
5b	IFA (IgG)	GP	Gulu	Fluorescence	Endpoint dilution
5c	VSV-PV neut.	GP	Gulu	CPE	Endpoint dilution
6	Auth. neut.	WV	Gulu	FFU	NT50
7a	ELISA (IgG)	GP	Boniface	OD	EU
7b	ELISA (IgG)	GP	Gulu	OD	EU
7c[1]	ELISA (IgG)	GP	Boniface	OD	AAU
7d	VSV-PV neut.	GP	Boniface	RLU	NT50
8	VLP neut.	GP	Gulu	RLU	NT50
9	LV-PV neut.	GP	Boniface	RLU	NT50
10a	Auth. neut.	WV	Boniface	FFU	Endpoint dilution
10b	IFA (IgG)	NP	Yambuku (Zaire)	Fluorescence	NT50
11a	VSV-PV neut.	GP	Boniface	FPFU	FRNT50
11b	ELISA (IgG)	GP	Gulu	OD	EU
12a[2]	ELISA (IgG)	GP[3]	Boniface	OD	Endpoint dilution
12b[2]	ELISA (IgG)	GP[3]	Boniface	OD	Endpoint dilution
13a	ELISA (IgG)	GP	Gulu	OD	EU
13b	VSV-PV neut.	GP	Boniface	RLU	NT50
13c	VSV-PV neut.	GP	Boniface	RLU	NT80
14	ELISA (IgG)	GP	Gulu	OD	EU
16	ELISA (IgG)	GP	Gulu	OD	Endpoint dilution
18	Auth. Neut. (PRNT)	WV	Gulu	CPE (Microscopy)	PRNT50
20a	ELISA (IgG)	GP	Boniface	OD	EU
20b	LV-PV neut.	GP	Boniface	RLU	IC50
21a	ELISA (IgG)	GP	Gulu	OD	EU
21b	LV-PV neut.	GP	Boniface	RLU	NT50
22	VSV-PV neut.	GP	Boniface	RLU	NT50
24	IFA (IgG)	GP	Gulu	Fluorescence	RFU
25	IFA (IgG)	WV	Gulu	Fluorescence	Endpoint dilution
26	IFA (IgG)	WV	Gulu	Fluorescence	Endpoint dilution
27a[4]	ELISA (IgG FcγR)	NP (aa38-351)	Gulu	OD	OD
27b[4]	ELISA (IgG FcγR)	NP (aa641-738)	Gulu	OD	OD

aa: amino acid; **AAU**: Antibody Activity Unit; **Auth. neut.**: Neutralisation assay using live authentic virus; **CPE**: Cytopathic Effect; **ELISA**: Enzyme-Linked ImmunoSorbent Assay; **EU**: ELISA unit; **FcγR**: Fc-gamma Receptor; **FFU**: Focus Forming Unit; **FPFU**: Fluorescent Plaque Forming Unit; **FRNA**: Foci Reduction Neutralisation Assay; **$FRNT_{50}$**: 50% Fluorescence reduction neutralisation titre; **GP**: Glycoprotein; **IFA**: Indirect Fluorescence Assay; **IgG**: Immunoglobulin G; **IgM**: Immunoglobulin M; **LV**: Lentivirus; **Neut**: Neutralisation assay; **NP**: Nucleoprotein; **NT_{50}**: 50% Neutralisation Titre; **NT_{80}**: 80%

Neutralisation Titre; **IC_{50}**: 50% Inhibitory Concentration; **OD**: Optical Density; **PFU**: Plaque Forming Unit; **PRNT**: Plaque Reduction Neutralisation Assay; **$PRNT_{50}$**: 50% neutralisation titre by Plaque Reduction Neutralisation Test; **PV**: Pseudotyped Virus; **RFU**: Ratio of Fluorescence Units; **RLU**: Relative Light Unit; **VLP**: Virus-Like Particles **VNT_{100}**: 100% Virus Neutralisation Titre; **VSV**: Vesicular Stomatitis Virus; Grey highlight indicates methods reporting qualitative results for the dilution(s) tested. **WV**: Whole Virus; [1] Assay 7c is a kit available commercially from Alpha Diagnostics (ELISA cat # AE-321620-1); [2] 12a and 12b are two data sets generated independently by two operators using the same assay; [3] GP does not contain the transmembrane domain; [4] Assays 27a and 27b are kits available commercially from Panadea Diagnostics (IgG FcγR ELISA reference ELG.xxx - pre-launch status, and ELG.009, respectively).

Table 4. Candidate International Standard for anti-SUDV antibody formulation review.

Sample name	SU-10, candidate IS
Produce code	24/124
Number of containers filled	1739
Mean fill mass (g)	0.27 (n=97)
CV of fill mass (%)	1.02
Mean residual moisture (%)	0.35 (n=12)
CV of residual moisture	38.73
Mean oxygen head space (%)	0.36 (n=12)
CV of oxygen head space (%)	41.62

Table 5. GM of anti-SUDV binding antibodies, as reported by the study participants.

			SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14
Assay	**Target**	**Lab**	neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3
IgG																
IFA	GP	5b[1]	Neg	Neg	Neg	Neg	32	Neg	Neg	254	32	25	1016	403	403	806
ELISA	GP	7a	Neg	101	46	Neg	237	159	20	267	94	172	800	4504	387	4164
ELISA	GP	7b	Neg	139	43	Neg	344	291	97	351	168	292	1389	6740	550	7718
ELISA	GP	7c	153	Neg	Neg	Neg	8	291	2646	49	32	372	1173	2641	445	2510
ELISA	GP	11b	Neg	431	234	Neg	632	540	210	1004	478	662	8451	14572	1565	17526
ELISA	GP	12a	800	1600	400	1600	2540	6400	12800	3200	2540	3200	32254	40637	6400	40637
ELISA	GP	12b	1008	1270	1600	800	3200	4032	8063	3200	800	2540	32254	40637	5080	51200
ELISA	GP	13a	Neg	138	34	Neg	215	240	75	453	140	263	4422	7855	822	9172
ELISA	GP	14	Neg	79	33	Neg	123	157	69	202	86	151	1346	2254	328	1893
ELISA	GP	16	Neg	317	178	Neg	317	317	Neg	504	Neg	283	3200	4525	713	5080
ELISA	GP	20a	Neg	73	23	Neg	157	99	27	164	45	98	605	980	113	957
ELISA	GP	21a	Neg	124	42	Neg	265	459	292	317	221	473	721	1570	377	1427
IFA	GP	24[1]	Neg	4	Neg	Neg	4	4	Neg	40	Neg	13	40	59	4	86
IFA	NP	10b[2]	Neg	>640	>640	>640	>640	>640	Neg	>640	160	>640	80	Neg	20	20
ELISA	NP	27a[3]	Neg	Pos	Pos	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg
ELISA	NP	27b[3]	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Equ	Neg	Neg	Neg	Neg	Neg	Neg
ELISA	WV	4	Neg	400	Neg	Neg	Neg	Neg	400	400	Neg	400	800	Neg	Neg	1008
IFA	WV	25[2,4]	Neg	Neg	>160	>160	>160	>160	Neg	>160	40	>160	>160	>160	>160	>160
IFA	WV	26[1,4]	Neg	Neg	160	1280	80	640	40	Neg	22	160	320[5]	320[5]	320[5]	320[5]
IgM																
IFA	GP	5a[1]	Neg	Neg	Neg	20	20	Neg	Neg	Neg	20	20	Neg	Neg	Neg	Neg

Equ: Equivocal; **ELISA**: Enzyme-Linked ImmunoSorbent Assay; **GP**: Glycoprotein; **IFA**: Indirect Fluorescence Assay; **IgG**: Immunoglobulin G; **IgM**: Immunoglobulin M; **Neg**: Negative; **NP**: Nucleoprotein; **Pos**: Positive; **WV**: Whole Virus. [1] Calculations performed using the ratio of reported values; [2] Laboratory for which calculation of the GM was not calculated as dilution did not reach an endpoint; [3] Laboratory for which calculation of the GM was not calculated because this diagnostic assay requires only one dilution (1/100) and the output is either positive, negative or equivocal; [4] An atypical staining pattern was observed for sample SU-11, SU-12, SU-13 and SU-14, see results section.

Table 6. Anti-SUDV binding antibody titres (IgG or IgM) expressed relative to the candidate IS, sample SU-10, with an arbitrary value of 1000 IU/mL.

Assay	Target	Lab	SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14
			neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3
IgG																
IFA	GP	5b[1]	-	-	-	-	1260	-	-	10079	1260	1000	40317	16000	16000*	32000
ELISA	GP	7a	-	641	282	-	1361	930	143	1583	549	1000	7955	45494	3666	42202
ELISA	GP	7b	-	473	132	-	1047	989	323	1170	568	1000	4630	22794	1877	26044
ELISA	GP	7c	436	-	-	-	118*	747	6510	154	-	1000	3087	7204	1132	6748
ELISA	GP	11b	-	624	342	-	846	731	299	1375	629	1000	11506	19795	2201	20193
ELISA	GP	12a	181	318	125	70	679	1256	2762	1010	300	1000	8257	19772	1505	24540
ELISA	GP	12b	222	333	133	66	583	1302	2636	1098	364	1000	15318	25304	2312	16303
ELISA	GP	13a	-	716	150	-	1082	1200	365	2213	677	1000	21564	41205	4243	48695
ELISA	GP	14	-	458	150	-	723	955	404	1281	621	1000	17336	24511	2360	29232
ELISA	GP	16	-	935	570	-	739	1100	-	1456	-	1000	10952	15167	1951	16676
ELISA	GP	20a	-	707	226	-	1791	919	256	1861	428	1000	9503	17523	1405	17944
ELISA	GP	21a	-	211	79	-	438	693	492	553	383	1000	4050	13218	868	12391
IFA	GP	24[1]	-	1000	-	-	1000	1000	-	10000	-	1000	3162	5623	1000	10000
IFA	NP	10b[2]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ELISA	NP	27a[3]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ELISA	NP	27b[3]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ELISA	WV	4[4]	-	937	-	-	-	-	1222	867	-	1000	1572	-	-	5794
IFA	WV	25[2]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
IFA	WV	26[1,5]	-	-	1000	8000	500	4000*	250	-	140	1000	2000	2000*	2000	2000
IgM																
IFA	GP	5a[1]	-	-	-	1000	1000	-	-	-	1000	1000	-	-	-	-

ELISA: Enzyme-Linked ImmunoSorbent Assay; **GP**: Glycoprotein; **IFA**: Indirect Fluorescence Assay; **IgG**: Immunoglobulin G; **IgM**: Immunoglobulin M; **n/**a: not applicable; **NP**: Nucleoprotein; **Pos**: Positive; **WV**: Whole Virus. *result identified as outlier by Grubbs' test applied to log-transformed estimates ($p<0.01$); [1] Calculation performed using the ratio of reported values; [2] Laboratory for which calculation of the GM was not calculated as dilution did not reach an endpoint [3] Laboratory for which calculation of the GM was not calculated as these diagnostic assays require only one dilution (1/100) and the output is either positive, negative or equivocal;[4] Calculations performed using parallel line analysis; [5] An atypical staining pattern was observed for sample SU-11, SU-12, SU-13 and SU-14, see results section.

Table 7. GM, median and inter-laboratory variability in the quantitative binding methods. A. Using the data reported by participants and relative to the candidate IS (SU-10). **B.** Using the data relative to the monoclonal antibody (SU-11) or cocktails of monoclonal antibodies (SU-12, SU-13, SU-14).

		SU-01	**SU-02**	**SU-03**	**SU-04**	**SU-05**	**SU-06**	**SU-07**	**SU-08**	**SU-09**	**SU-10**	**SU-11**	**SU-12**	**SU-13**	**SU-14**	**Median of %GCV fold red.[1]**
		neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3	
As reported	GM	498	171	99	425	144	312	254	353	118	215	1584	2798	462	2884	
	Median	800	138	46	1012	215	291	143	334	94	273	1173	3449	424	2510	
	% GCV	179	377	275	684	564	492	784	254	335	364	458	532	472	478	
	N	3	12	11	4	15	13	12	14	13	16	15	14	14	15	
Relative to SU-10	GM	260	555	216	438	760	1077	627	1438	503	1000	7378	15525	2151	16018	
	Median	222	632	150	535	846	989	385	1328	558	1000	8257	18648	1975	17944	
	% GCV	58	63	112	913	90	56	228	185	77	0	151	126	108	132	
	Fold red.	3.1	6.0	2.5	0.7	6.3	8.8	3.4	1.4	4.4	n/a	3.0	4.2	4.4	3.6	**3.4**
	N	3	12	11	4	15	13	12	14	12	16	15	14	14	15	
Relative to SU-10*	GM					867	966						18175	1844		
	Median					923	972						19772	1951		
	% GCV					49	23						80	59		
	Fold red.					11.5	21.4						6.7	8.0		**3.4**
	N					14	12						13	13		

Fold red.: Ratio of the %GCV from reported data against %GCV from relative data **GM**: Geometric Mean**; %GCV**: % Geometric Coefficient of Variation; **N**: Number of assays; *excluding results identified as outliers in Table 6. A colour scale gradient is used to represent the %GCV fold reduction where green, red and white shows increase, decrease or no change in data harmonisation, respectively. [1] Median of %GCV fold reduction calculated for SU-01 to SU-10.

		SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14	Median of %GCV fold red.[1]
		neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3	
Relative to SU-11	GM	36	72	25	53	90	148	94	178	49	136	1000	1884	261	2171	
	Median	22	64	24	8	78	117	96	135	45	121	1000	1811	206	2186	
	% GCV	236	165	225	4223	120	162	382	180	67	151	0	93	76	79	
	Fold red.	0.8	2.3	1.2	0.2	4.7	3.0	2.1	1.4	5.0	2.4	n/a	5.7	6.2	6.1	**2.2**
	N	3	12	11	3	14	13	12	14	11	15	15	14	14	15	
Relative to SU-11*	GM			19			119		142							
	Median			22			109		133							
	% GCV			97			79		89							
	Fold red.			2.8			6.2		2.9							**2.6**
	N			10			12		13							
Relative to SU-12	GM	17	26	12	33	48	70	35	82	26	64	531	1000	139	1110	
	Median	9	19	6	4	39	52	17	52	25	54	552	1000	100	1062	
	% GCV	201	121	308	6250	119	215	399	240	84	126	93	0	142	32	
	Fold red.	0.9	3.1	0.9	0.1	4.7	2.3	2.0	1.1	4.0	2.9	4.9	n/a	3.3	14.9	**2.1**
	N	3	11	11	3	14	13	11	13	11	14	14	14	14	14	
Relative to SU-12*	GM	9	22	8			53		52		55					
	Median	9	18	6			52		51		51					
	% GCV	n/a	66	101			77		56		80					
	Fold red.	59.7	5.7	2.7			6.4		4.5		4.6					**4.5**
	N	2	10	10			12		11		13					
Relative to SU-13	GM	165	277	106	174	346	584	304	691	191	465	3830	7216	1000	8007	
	Median	120	243	83	46	375	564	172	625	200	506	4875	10048	1000	10738	
	% GCV	111	79	116	1426	111	75	343	158	78	108	76	142	0	122	
	Fold red.	1.6	4.8	2.4	0.5	5.1	6.6	2.3	1.6	4.3	3.4	6.0	3.7	n/a	3.9	**2.9**
	N	3	11	11	3	14	13	11	13	11	14	14	14	14	14	
Relative to SU-13*	GM								583		542				10689	
	Median								624		513				11494	
	% GCV								18		59				35	
	Fold red.								14.1		6.2				13.7	**4.5**
	N								10		13				12	
Relative to SU-14	GM	19	29	12	36	43	66	40	77	24	62	461	901	125	1000	
	Median	14	19	7	4	38	51	27	56	22	56	457	943	94	1000	
	% GCV	206	129	312	5858	102	213	431	169	68	132	79	32	122	0	
	Fold red.	0.9	2.9	0.9	0.1	5.5	2.3	1.8	1.5	4.9	2.8	5.8	16.6	3.9	n/a	**2.1**
	N	3	12	11	3	14	13	12	14	11	15	15	14	14	15	
Relative to SU-14*	GM			8			49		64					94		
	Median			7			51		45					87		
	% GCV			103			68		99					35		
	Fold red.			2.7			7.2		2.6					13.5		**2.7**
	N			10			12		13					12		

Fold red.: Ratio of the %GCV from reported data against %GCV from relative data **GM**: Geometric Mean**; %GCV**: % Geometric Coefficient of Variation; **n/a**: not applicable; **N**:

Number of assays; *excluding results identified as outliers in Table 6. A colour scale gradient is used to represent the %GCV fold reduction where green, red and white shows increase, decrease or no change in data harmonisation, respectively. [1] Median of %GCV fold reduction calculated for SU-01 to SU-10.

Table 8. GM, median and inter-laboratory variability in the quantitative binding methods detecting anti-SUDV GP antibodies only, using the data reported by participants and relative to the candidate IS (SU-10), the monoclonal antibody (SU-11) or cocktails of monoclonal antibodies (SU-12, SU-13, SU-14).

		SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14	Median %GCV fold red.[1]
		neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3	
As reported	GM	498	158	95	295	150	293	291	349	135	211	1888	3306	476	3703	
	Median	800	138	45	800	226	291	154	317	117	273	1346	4504	445	4164	
	% GCV	179	403	299	954	608	532	917	273	325	414	493	509	513	470	
	N	3	12	11	4	15	13	12	14	13	16	15	14	14	15	
Relative to SU-10	GM	260	529	185	166	783	966	643	1495	565	1000	9188	18175	2163	20328	
	Median	222	624	150	70	923	972	385	1375	568	1000	9503	19772	1951	20193	
	% GCV	58	62	79	373	92	23	252	194	54	0	117	80	114	76	
	Fold red.	3.1	6.5	3.8	2.6	6.6	23.1	3.6	1.4	6.0	n/a	4.2	6.4	4.5	6.2	**4.5**
	N	3	11	10	3	14	12	10	13	11	14	13	13	13	1	
Relative to SU-11	GM	36	60	19	6	84	119	74	163	48	109	1000	1978	235	2213	
	Median	22	54	22	6	74	109	48	133	41	105	1000	1844	197	2186	
	% GCV	236	111	97	n/a	115	79	379	176	70	117	0	93	54	76	
	Fold red.	0.8	3.6	3.1	n/a	5.3	6.7	2.4	1.6	4.6	3.5	n/a	5.5	9.5	6.2	**3.6**
	N	3	11	10	2	13	12	10	13	10	13	13	13	13	12	
Relative to SU-12	GM	17	26	8	3	42	53	31	82	23	55	505	1000	119	1118	
	Median	9	19	6	3	34	52	16	52	25	51	542	1000	96	1100	
	% GCV	201	121	101	n/a	92	77	413	240	71	80	93	0	102	33	
	Fold red.	0.9	3.3	3.0	n/a	6.6	6.9	2.2	1.1	4.6	5.2	5.3	n/a	5.0	14.2	**4.0**
	N	3	11	10	2	13	12	10	13	10	13	13	13	13	13	
Relative to SU-13	GM	165	277	91	36	355	527	333	691	212	462	4247	8401	1000	9396	
	Median	120	243	80	37	379	563	177	625	231	513	5082	10386	1000	11475	
	% GCV	111	79	83	n/a	116	55	366	158	64	114	54	102	0	73	
	Fold red.	1.6	5.1	3.6	n/a	5.2	9.7	2.5	1.7	5.1	3.6	9.1	5.0	n/a	6.4	**4.4**
	N	3	11	10	2	13	12	10	13	10	13	13	13	13	13	
Relative to SU-14	GM	19	25	8	3	38	49	30	74	21	49	452	894	106	1000	
	Median	14	18	7	3	36	51	15	45	22	50	457	910	87	1000	
	% GCV	206	93	103	n/a	66	68	444	175	49	76	76	33	73	0	
	Fold red.	0.9	4.3	2.9	n/a	9.2	7.8	2.1	1.6	6.6	5.4	6.5	15.4	7.0	n/a	**4.9**
	N	3	11	10	2	13	12	10	13	10	13	13	13	13	13	

Fold red.: Ratio of the %GCV from reported data against %GCV from relative data **GM**: Geometric Mean**; %GCV**: % Geometric Coefficient of Variation; **N**: Number of assays. A colour scale gradient is used to represent the %GCV fold reduction where green, red and white shows increase, decrease or no change in data harmonisation, respectively. [1] Median of %GCV fold reduction calculated for SU-01 to SU-10.

Table 9. Inter assay variability for quantitative binding assays, as expressed in ratio of maximum/minimum quantification across the three independent repeats performed. Using the three quantifications obtained during the three independent assay repeats, the ratio of the maximum and minimum values was calculated as an indicator of the assay-to-assay variability, and represented as a heat map, for the reported (**A**) or relative (**B**) quantifications. For each lab, the median max/min ratio was calculated for samples SU-01 to SU-09. In Table 9B, increase of median max/min ratio between the reported and relative data is indicated in yellow. Decrease or no change in average or median of max/min ratio between the reported and relative data is indicated in blue.

	SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14	
Lab	neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3	**Median**
4[2]	-	1.0	-	-	-	-	1.0	1.0	-	1.0	4.0	-	-	4.0	1.0
5a[1]	-	-	-	1.0	1.0	-	-	-	1.0	1.0	-	-	-	-	1.0
5b[1]	-	-	-	-	4.0	-	-	8.0	2.0	2.0	2.0	2.0	2.0	4.0	2.0
7a	-	1.9	1.5	-	1.6	1.1	1.2	1.1	1.2	1.1	1.6	1.5	1.8	1.8	1.5
7b	-	1.7	1.2	-	1.5	1.1	1.2	1.2	1.1	1.2	1.1	1.1	1.1	1.1	1.2
7c	1.2	-	-	-	1.6	1.1	1.1	1.7	1.0	1.2	1.2	1.4	1.1	1.3	1.2
11b	-	1.3	1.4	-	1.1	1.1	1.2	1.4	1.4	1.3	1.5	1.6	2.2	1.8	1.4
12a	8.0	8.0	16.0	1.0	16.0	8.0	4.0	8.0	4.0	16.0	2.0	2.0	8.0	2.0	8.0
12b	8.0	4.0	1.0	1.0	16.0	8.0	8.0	16.0	16.0	16.0	4.0	2.0	4.0	1.0	6.0
13a	-	1.4	1.2	-	1.3	1.4	1.2	1.2	1.1	1.0	1.3	1.2	1.4	1.3	1.3
14	-	1.3	1.0	-	1.4	1.2	1.4	1.5	1.4	1.4	3.2	6.3	1.6	3.1	1.4
16	-	2.0	1.4	-	2.0	1.4	-	2.0	-	2.0	2.0	2.8	2.8	2.0	2.0
20a	-	1.2	1.4	-	1.1	1.1	1.1	1.2	1.1	1.0	1.3	1.1	1.2	1.3	1.2
21a	-	1.5	1.3	-	1.1	1.5	1.6	1.3	1.2	1.8	1.4	1.7	1.1	1.2	1.3
24[1]	-	1.0	-	-	1.0	1.0	-	1.0	-	10.0	10.0	3.2	1.0	10.0	1.0
26[1]	-	-	1.0	1.0	1.0	1.0	1.0	-	1.4	1.0	1.0	1.0	1.0	1.0	1.0

Lab	SU-01 neg	SU-02 mid1	SU-03 low1	SU-04 unkn	SU-05 hig1	SU-06 liq ISc	SU-07 low2	SU-08 hig2	SU-09 mid2	SU-10 F/D ISc	SU-11 mAb	SU-12 cmAb1	SU-13 cmAb2	SU-14 cmAb3	Median
4[2]	-	1.0	-	-	-	-	1.0	1.0	-	1.0	1.0	-	-	1.0	1.0
5a[1]	-	-	-	1.0	1.0	-	-	-	1.0	1.0	-	-	-	-	1.0
5b[1]	-	-	-	-	2.0	-	-	16.0	2.0	1.0	2.0	4.0	4.0	4.0	3.0
7a	-	1.2	1.4	-	1.2	1.1	1.2	1.1	1.1	1.0	1.1	1.3	1.1	1.3	1.1
7b	-	1.3	1.3	-	1.2	1.1	1.3	1.1	1.2	1.0	1.3	1.6	1.5	1.4	1.3
7c	1.1	-	-	-	1.2	1.1	1.2	1.9	1.0	1.0	1.2	1.5	1.3	1.3	1.2
11b	-	1.1	1.4	-	1.1	1.3	1.3	1.4	1.3	1.0	1.6	2.0	2.4	1.7	1.4
12a	1.6	1.2	1.7	1.0	1.4	1.3	1.5	1.1	1.5	1.0	1.1	1.5	2.1	6.5	1.5
12b	1.7	1.2	1.0	1.0	1.4	2.1	1.8	1.5	1.2	1.0	3.2	2.1	1.9	7.2	1.6
13a	-	2.1	2.2	-	1.9	2.2	2.0	1.9	1.9	1.0	2.0	2.1	1.9	1.8	2.0
14	-	1.4	1.0	-	1.4	1.3	1.6	1.3	1.4	1.0	2.2	2.0	2.2	2.6	1.4
16	-	2.4	2.1	-	3.5	2.9	-	3.2	-	1.0	1.5	1.3	1.8	2.0	2.1
20a	-	1.2	1.1	-	1.2	1.4	1.1	1.2	1.2	1.0	1.3	1.9	1.3	1.7	1.2
21a	-	4.5	3.4	-	3.0	2.6	1.8	2.3	2.5	1.0	2.8	5.8	1.5	3.2	2.7
24[1]	-	1.0	-	-	1.0	1.0	-	1.0	-	1.0	1.0	3.2	1.0	1.0	1.0
26[1]	-	-	1.0	1.0	1.0	1.0	1.0	-	1.4	1.0	1.0	1.0	1.0	1.0	1.0

Table 10. GM of anti-SUDV neutralising antibodies, as reported by the participants.

Assay	Lab	SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14
		neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3
Auth. virus	1	Neg	12	Neg	Neg	87	64	Neg	51	14	44	55	119	11	102
Auth. virus	2	Neg	Neg	Neg	Neg	28	36	Neg	22	Neg	46	NT	NT	NT	NT
Auth. virus	6	Neg	Neg	Neg	Neg	14	10	Neg	13	Neg	10	5	5	Neg	8
Auth. virus	10a	Neg	Neg	Neg	Neg	16	10	Neg	10	Neg	10	Neg	20	Neg	20
Auth. virus	18	Neg	Neg	Neg	187	60558	8439	Neg	21004	63	20	Neg	Neg	Neg	Neg
LV-PV	3	Neg	27	25	Neg	98	266	Neg	86	25	222	130	302	28	640
LV-PV	9	Neg	156	171	46	74	185	49	166	141	239	582	489	30	366
LV-PV	20b	7	273	120	24	1579	1482	Neg	693	129	1697	1045	1750	100	2165
LV-PV	21b	19	19	75	21	27	57	19	57	Neg	26	258	248	24	259
VSV-PV	5c	Neg	Neg	Neg	Neg	32	Neg	Neg	Neg	13	25	13	28	Neg	25
VSV-PV	7d	Neg	51	Neg	Neg	320	307	Neg	205	34	495	940	1321	101	957
VSV-PV	11a	Neg	42	Neg	Neg	231	271	Neg	216	Neg	286	1514	1713	195	1917
VSV-PV	13b	Neg	65	18	Neg	313	191	Neg	153	41	589	1165	677	149	793
VSV-PV	13c	Neg	18	Neg	Neg	145	92	Neg	83	11	104	302	329	63	364
VSV-PV	22	Neg	106	Neg	Neg	658	652	Neg	654	119	716	782	844	61	1223
VLP	8[1]	Neg	Neg	44	Neg	46	52	Neg	50	47	47	Neg	86	Neg	78

Auth. virus: Authentic virus; **LV**: Lentivirus; **Neg**: Negative; **Neut**: Neutralisation assay; **NT**: Not Tested; **PV**: Pseudotyped Virus; **VLP**: Viral Like Particle; **VSV**: Vesicular Stomatitis Virus. [1]All samples were found negative, as below the positivity threshold of this assay, set by the manufacturer at NT_{50}>170. However, laboratory 8 reported a value between the positivity threshold and the reciprocal of the lowest dilution tested (1/20), reported in Table 10, suggesting that a low neutralising activity was detected.

Table 11. Anti-SUDV neutralising antibody titres expressed relative to the candidate IS, sample SU-10 which was given an arbitrary value of 1000 IU/mL.

Assay	Lab	SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14
		neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3
Auth. Vir.	1	-	268	-	-	2000	1470	-	1162	313	1000	1258	2718	250	2333
Auth. Vir.	2	-	-	-	-	545	841	-	530	-	1000	-	-	-	-
Auth. Vir.	6	-	-	-	-	1247	919	-	1148	-	1000	537	471	-	827
Auth. Vir.	10a	-	-	-	-	1587	1000	-	1000	-	1000	-	2000	-	2000
Auth. Vir.	18	-	-	-	9362	3025241 *	421579*	-	1049275*	83	1000	-	-	-	-
LV-PV	3	-	122	111	-	441	1200	-	386	113	1000	587	1360	125	2883
LV-PV	9	-	653	716	193	310	771	204	692	587	1000	2432	2043	126	1528
LV-PV	20b	4	161	71	14	931	873	-	409	76	1000	616	1032	59	1276
LV-PV	21b	742	729	2897	796	1039	2172*	739	2196	-	1000	9905	9518	932	9926
VSV-PV	5c	-	-	-	-	1260	-	-	-	561	1000	500	1122	-	1000
VSV-PV	7d	-	103	-	-	646	620	-	414	66	1000	1899	2669	204	1934
VSV-PV	11a	-	147	-	-	808	947	-	755	-	1000	5293	5990	681	6703
VSV-PV	13b	-	104	15	-	581	310*	-	278	56	1000	2740	1452	271	2434
VSV-PV	13c	-	224	-	-	2681	935	-	1036	211	1000	2344	6283	1359	4315
VSV-PV	22	-	149	-	-	920	911	-	914	167	1000	1093	1180	85	1709
VLP	8	-	-	965	-	975	1101	-	1059	1009	1000	-	1804	-	1587

Auth. virus: Authentic virus; FRNT: Foci Reduction Neutralisation Test; **LV**: Lentivirus; **Neg**: Negative; **Neut**: Neutralisation assay; **PV**: Pseudotyped Virus; **VLP**: Viral Like Particle; **VSV**: Vesicular Stomatitis Virus. *Result identified as outlier by Grubbs' test applied to Log-transformed estimates ($p<0.01$).

Table 12. GM, median and inter-laboratory variability in the neutralisation methods.

		SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14	Median of %GCV fold red.[1]
		neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3	
As reported	GM	11	48	56	46	143	149	31	126	40	98	237	223	55	245	
	Median	11	46	58	34	92	185	31	86	41	70	419	315	62	365	
	% GCV	n/a	176	142	172	719	497	n/a	558	154	400	535	500	149	492	
	N	2	10	6	4	16	15	2	15	11	16	12	14	10	14	
Relative to SU-10	GM	53	205	250	379	1518	1393	388	1200	187	1000	1560	2057	250	2239	
	Median	373	155	414	495	953	935	472	914	167	1000	1579	1902	227	1967	
	% GCV	n/a	103	602	1401	717	413	n/a	604	171	0	160	125	185	100	
	Fold red.	n/a	1.7	0.2	0.1	1.0	1.2	n/a	0.9	0.9	n/a	3.3	4.0	0.8	4.9	**0.9**
	N	2	10	6	4	16	15	2	15	11	16	12	14	10	14	
Relative to SU-10*	GM					915	945		740							
	Median					931	927		835							
	% GCV					77	24		76							
	Fold red.					9.3	20.7		7.3							**1.7**
	N					15	12		14							
Relative to SU-11	GM	22	105	101	53	582	528	79	415	136	641	1000	1337	128	1491	
	Median	41	116	189	79	796	399	79	442	153	661	1000	1268	103	1551	
	% GCV	n/a	128	427	104	221	171	n/a	149	222	160	0	65	96	69	
	Fold red.	n/a	1.4	0.3	1.7	3.3	2.9	n/a	3.7	0.7	2.5	n/a	7.7	1.6	7.1	**2.1**
	N	2	10	5	3	12	11	2	11	9	12	12	12	10	12	
Relative to SU-11*	GM			208	80											
	Median			241	80											
	% GCV			56	n/a											
	Fold red.			2.5	n/a											**2.5**
	N			4	2											
Relative to SU-12	GM	17	80	122	48	461	433	88	352	110	486	748	1000	97	1088	
	Median	41	83	193	84	484	500	89	339	99	527	798	1000	92	1021	
	% GCV	n/a	114	329	192	146	117	n/a	123	201	125	65	0	55	42	
	Fold red.	n/a	1.5	0.4	0.9	4.9	4.2	n/a	4.5	0.8	3.2	8.2	n/a	2.7	11.7	**2.4**
	N	2	10	6	3	14	13	2	13	10	14	12	14	10	14	
Relative to SU-13	GM	226	821	1013	683	3406	3656	1135	2745	780	4006	7788	10310	1000	11241	
	Median	430	877	1195	855	2815	4459	1209	2718	1077	4449	9710	10867	1000	10245	
	% GCV	n/a	197	490	157	148	185	n/a	142	225	185	96	55	0	78	
	Fold red.	n/a	0.9	0.3	1.1	4.9	2.7	n/a	3.9	0.7	2.2	5.6	9.1	n/a	6.3	**1.6**
	N	2	10	5	3	10	10	2	10	8	10	10	10	10	10	
Relative to SU-14	GM	15	73	102	48	424	392	100	319	105	447	671	919	89	1000	
	Median	39	63	174	80	576	500	104	320	78	509	645	979	98	1000	
	% GCV	n/a	125	488	262	139	93	n/a	112	237	100	69	42	78	0	
		n/a	1.4	0.3	0.7	5.2	5.3	n/a	5.0	0.6	4.0	7.8	11.9	1.9	n/a	**2.7**
	N	2	10	6	3	14	13	2	13	10	14	12	14	10	14	

Fold red.: Ratio of the %GCV from reported data against %GCV from relative data **GM**: Geometric Mean**; %GCV**: % Geometric Coefficient of Variation; **N**: Number of assays. A

colour scale gradient is used to represent the %GCV fold reduction where green, red and white shows increase, decrease or no change in data harmonisation, respectively. [1] Median of %GCV fold reduction calculated for SU-01 to SU-10.

Table 13. Inter assay variability for neutralisation assays, as expressed in ratio of maximum/minimum quantification across the three independent repeats performed. Using the three quantifications obtained during the three independent assay repeats, the ratio of the maximum and minimum values was calculated as an indicator of the assay-to-assay variability, for the reported (**A**) or relative (**B**) quantifications. For each lab, the median max/min ratio was calculated for samples SU-01 to SU-09. Increase of median max/min ratio between the reported and relative data is indicated in yellow. Decrease or no change in average or median of max/min ratio between the reported and relative data is indicated in blue.

	SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14	
Lab	neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3	**Median**
1	-	1.6	-	-	1.6	1.6	-	1.6	1.6	1.6	2.5	1.3	1.3	1.6	1.6
2	-	-	-	-	4.0	4.0	-	2.0	-	4.0	-	-	-	-	4.0
3	-	1.5	3.4	-	1.8	3.7	-	1.4	2.9	2.0	1.6	2.2	3.7	1.6	2.0
5c	-	-	-	-	2.0	-	-	-	2.0	8.0	2.0	4.0	-	8.0	3.0
6	-	-	-	-	2.1	1.9	-	2.0	-	1.3	3.3	2.7	-	1.3	2.0
7d	-	1.5	-	-	2.2	3.0	-	14.0	1.8	2.5	2.2	1.9	2.8	2.3	2.3
8	-	-	1.8	-	1.7	1.4	-	1.3	1.5	1.5	-	2.3	-	2.3	1.6
9	-	3.6	4.3	3.7	2.2	6.8	6.0	3.6	2.4	1.1	1.3	1.5	1.6	4.7	3.6
10a	-	-	-	-	4.0	1.0	-	1.0	-	1.0	-	4.0	-	4.0	2.5
11a	-	2.4	-	-	2.9	1.5	-	1.4	-	1.7	2.2	3.1	1.4	1.6	1.7
13a	-	1.5	3.3	-	4.0	1.7	-	1.6	2.7	2.1	2.6	1.6	2.2	1.5	2.1
13b	-	3.7	-	-	3.0	2.4	-	1.6	1.8	2.3	1.5	2.9	2.5	2.0	2.3
18	-	-	-	12.2	4.8	83.6	-	16.7	2.1	72914	-	-	-	-	14.4
20b	29.7	1.5	1.2	7.3	2.4	2.1	1.0	11.5	14.0	1.8	1.5	1.3	3.4	1.5	1.9
21b	1.1	3.2	1.6	1.5	2.1	1.9	1.2	1.2	-	1.7	2.2	1.8	1.2	1.1	1.6
22	-	4.1	-	-	3.0	2.4	-	3.9	2.2	1.7	2.5	1.6	3.7	1.8	2.5

	SU-01	SU-02	SU-03	SU-04	SU-05	SU-06	SU-07	SU-08	SU-09	SU-10	SU-11	SU-12	SU-13	SU-14	
Lab	neg	mid1	low1	unkn	hig1	liq ISc	low2	hig2	mid2	F/D ISc	mAb	cmAb1	cmAb2	cmAb3	**Median**
1	-	1.6	-	-	1.0	2.0	-	2.0	2.6	1.0	1.6	2.0	2.1	2.0	2.0
2	-	-	-	-	1.2	1.4	-	1.7	-	1.0	-	-	-	-	1.3
3	-	2.5	5.3	-	1.9	4.1	-	2.3	4.7	1.0	2.2	3.7	7.6	2.1	2.5
5c	-	-	-	-	2.0	-	-	-	4.0	1.0	2.0	5.7	-	5.7	3.0
6	-	-	-	-	1.1	1.0	-	1.2	-	1.0	1.2	1.8	-	1.0	1.1
7d	-	2.6	-	-	1.9	2.3	-	7.8	1.5	1.0	3.3	1.2	1.2	1.9	1.9
8	-	-	1.7	-	1.5	1.4	-	1.3	1.4	1.0	-	1.6	-	1.7	1.5
9	-	3.8	4.5	3.8	2.3	7.4	6.5	3.8	2.7	1.0	1.2	1.4	1.5	4.5	3.8
10a	-	-	-	-	4.0	1.0	-	1.0	-	1.0	-	4.0	-	4.0	2.5
11a	-	1.4	-	-	1.4	1.2	-	1.2	-	1.0	1.7	1.9	1.3	1.3	1.3
13a	-	1.7	1.0	-	1.0	2.2	-	2.3	1.0	1.0	1.5	1.9	1.0	1.0	1.0
13b	-	2.8	1.0	-	1.0	1.9	-	1.2	1.0	1.0	1.2	1.5	1.0	1.0	1.0
18	-	-	-	134764	348945	5780122	-	21818	1.1	1.0	-	-	-	-	78291
20b	16.9	2.7	1.8	4.3	1.3	1.4	1.0	12.6	7.9	1.0	1.6	1.9	4.9	1.3	1.9
21b	1.8	4.3	1.4	1.6	2.1	1.3	1.6	1.7	-	1.0	2.9	1.2	1.5	1.9	1.6
22	-	1.9	-	-	1.9	1.6	-	1.4	1.1	1.0	1.2	1.2	1.4	1.2	1.3

Figure 1. Evaluation of thermal degradation of the candidate International Standard for anti-SUDV antibodies.
Freeze-dried ampoules of sample SU-10, MHRA code 24/124 were stored at five different temperatures (-20°C, 4°C, 20°C, 37°C and 45°C). At each time point, three ampoules were retrieved and reconstituted with 0.25 mL of molecular grade water. Each vial was assessed in duplicate by anti-SUDV GP IgG ELISA (**A**) and VSV-PV based neutralisation assay (**B**). Data are reported relative to the sample kept at a storage temperature of -20°C. Each dot represents the GM of two independent assays for the three ampoules. The error bars indicate standard deviations across the three ampoules. All assays were performed twice. **EU**: ELISA Unit; **NT_{50}**: 50% Neutralisation Titre.

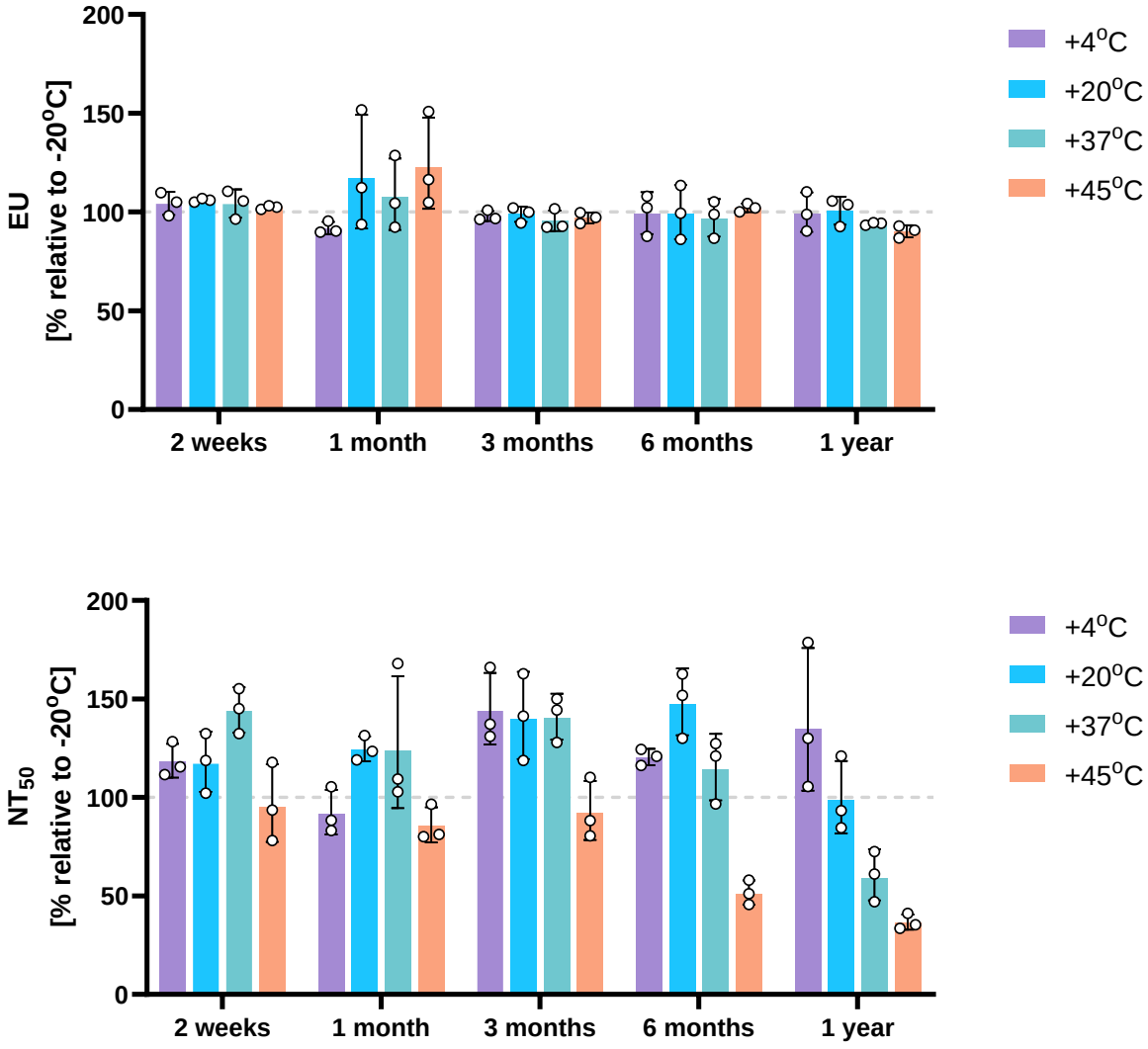
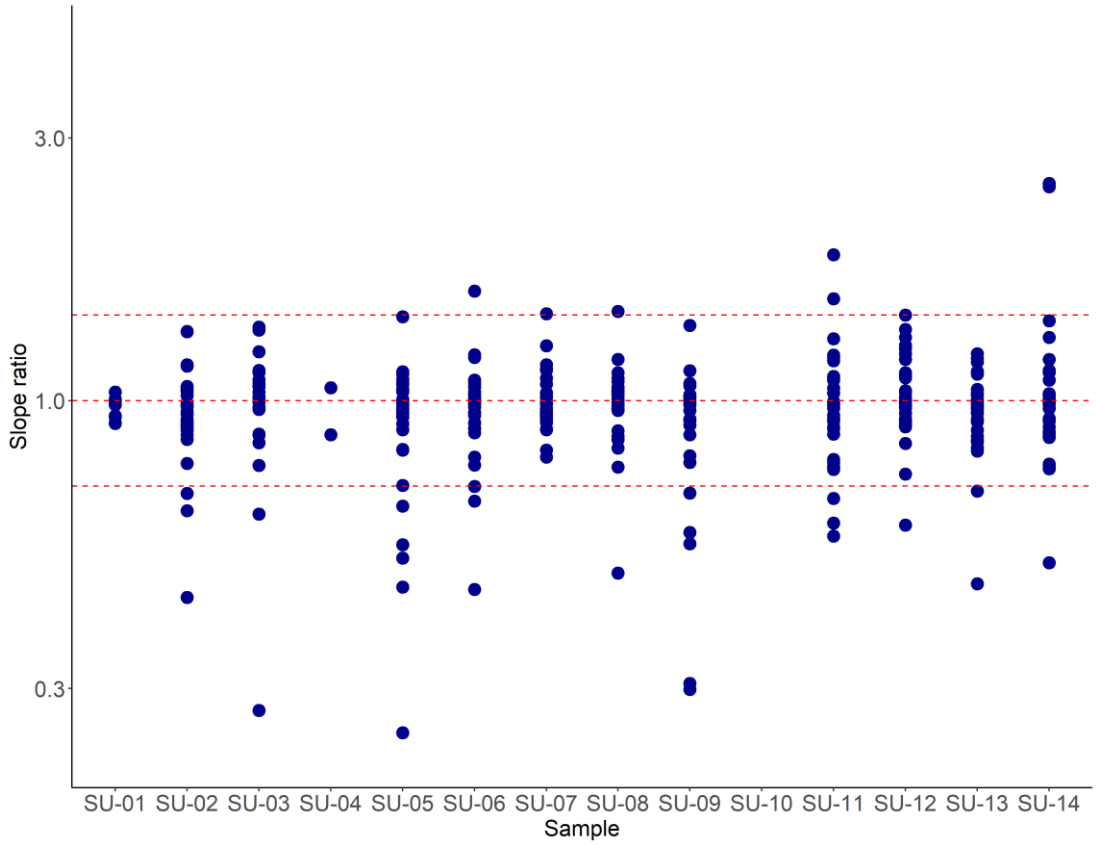


Figure 2. Harmonisation of binding antibody titres when reported as relative to the candidate IS or samples containing mAbs.
A. Binding antibody titres reported by participants. **B**-**F**. Binding antibody titres expressed as relative to the candidate International Standard (**B**), sample SU-11 (**C**), SU-12 (**D**), SU-13 (**E**) or SU-14 (**F**), with an arbitrary assigned unitage of 1000 IU per mL.

Figure 3. Harmonisation of neutralising antibody titres when reported as relative to the candidate IS or samples containing mAbs.
A. Neutralising antibody titres reported by participants. **B**-**F**. Neutralising antibody titres expressed as relative to the candidate International Standard (**B**), sample SU-11 (**C**), SU-12 (**D**), SU-13 (**E**) or SU-14 (**F**), with an arbitrary assigned unitage of 1000 IU per mL.

Figure 4. Slope ratio between the dose response curve obtained from the candidate WHO IS (SU-10) and individual samples, for quantitative binding assays.



Appendix 1. Collaborative study participants

(in alphabetical order by country, and by Institution within the same country)

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Appendix 2. Collaborative Study Protocol

Medicines & Healthcare products Regulatory Agency

WHO International Standard for anti-Sudan Virus Antibodies

Collaborative Study CS742

STUDY PROTOCOL

This multi-center International collaborative study aims to evaluate candidate preparations to serve as the First WHO International Standard (IS) for anti-Sudan virus (SUDV) antibodies and is organized by the MHRA on behalf of the World Health Organization (WHO). The study has been facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI) which sponsored the sourcing of candidate materials and the National Institute of Allergy and Infectious Diseases (NIAID) and Battelle, which sponsored the development of the WHO IS. ISs are recognized as the highest order of reference materials for biological substances, and they are assigned potencies in International Units (IU). The IS is used to quantify the amount of biological activity present in a sample in terms of the IU, making assays from different laboratories comparable. This makes it possible to better define parameters such as the analytical sensitivity of tests or clinical parameters such as protective levels of antibody. The availability of an IS for anti-SUDV antibodies will facilitate the standardisation of serological assays used to establish infection, epidemiology and vaccine responses. The establishment of such a standard will follow published WHO guidelines and be submitted for formal establishment by the WHO Expert Committee on Biological Standardization (ECBS) [1].

Aims

The aims of this WHO International collaborative study are to:

- assess the suitability of the candidate antibody preparations to serve as the International Standard with an assigned unitage per ampoule for use in the harmonisation of serology assays detecting anti-SUDV antibodies.
- characterise the antibody preparation in terms of reactivity/specificity in different assay systems
- assess commutability i.e. to establish the extent to which each preparation is suitable to serve as a standard for the variety of different samples and assay types
- recommend to the WHO ECBS, the antibody preparation(s) found to be suitable to serve as the standard and propose an assigned unit

Study Samples

All samples will be provided coded and blinded as described in Table 1. Laboratories will receive at least 3 sets of study samples which should allow for 3 independent assays.

The samples are individual or pools of serum, obtained from healthy donors who have been exposed to SUDV during the 2000 or 2012 SUDV outbreaks in Uganda. These samples have been kindly donated by Integrum Scientific, LLC, in partnership with the Uganda Virus Research Institute (UVRI). Anti-SUDV antibody negative samples were kindly provided by the National Health Service Blood and Transplant (NHSBT), UK.

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All samples collected by UVRI were tested and confirmed negative for SUDV RNA. All samples were tested for HIV-1, HBV and HCV markers and were found negative for HIV antigen (Ag) and HCV antibodies (Ab) using the Diasorin Liaison XL and Roche Cobas 6000 platforms. Samples SU-03 and SU-08 were found positive for HIV Ab and samples SU-05 and SU-09 were found positive for HBsAg. Importantly, all samples have been treated using a validated solvent-detergent treatment (Appendix I) which has been demonstrated to inactivate HIV, HBV and HCV [2]. All samples will therefore be shipped as non-infectious.

Sample SU-10 is a freeze-dried preparation, filled in 0.25 mL aliquots into 2.5 mL ampoules. This sample requires reconstitution by adding 0.25 mL of sterile deionised water (see instruction for use). The remaining samples are all liquid frozen and filled in 0.1 mL aliquots into screw cap tubes.

Four samples consist of serum/plasma spiked with a mix of monoclonal antibodies kindly provided by the University of Oxford. In order to receive these samples, please request your Technology Transfer Office to complete and sign the Material Transfer Agreement referenced "MHRA Research Reagents MTA CS742". To avoid potential long delays and expedite the initiation if this study, this MTA is non-negotiable. If your organisation is unable to sign this MTA as presented, but still would like to participate to our study, we will omit these 4 samples in the panel supplied. Characterisation of these 4 samples would be very valuable and we hope you will be able to accept the MTA as is, and test these 4 samples during this collaborative study.

Table 1. CS742 Study Sample Panel.

Sample Code	Formulation	Volume (mL) or mass (g)	Vial	Storage Temperature
CS742 SU-01	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-02	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-03	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-04	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-05	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-06	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-07	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-08	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-09	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-10	Freeze-dried (serum)	0.25	DIN Ampoule	-20°C
CS742 SU-11	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-12	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-13	Liquid frozen	0.1	Screw Cap Vial	-80°C
CS742 SU-14	Liquid frozen	0.1	Screw Cap Vial	-80°C

CAUTION: These preparations are not for administration to humans or animals in the human food chain. As with all materials of biological origin, the material should be regarded as potentially hazardous to health.

Assay Methods

For testing the study samples, participants are requested to use the method(s) in routine use in their laboratory for the detection of antibodies to SUDV. Laboratories may use multiple

methods to test the study materials, provided that the study design (see below) is followed for each method.

Design of study

Participants are requested to:

- Perform 3 independent tests on different days or by different operators for detection of antibodies against SUDV
- Reconstitute the freeze-dried sample according to the Instructions for Use (IFU) supplied with the sample shipment. Use a freshly reconstituted sample for each independent test
- For the liquid frozen samples, use a freshly thawed aliquot for each independent test. Each sample should be thawed at room temperature or 37°C and used immediately or placed on ice until use
- Due to limited volumes for some of the collaborative study samples, we may ask participants to use the same set of samples for multiple methods (*e.g.* using 20µL for ELISA and 50µL for neutralisation assay from the same 100µL aliquot). It is recommended to use reconstituted or thawed samples on the same day, but if this is not practical, they can be stored in a fridge for up to 5 days, if kept sterile.
- For each independent test, prepare a series of dilutions for each of the coded samples, using the sample matrix specific to the individual assay(s) (e.g. plasma, serum, buffer, media). **The optimal dilution range should cover at least 5 to 6 points including one point beyond the endpoint dilution.** Adjust dilutions accordingly for subsequent assays if needed. Record in the Excel spreadsheet changes to the dilutions tested.
- It is requested that samples are tested in duplicate, although additional replicates may be performed where sample volumes are sufficient and recorded within the Excel reporting sheet.
- If feasible, include all study samples in each assay so that the concentration of antibodies relative to one another may be calculated. Please note in the reporting sheet, if it is not practical to test all samples concurrently, indicate which samples were tested concurrently.

Results and data analysis

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

- Within the Excel reporting sheet, under 'Results Summary' record the qualitative (+ / -) and quantitative (endpoint titre/IC50 etc.) result for each of the coded study samples as per analysis in your laboratory. It is important for us to know whether the samples are considered 'positive' in each assay.
- Use the 'Raw data' section of the Excel reporting sheet to record for each dilution the raw assay readout (e.g. absorbance O.D./RLU/plaques/GFP%, etc.). 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 or an assay provides a readout against multiple targets, complete one reporting sheet per method/target.

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- Record in the Excel reporting sheet any deviations from the study protocol and complete all fields requesting additional method details.

The confidentiality of each laboratory is assured with each participant being anonymous to the other laboratories. Analysis of the study will assess the potencies of each material relative to each other, and the performance within the different assay methods.

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 most appropriate antibody preparation to serve as the First WHO IS for anti-SUDV antibodies. Participants' comments will be included in the report prior to submission to the WHO ECBS. Study participants will be notified of the outcome of the study after the WHO ECBS meeting.

Participation in the collaborative study is conducted under the following conditions:

- The study samples have been prepared from materials provided by donors and therefore must be treated as proprietary. The materials must not be used for any other purpose other than for this study;
- 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 organization/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, 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 of this study is at the participant's 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.

Deadline for completed results spreadsheets is <u>10 weeks</u> from receipt of study materials. If it is not practical to return results within 10 weeks, please inform Yann Le Duff.

All completed results spreadsheets should be returned electronically to yann.leduff@mhra.gov.uk.

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References:

[1] WHO, Recommendations for the preparation, characterization and establishment of international and other biological reference standards. WHO Technical Report Series, No. 932., in Expert Committee on Biological Standardization. 2006.

[2] Dichtelmüller et al., Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion, 49(9): p. 1931-43, 2009.

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Appendix I

Solvent Detergent Treatment for inactivation of HIV-1 in Plasma as an Indicator of Function in the Treatment of convalescent Ebola Patient plasma.

Introduction

Plasma from convalescent patients may contain blood borne viruses capable of replication which can pose a risk to people handling samples. To ensure the inactivation of enveloped viruses which may be in samples they can be treated with a mixture of a solvent and a detergent. This treatment has been shown to be effective against a number of viruses and has been used by blood services for the treatment of plasma packs. In this instance treatment is being done to reassure end users of double PCR negative convalescent Ebola plasma that no live virus is present.

This report describes the treatment of plasma spiked with HIV-1 IIIB for the inactivation of the virus and inhibition of infection on a T cell line, to prove the treatment process is effective.

For further reading see:

Dichtelmüller, H. O., Biesert, L., Fabbrizzi, F., Gajardo, R., Gröner, A., von Hoegen, I., Jorquera, J. I., Kempf, C., Kreil, T. R., Pifat, D., Osheroff, W. and Poelsler, G. (2009), Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion, 49: 1931–1943.

Method

Solvent detergent treatment

Plasma samples are treated by the addition of 1% v/v Tributyl phosphate (TBP) and 1% v/v Triton X-100, and incubated at 30°C for 3 hours, with mixing every 15 minutes. 10% v/v soybean oil is added and samples mixed for 30 minutes at room temperature to emulsify the detergent. This is then centrifuged at 3000rpm for 30 minutes at room temperature to separate the oil/detergent layer from the plasma. The plasma is removed from below the oil layer without disturbing the interface, and the solvent removed using C18 reverse phase chromatography columns (which are activated using methanol, and rinsed in sterile water first). Using a vacuum manifold, 20 ml of oil free plasma sample is added to each column and run through at 0.2bar. This was repeated twice for each sample to ensure that the solvent was fully removed.

Infectivity assay

Normal human plasma was spiked with HIV-1 IIIB grown on C8166 cells, and then split into two samples. One sample was solvent detergent treated as per the method described, and the second left untreated.

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The two samples were titrated 10 fold down to a dilution of 10 9 on C8166 cells in duplicate 24 well plates. Each plate had 4 replicates of each titration, as well as 4 wells of negative control cells which were mock infected with media alone. The cells were incubated and grown for 14 days. During the course of the test the cells were checked for cytopathic effect (CPE) visually and the wells showing CPE were recorded. For the detection of gag protein as a marker of active replication when high levels of CPE were seen for this study this was at 14 days post infection) media was removed and samples of all cell wells were taken and lysed in NP-40 detergent with 1% trypan blue.

After 48 hours these samples were tested with an in house antigen capture assay for HIV-1 Gag p24 protein (see appendix 2.3)

Results

Both the visual CPE recording and gag antigen detection methods demonstrated that the cells were only infected by the untreated HIV- spiked plasma.

Visually the cells from the treated samples were all healthy and growing after 12 days, with no apparent CPE and no evidence of viral infection. By comparison the cells from the treated samples were very damaged and sparse by 12 days with evidence of CPE that gave a calculated TCID50 of approximately 6.5 (see appendix 2.2 for method of calculation)

The antigen capture assay appears less sensitive than visual CPE, but gives a definite non biased measure of viral infection.

Figure 1 shows the treated samples have no measurable Gag protein after 14 days at any dilution of the virus in any of the 8 eight replicates tested. The untreated samples shown in figure 2 however have p24 which is measurable in some wells down to a dilution of 10-3 in some case. Calculating the TCID50 in the same manner as for the visual method gave a result from the ACA of approximately 3.5.

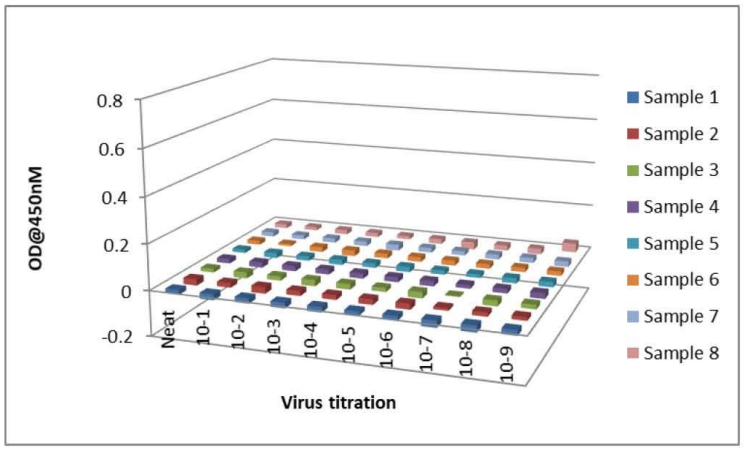

Figure 1. Titration of solvent detergent treated HIV-1 IIIB on C8166 cells. Eight samples of each dilution were tested across two 24 well plates. Cells were incubated with virus for 14 days, with media changes on days 7 and 10.

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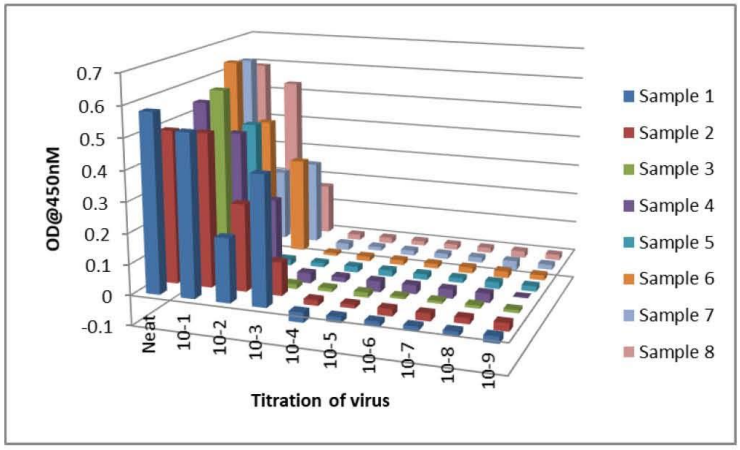

Figure 2. Titration of untreated HIV-1 IIIB on C8166 cells. Eight samples of each dilution were tested across two 24 well plates. Cells were incubated with virus for 14 days, with media changes on days 7 and 10.

Conclusions

There is no evidence of HIV infection in cells exposed to virus in plasma after solvent detergent treatment either by visual inspection or by gag p24 antigen capture.

The level of infection seen in the titration of the non-treated samples shows that the method reduced infectivity by at least 3 logs, and up to possibly 6 logs.

Appendix 3. Proposed Instruction for Use (24/124_BA)

WHO International Standard
First WHO International Standard for Sudan virus antibodies for binding assays (human serum)
NIBSC code: 24/124_BA

Instructions for use

1. INTENDED USE

The First WHO International Standard for Sudan virus (SUDV) antibodies for binding assays (human serum), is the freeze-dried equivalent of 0.25 mL of pooled serum obtained from 14 individuals who recovered from Sudan Virus Disease (SVD). The preparation has been evaluated in a WHO International collaborative study [1]. The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting anti-SUDV GP binding IgG. The preparation has been solvent detergent treated to minimise the risk of the presence of enveloped viruses [2].

2. CAUTION

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-HIV Ab, HIV Ag, HCV RNA and anti-HCV Ab. 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 SUDV antibodies for binding assays (human serum) is 250 IU/ampoule for anti-glycoprotein IgG. These values have been arbitrarily chosen and do not reflect the proportion of the antibody activities 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 for anti-glycoprotein IgG.

4. CONTENTS

Country of origin of biological material: Uganda. Each ampoule contains the freeze-dried equivalent of 0.25 mL of pooled human sera.

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.

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] Le Duff *et al*. Collaborative Study for the Establishment of the First WHO International Standard for Sudan virus antibodies. 2025 WHO Expert Committee on Biological Standardization. WHO/BS/2025.XXX
[2] Dichtelmüller, H.O., et al., Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion, 2009. 49(9): p. 1931-43.

10. ACKNOWLEDGEMENTS

We would like to wholeheartedly thank the anonymous donors of the serum samples for their consent which has allowed this study to be undertaken. We would like to express our gratitude to Julius Lutwama and his team at the Uganda Virus Research Institute (UVRI), Uganda, Wendy Boone and the team at Integrum Scientific, USA. We would like to thank the team at Battelle for their valuable contribution throughout the project and the teams at the Coalition for Epidemic Preparedness Innovation (CEPI), at the National Institute of Allergy and Infectious

Diseases (NIAID) and at the Biomedical Advanced Research and Development Authority (BARDA) for providing advice during this study. We would like to acknowledge the support from CEPI (project ID PRJ-6859) for the collection of samples and from the NIAID, (task Order No. 75N93023F00001 / TO V17), for the preparation of the International Standard and the evaluation of the candidate IS through the collaborative study. We gratefully acknowledge the important contributions of the collaborative study participants. We would also like to thank MHRA Manufacturing and Logistics teams for the formulation and distribution of materials.

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.

WHO/BS/2023.2450 Page 33 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): 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: 0.25 g Toxicity Statement: Non-toxic Veterinary certificate or other statement if applicable. Attached: Not Applicable WHO/BS/2023.2450 Page 34

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 (24/124_NT)

WHO International Standard
First WHO International Standard for Sudan virus antibodies for neutralisation assays (human serum)
NIBSC code: 24/124_NT

Instructions for use

18. INTENDED USE

The First WHO International Standard for Sudan virus (SUDV) antibodies for neutralisation assays (human serum), is the freeze-dried equivalent of 0.25 mL of pooled serum obtained from 14 individuals who recovered from Sudan Virus Disease (SVD). The preparation has been evaluated in a WHO International collaborative study [1]. The intended use of the International Standard is for the calibration and harmonisation of neutralisation assays against SUDV. The preparation has been solvent detergent treated to minimise the risk of the presence of enveloped viruses [2].

19. CAUTION

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-HIV Ab, HIV Ag, HCV RNA and anti-HCV Ab. 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.

20. UNITAGE

The assigned potency of the WHO International Standard for SUDV antibodies for neutralising assays (human serum) is 250 IU/ampoule for neutralising antibodies. These values have been arbitrarily chosen and do not reflect the proportion of the antibody activities 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 for neutralising antibodies.

21. CONTENTS

Country of origin of biological material: Uganda. Each ampoule contains the freeze-dried equivalent of 0.25 mL of pooled human sera.

22. 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.

23. DIRECTIONS FOR OPENING

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24. 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.

25. STABILITY

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26. REFERENCES

[1] Le Duff *et al*. Collaborative Study for the Establishment of the First WHO International Standard for Sudan virus antibodies. 2025 WHO Expert Committee on Biological Standardization. WHO/BS/2025.XXX
[2] Dichtelmüller, H.O., et al., Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion, 2009. 49(9): p. 1931-43.

27. ACKNOWLEDGEMENTS

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28. FURTHER INFORMATION

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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

29. 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

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31. MATERIAL SAFETY SHEET

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

WHO/BS/2023.2450 Page 33 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): 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.

32. 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.

33. 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: 0.25 g Toxicity Statement: Non-toxic Veterinary certificate or other statement if applicable. Attached: Not Applicable WHO/BS/2023.2450 Page 34

34. 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.