

**EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 18 October to 21 October 2021****Establishment of the first WHO International Standard and Reference
Panel for anti-Lassa Fever virus antibody**

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NOTE:

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Summary

Lassa virus (LASV) is a zoonosis endemic in several Western African countries, causing seasonal outbreaks. LASV has been identified by the WHO R&D Blueprint as one of the top ten priority pathogens for its outbreak potential. Vaccines and treatments are in development and reliable assays are needed for their evaluation. The availability of an International Standard (IS) for antibody would facilitate the standardisation of LASV serological assays and enable the development of vaccines and therapeutics, as well as contributing to improving our understanding of virus epidemiology. In this report a pool of plasma from Lassa fever recovered patients was evaluated in 36 methods by 17 laboratories worldwide for its suitability as an International Standard for anti-LASV antibody. These include neutralisation assays, either using live LASV or pseudotyped virus systems, and binding assays directed against the glycoprotein, the nucleoprotein or a combination of them. The candidate preparation sample L4, NIBSC code 20/202, was assessed as part of a blinded sample panel which also included the candidate WHO International reference panel, a working standard for LASV antibody (made available at the beginning of 2020 to vaccine developers) and two cocktails of monoclonal antibodies. The candidate International Reference panel includes two high antibody titre preparations from Nigeria (20/228) and Sierra Leone (20/244), a mid titre pool from Nigeria (20/226), convalescent plasma from one individual with mid titre neutralising, but high titre binding antibodies (20/204), and three low titre pools from Nigeria (20/226) and Sierra Leone (20/246, 20/248). The ranking of the panel was similar but not identical between participants even within the same type of method. The low titre preparations represented a challenging sample for some of the assays to detect, while the high titre pools were consistently detected in all assays. This makes the candidate reference panel a useful tool for the development and assessment of serological assay sensitivity for LASV antibodies. The candidate International Standard 20/202 was found fit for purpose in all the assays. Expressing LASV antibody titres of the collaborative study samples as relative to the candidate IS, sample L4, affords a reduction in

the inter-laboratory variability and allows for better comparability of the results for both neutralisation and binding methods. As shown by some reference panel members, the composition of neutralising and binding antibodies may vary. Therefore we propose that sample L4, NIBSC code 20/202, serves as the IS for different methods with an arbitrary assigned unitage of 25 IU/ampoule for neutralising activity, 250 IU/ampoule for anti-GP binding activity and 250 IU/ampoule for anti-NP binding activity. Secondary reagents should be calibrated to the IS in the type of assay they are used and may have different potencies of neutralising and binding antibody.

Introduction

Lassa fever virus (LASV) is the aetiological agent of Lassa fever transmitted to humans by infected *Mastomys* spp. rats, as well as person-to-person contact with contaminated body fluids. The virus is endemic in western Africa where it causes recurrent outbreaks. While it has been reported that 80% of infections may be asymptomatic, it can result in severe haemorrhagic fever [1]. The case fatality rate (CFR) in these severe cases, has been reported to be up to 60-70% in previous outbreaks within Nigeria [2] and Sierra Leone [3]. The disease incidence has been increasing each year, with more than 800 confirmed cases within Nigeria in 2019 with a CFR of 21% [4]. The most recent outbreak was recorded in Nigeria in February to April 2020 with 963 confirmed cases and 188 deaths (CFR=19.5%) [5]. There is a high level of sequence diversity amongst LASV strains, which have been grouped phylogenetically into 7 lineages [6-8]. The different lineages are spatially segregated, with lineage II and III strains circulating within Nigeria and lineage IV within Sierra Leone, as well as Guinea and Liberia.

As identified in the WHO R&D Blueprint, there is a lack of, and therefore a need, to develop effective treatments and vaccines against LASV [9,10]. Analysis of vaccine potency and efficacy will include the requirement of assays to measure the antibody response. The availability of an antibody reference reagent for these assays early in this process is of value, allowing assay harmonisation by calibration to the reference which is given an arbitrary unitage. It assists better definition of parameters such as analytical sensitivity of tests or clinical parameters such as protective levels of antibody. The reference material will enable the comparison of Lassa serological data reported between laboratories and at different stages of vaccine efficacy clinical trials. Currently, the Coalition for Epidemic Preparedness Innovations (CEPI) is funding the development of six vaccines against LASV [11].

WHO International Standards (IS) are recognised as the highest order of reference materials for biological substances and they are assigned potencies in International Units (IU). International Standards are used to quantify the amount of biological activity present in a sample in terms of the IU, allowing assays from different laboratories to be compared and the results rendered comparable. The availability of an IS for anti-LASV antibodies would facilitate the standardisation of serological assays used for detection of exposure to LASV and in vaccinology studies to measure antibodies elicited by human vaccination. In October 2018, the WHO ECBS endorsed the preparation of the International Standard for anti-Lassa virus antibodies [12].

Within this Collaborative study, pools of convalescent sera or plasma collected from either Sierra Leone or Nigeria with different anti-LASV antibody titres, including a negative control, were tested by participants in serological assays that are in routine use in their laboratories. A

candidate preparation to serve as the First WHO International Standard for anti-LASV antibodies and a Reference Panel have been evaluated. The Collaborative study was organised by NIBSC in collaboration with the WHO, and has been facilitated by CEPI, who conducted outreach to scientists in Lassa fever-affected countries, and subsequently sponsored the sourcing and formulation of the candidate material.

The aims of this WHO International collaborative study are to:

- Assess the suitability of the candidate to serve as the International Standard for anti-LASV antibody with an assigned unitage per ampoule for use in the harmonisation of Lassa fever serology assays.
- Characterise the antibody preparations in terms of reactivity/specificity in different assay systems, to serve as a reference panel for anti-Lassa virus antibodies.
- Assess each preparation's potency i.e. readout in a range of typical assays performed in different laboratories.
- Recommend to the WHO ECBS the antibody preparation found to be suitable to serve as the International Standard and propose an assigned unit
- Advise WHO ECBS on the establishment of a WHO Reference Panel for anti-Lassa virus antibody.

Materials and Methods

Ethical statement

Convalescent plasma and serum from Lassa fever survivors was kindly donated by Prof. Robert Garry (University of Tulane, USA) on behalf of the Viral Hemorrhagic Fever Consortium and Dr. Ephraim Ogbaini-Emovon (Irrua Specialist Teaching Hospital, Nigeria) and Prof. Stephan Günther (Bernhard Nocht Institute for Tropical Medicine, Germany). Collection of human plasma or serum was approved by the following organization: Tulane University's Human Research Protection Program, the Sierra Leone Ethics and Scientific Review Committee, ISTH Research and Ethics Committee.

Study samples

Candidate WHO International Standard for anti-Lassa virus antibody

The candidate International Standard for anti-LASV antibody (NIBSC code 20/202) is a freeze-dried preparation of a pool of plasma from six Lassa fever-recovered patients from Nigeria. Prior to receipt at NIBSC, to fulfil local health and safety requirements, the source material was tested for presence of LASV RNA using the Qiagen viral RNA mini extraction kit followed by the Altona RealStar Lassa Real-Time RT-PCR 2.0 within containment level 4 facilities at Public Health England (Colindale, UK) and found negative. Donations from each patient were processed at NIBSC using a solvent-detergent treatment to minimise the risk of the presence of enveloped viruses [13,14]. Additionally, they were tested for known blood borne virus markers (HIV antibodies, HBsAg and HCV RNA) and found to be negative. Approximately 3500 2.5mL glass DIN ampoules containing 0.25 mL pooled plasma were lyophilised on 4th September 2020.

Freezing was performed in a CS100 freeze drier to -50°C for 4 hours. Primary drying was performed at -35°C for 40 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. Ampoules were back filled with dry nitrogen to atmospheric pressure and sealed.

Candidate WHO Reference Panel for anti-Lassa virus antibody

The seven candidate panel members comprise freeze-dried preparations of pools of convalescent plasma from Lassa fever-recovered patients from either Sierra Leone or Nigeria. Prior to receipt at NIBSC, the source material was tested for presence of LASV RNA using the Qiagen viral RNA mini extraction kit followed by the Altona RealStar Lassa Real-Time RT-PCR 2.0 within containment level 4 facilities at Public Health England (Colindale, UK) and found negative. Donations from each patient were processed at NIBSC using a solvent-detergent treatment to minimise the risk of the presence of enveloped viruses [13,14]. Additionally, they were tested for known blood borne virus markers (HIV antibodies, HBsAg and HCV RNA). All panel members were found negative for HCV RNA, 20/246 mid/low titre from Sierra Leone was found positive for HIV antibodies, and three panel members (20/244, 20/246 and 20/248) were positive for HBsAg. As the material was solvent-detergent treated, the panel members were distributed as non-infectious. The reference panel was composed of two high titre samples, NIBSC code 20/228 from Nigeria and 20/244 from Sierra Leone; a mid-titre sample from Nigeria, 20/226, and a high binding, mid neutralising antibody titre member from Nigeria 20/224. The candidate reference panel also include low antibody titre preparations, 20/246 from Sierra Leone, and 20/222 from Nigeria; and a very low titer convalescent plasma pool from Sierra Leone 20/248. The definition of high, medium and low titre were relative to these group of samples as obtained in the assays (PV-neutralisation and ELISA) performed at NIBSC. Approximately 600 2.5mL glass DIN ampoules containing 0.25 mL pooled plasma for each panel member were lyophilised between 8th October and 27th November 2020 using the same 4-day cycle as per the candidate International Standard described above.

Additional samples included in the collaborative study

A LASV-antibody negative serum pool from Sierra Leone was provided as negative control as a liquid preparation, frozen and filled in 0.1mL aliquots in screw cap tubes.

Two mixtures of human anti-LASV monoclonal antibodies (mAb) isolated from Lassa fever survivors were kindly provided by Dr Luis Branco (Zalgen Labs, USA) [15]. LASV mAb mixtures contained both IgG and IgM against LASV nucleoprotein (NP) and glycoprotein (GP); mAbs cocktail 1 contained a mix of seven mAbs, while mAbs cocktail 2 was a reduced mix limited to only four mAbs of the mix 1 (Appendix 2). The mAb were produced in HEK-293T/17 or NS0 cells and purified by affinity chromatography. The purity by SDS-PAGE was higher than 90%. Both mAb cocktails were formulated in PBS with 0.1% bovine serum albumin, pH 7.4 at the final concentration of 0.25 mg/mL of each antibody. These samples were provided frozen and filled in 0.1mL aliquots in screw cap tubes.

Finally, the working standard LASV-3, developed in 2019 in a joint effort between NIBSC and CEPI (Appendix 3) was included as an additional sample in the collaborative study. This is a pool of convalescent serum from Lassa fever recovered patients from Nigeria with a high antibody titre. It was available to CEPI partners to assist in the development of serological

assays for anti-LASV antibodies. The inclusion of LASV-3 in this study will allow assignment of a unitage relative to the International Standard. This will enable users to back-calibrate their assays where the reagent has been used.

Coded study samples

Table 1 lists the collaborative study samples, provided coded and blinded to the participants. For each method in use in their laboratory, participants received 3 sets of the 12 samples, plus one additional set as a spare. One participant did not receive sample L9, as the stock was low. Samples were labelled CS686 Sample L1 to L12. Samples were shipped on dry ice under NIBSC reference CS686.

Participants

Twenty-two laboratories agreed to participate in the study; however one participant did not received the collaborative study samples due to delays issuing the import permit. Four laboratories did not return results in time for inclusion in the collaborative study report. The seventeen participants providing results were from seven countries: France (2), Germany (2), Italy (2), Japan (1), Switzerland (1), United Kingdom (4) and United States of America (5). All laboratories are referred to by a code number randomly allocated and not reflected in the order presented in Appendix 1. Participating organisations included vaccine developers, national control/reference laboratories, other government institution, diagnostics laboratories, kit manufacturers, non profit research organization and academic laboratories.

Study design

The collaborative study protocol is given in Appendix 4. The study took place between January and May 2021 during the COVID-19 pandemic, which added delays in the shipping of the study samples and may have contributed to some participants not returning their data in time to be included in this report. Participants were requested to test the study samples using their established method(s) for the detection of antibodies against Lassa virus. Participants were asked to perform three independent assays, and for each assay at least two independent serial dilutions of the study samples. 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 12 weeks of receipt of materials.

Assay methods

Assays used by the participants are summarized in Table 2. Where laboratories performed multiple assay methods or had multiple targets, laboratory codes are followed by a letter indicating the different methods e.g. laboratory 1a, 1b. Two participants returned three sets of data in partnership under one laboratory code, which explains why there are 16 laboratories and 17 participants.

Thirty-six methods were used to evaluate the samples. Neutralising antibodies were quantified in sixteen neutralisation assays using either live virus (n=8) or pseudotyped virus (PV) assays (n=8); the latter included non-replicative vesicular stomatitis virus (VSV)-based pseudotypes (n=3) , replication competent chimeric VSV (n=3) and Human Immunodeficiency virus (HIV)-

based pseudotyped virus (n=2). In all the PV-based neutralisation assays the glycoprotein (GP) used derived from the Josiah isolate, lineage IV, except for one laboratory which used the GP from AV isolate (lineage V). In the live virus neutralisation assay, six laboratories used the Josiah isolate, the other two isolates used were 101LV18 (lineage II) and GA391 (lineage III). Results were returned for sixteen ELISA methods, this include commercially available (n=8) and in house methods (n=9) with colorimetric readouts. Binding antibodies titres were also measured by immunofluorescence assays (n=3). Thirteen methods detected immunoglobulin G, and five methods were specific for immunoglobulin M. One laboratory used a Double Antigen Binding Assay (DABA) which detect all class of antibodies. One laboratory tested the sample by surface plasmon resonance, which detects all classes of antibodies.

Stability study of the candidate International Standard

Stability of the lyophilised ampoules of the candidate International Standard sample L4, NIBSC code 20/202, was assessed in an accelerated thermal degradation study. Fifteen ampoules of sample 20/202 were stored at each of the following temperatures -20, +4, +20, +37 and +45°C. Three ampoules for each temperature were retrieved at the following time points: one month, three months, and 6 months. The final three ampoules for each temperature will be retrieved at one year (19th October 2021) time point. The potency of the preparations relative to the baseline, -20°C sample, were assessed by pseudotyped based neutralisation assay using a vesicular stomatitis virus (VSV) vector expressing the GP from the Josiah isolate (lineage IV). Relative potencies were calculated by sigmoid curve analysis using European Directorate for the Quality of Medicines & Healthcare (EDQM) software CombiStats™ [16]. The stability of the candidate material was assessed using the Arrhenius model for accelerated degradation studies with potencies expressed relative to the samples stored at -20°C [17]. Due to the increased workload because of the concurrent COVID-19 pandemic, the predicted stability of the individual members of the candidate Reference Panel is inferred from the stability of the candidate International Standard as these samples were freeze dried using the same protocols.

LASV GP-pseudotyped VSV-based neutralisation assay

The potency of neutralising anti-LASV antibodies in the samples was assessed using non-replicative, VSV particles expressing LASV GP (Josiah isolate) on their surface and containing firefly luciferase as a marker gene for infection. Briefly, 96-well plates were seeded with 40,000 VERO E6 cells per well 2-4 hours before the neutralisation assay in 0.1 mL/well Dulbecco's modified essential medium, supplemented with 10%(v/v) foetal calf serum and penicillin/streptomycin. Samples were 2-fold serially diluted in culture medium starting at 1/20. LASV- pseudotyped VSV was added to each well at a concentration of 400 TCID₅₀/well and incubated for 1 hour at 37°C. The antibody-pseudotyped virus complex was then added to the cells, and incubated for 24 hours. Infection was detected by adding the firefly luciferase substrate (Promega BrightGlo) and luminescence was recorded by GloMax Navigator (Promega).

Statistical methods

For the neutralisation assays, the geometric mean (GM) of the potency of each sample was calculated from the endpoint titres or 50% reduction neutralisation titres (NT₅₀) 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 the software CombiStats™. 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 0.80 – 1.25. Relative potency estimates from all valid assays were combined to generate an unweighted GM for each laboratory and assay type, with these laboratory means being used to calculate overall unweighted geometric means for each analyte.

Variability between laboratories has been expressed using geometric coefficients of variation ($GCV = [10^s - 1] \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates) and the ratio of the upper quartile to lower quartile of the estimates. Variability was also assessed by calculating the percentage of laboratory GM estimates within 2-fold of the overall sample median.

Results

Production of the candidate WHO IS and Reference panel

In September 2020, NIBSC filled and freeze-dried the candidate International Standard, NIBSC code 20/202 and the Reference Panel members (NIBSC codes 20/204, 20/226, 20/228, 20/244, 20/246, 20/248) using documented procedures. The product summary for the candidate WHO IS, sample L4, is shown in Table 3, and for the Reference Panel in Table 4. The mean residual moisture of the two panel members 20/228 (L2, high titer) and 20/246 (L1, mid/low) were higher than the ideal 1%, but a higher moisture content can be acceptable if the final product is proven stable [18]. The residual oxygen content of the candidate IS and the reference panel members fell below the NIBSC working limit of 1.1%. All microbiological tests for bacterial and mould/yeast colony count returned negative. The blood virology report for the samples from Sierra Leone 20/244, 20/246 and 20/248 were all positive for HBsAg, and samples 20/246 was also positive for anti-HIV antibody. As all the samples were solvent-detergent treated, they are shipped as non-infectious, although all material of human origin should be treated with caution in accordance with local regulations.

Collaborative study data received

The collaborative study under NIBSC reference CS686 started on 25th January 2021. The first set of results were received on 23rd February 2021 and the last data on 11th June 2021. Seventeen participants returned results from 36 methods (Table 2). These methods comprised 8 live virus and 8 pseudotyped virus neutralisation assays, 15 direct or indirect ELISAs, one double antigen bridging assay (DABA), 3 immunofluorescence assays, and one surface plasmon resonance method.

Two participants returned three sets of data under one laboratory code. Lab 13 did not receive sample L9 due to low stock. All laboratories returned results from three independent assays, with the exception of lab 8, which performed the test twice and lab 14a which performed one experiment, due to time constraints.

Potency of neutralising antibodies in the collaborative study samples were assessed by live and pseudotyped virus neutralisation assays. For the live neutralisation assay, 6 out of 8 laboratories used the Josiah isolate, lineage IV. The two remaining laboratories used the GA391, lineage III (lab 4b) and 101LV18, lineage II (lab 9b). The majority of the participants performed a focus forming reduction neutralisation assay (lab 4a, 5a, 9b, 9c, 13, 14c) and the antibody titre was calculated as 50% neutralisation titer. Lab 8a and 15a reported the data as an endpoint titre, expressed as the inverse of the highest dilution factor which produced a positive result by observing cytopathic effect.

Three systems were used for the pseudotyped virus neutralisation assays. Two versions of a VSV-based pseudotype were employed: a non-replicative VSV system, where the VSV glycoprotein gene is replaced with the firefly luciferase gene (lab 2b, 2c, 5b). and a replication-competent VSV either expressing green fluorescent protein (lab 11c and 12) or without a marker gene (lab 15b). The remaining two laboratories, 1 and 2a, used a non-replicative lentiviral vector carrying the LASV GP on their surface and firefly luciferase as the reporter gene. In all cases, the data were reported as 50% neutralisation titre. Lab 2 was the only lab using GP derived from two LASV isolates Josiah (2b) and AV, lineage V (2c). The GP used for pseudotyping by all other labs derived from the Josiah isolate.

Neutralisation assays

Table 5 shows the geometric means of the neutralisation results as provided by the participants. The neutralisation assays are divided based on whether a live virus or a pseudotyped virus was used. All the laboratories correctly identified sample L11 as negative. The candidate International Standard sample L4 was detected in all assays. For the candidate Reference Panel, the low titer sample L8 from Sierra Leone and L3 from Nigeria were detected in 9 and 10 out of 16 assays, respectively; the low/mid titer sample L1 from Nigeria was scored positive in 12 out of 16 assays. Sample L1 which tested positive for anti-HIV antibodies was still the lowest sample after L8 in the HIV-based pseudotyped neutralisation assays, suggesting that the presence of anti-HIV antibodies do not generate false results in a neutralisation assay for LASV. Three laboratories failed to detect neutralising antibodies in the mid titer sample from Nigeria L5 and the mid titer sample L6 and high titer sample L7 from Sierra Leone were not detected in just one assay (lab 8a). The high titer sample L2 from Nigeria was scored positive in all assays. The ranking of the Reference Panel varied across assays with no identifiable link to a type of assay or lineage of the virus stock/GP used (Figure 1). Sample L7, had the highest titre in 53% of the assays, and was ranked second in 40% of the assays, with one lab unable to detect neutralising antibodies in the sample. In the majority of the assays (60%), sample L2 from Nigeria was scored the second highest titre, and 33% of the laboratories ranked it as the highest titre sample. Lab 5a scored sample L6 the highest followed by L7 and L2, but 47% of the laboratories ranked L6 as the third highest sample and 27% as fourth. Similar proportions were observed for L5, which was mainly scored as fourth ranking sample (47% of labs) and L1 as fifth ranking sample (40% of labs). Samples L3 and L8 were the lowest ranked samples with identical numbers of labs scoring them negative, with L3 overall ranking slightly higher. The two monoclonal antibody cocktails were the highest titre samples in all the assays. The working standard made available to CEPI partners at the beginning of 2020, sample L9, was scored negative in 4 out of 16 assays, and in most assays had an antibody titre similar to the mid titer sample L6.

Neutralising antibody titres expressed relative to the candidate International Standard

The neutralisation titres for each sample were expressed relative to sample L4, the candidate IS (Table 6). For each sample, the spread of the results between participants was wider when reported as absolute titres than when expressed relative to the candidate IS (Figure 2). To ease the comparison in the figure the candidate International Standard was arbitrarily assigned a unitage of 100 International units/mL (IU/mL) to allow the data to be plotted on the same y-axis scale. The increased agreement of the results was seen when analysing the live virus neutralisation assays (Fig 1A-B) or pseudotyped virus neutralisation assays alone (Fig 1C-D) or combining the two (Fig 1E-F). The reduction in the inter-laboratory variation by expressing the titres relative to the candidate International Standard is also summarised in Table 7. Three parameters were analysed for the titres as reported by the participants and the value relative to the candidate IS: 1) the percentage of the coefficient of variation (%GCV; the lower the percentage, the smaller the difference between laboratories); 2) the percentage of laboratories with a GM within 2-fold of the median (Lab GM:Median<2, the higher the percentage, the greater the agreement between labs); 3) the ratio of upper and lower quartile which represents the inter-quartile range of potencies (UQ/UL, values closer to 1 represent a smaller range of potencies and better agreement between labs). For all three parameters and for every sample, expressing the data relative to sample L4 gave a reduction in %GCV, an increase in GM:Median<2 and decrease in UQ/LQ; all indicative of a reduction in the inter-laboratory variation.

Binding antibody immunoassays

The geometric means of the binding immunoglobulin G titres as reported by the participants are summarised in Table 8. The two main formats used were enzymatic immunoassay (EIA) and immunofluorescence assay (IFA). The two target antigens were LASV glycoprotein (GP) or nucleoprotein (NP). In the IFA methods, one laboratory, lab 8b, used the whole virus lysate as antigen, while lab 3c used a combination of GP/NP and lab 4c used an anti-NP specific assay only. For the EIA methods, seven laboratories targeted GP and three laboratories NP. All the participants reported the results as the inverse of the highest positive dilution factor. All the laboratories correctly scored negative sample L11. The candidate International Standard sample L4 was detected in all the assays. Based on the absolute titres returned by the participants, most of the convalescent plasma samples were ranked similarly in the EIA for both GP and NP and in the IFA (Figure 3). Sample L6, with a mid level of neutralising antibodies contained the highest level of binding antibodies, followed by sample L7, high titre from Sierra Leone; samples L2 (high, Nigeria) and L4 (WHO IS) scored similarly, both ranking in the middle. Among the low neutralising antibody titre samples, L3 was slightly higher than L1 and sample L8 was the lowest titre sample. Sample L9 ranked in the middle in the anti-GP EIA and IFA methods but was the highest for anti-NP antibody content. Sample L5 which contained mid/low levels of neutralising antibodies, had the highest titer of anti-GP antibodies among the convalescent plasma pools, and a mid titre for anti-NP antibodies. The titres of the two mAbs cocktails L10 and L12 were in most cases the highest by over 10-fold, with only one exception for sample L10 (lab 16). Sample L12, a cocktail of seven antibodies, had a higher potency than sample L10 (a mix of four antibodies) in all assays (Appendix 2).

Binding antibody titres expressed as relative to the candidate International Standard

LASV antibody titres from the binding antibody methods were expressed relative to the sample L4, IS (Table 9). Two laboratories 4c and 8b did not report their results or did not perform serial dilutions of the samples and the calculation of the relative potency by parallel line analysis was not possible; in this case, the ratio between the value provided by the participant for the sample over sample L4 was calculated (in red, Table 9) but their data were excluded from further statistical analysis. A small number of results were excluded for non-parallelism (NP, table 9) using the criteria described in the Statistical Methods for the analysis of this study. In one case, samples L7, L8, L9 were run on a different plate than the candidate IS, L4 and the relative potency calculation was not possible (n/a Lab 6). Although in this study all the participants had expressed the binding antibody titre as the inverse of the highest dilutions above cut-off, still the absolute titres were around 200-fold different (*e.g.* Lab 6 vs lab 11b, Table 8). Reporting the data as relative to the candidate IS, sample L4, reduced this difference to less than 6-fold (Figure 4). To ease the comparison in the figure the candidate International Standard was arbitrarily assigned a unitage of 1000 International units/mL (IU/mL) to allow the data to be plotted using the same y-axis scale. Tables 10 and 11 show the reduced inter-laboratory variation for each sample as %GCV, Lab GM:Med <2 and UQ/LQ for the EIA methods for anti-GP and anti-NP, respectively. For the IFA, as only one laboratory returned results suitable for the calculation of the relative titre by parallel line analysis, there were not enough datasets to perform statistical analysis. Table 12 combines the data from all of the binding assays, showing an evident reduction of inter-laboratory variation for the samples. For six out of eight pools of convalescent plasma, 100% of laboratories showed a geometric mean potency within 2-fold of the median when reported as relative to the candidate International Standard.

As per the neutralising antibody titres, there was no clear pattern associated with the different lineages. Lab 11 generated the highest absolute antibody titres for anti-GP antibodies, with similar titres when using a GP derived from lineage II or lineage IV (Table 8 and Figure 4). Lab 6, using a GP derived from lineage III, obtained the lowest absolute titres, but in every case, expressing the potencies as relative to sample L4 produced similar values within each binding assays.

Determination of IgM in the collaborative study samples

Five laboratories lab 3d, 3e, 3f, 8c, 14a used assays for the detection of IgM (Table 13). Lab 3 used an EIA for the detection of anti-GP (3d), anti-NP (3e) or a combination of GP/NP (3f); lab 8 and 14 determined anti-LASV IgM in their IFA against a whole virus lysate. All the assays correctly identified sample L11 as negative. The cocktail of mAbs were scored highly positive in all the assays. The high titre pools of convalescent plasma samples L2, L7 and the candidate International Standard L4 were negative in all assays. The results for the other Reference Panel members were inconsistent between laboratories. The mid titre sample L6 was positive in all the EIA (lab 3d, 3e, 3f) but negative in both the IFA (lab 8c and 14a). Samples L1 and L5 were only positive in the EIA for anti-GP and for the anti-GP/NP; the low titre samples L3 and L8 were only positive in the anti-GP EIA. Only sample L9, the CEPI working standard, was positive in one of the two laboratories performing the IFA (14a) and also in the anti-NP and anti-GP/NP EIA (lab 3e and 3f).

Surface Plasmon Resonance

Lab 16b analysed the panel of samples by surface plasmon resonance (SPR) targeting the GP protein of LASV (Josiah isolate) (Figure 5a). The samples were ranked similarly to the anti-GP EIA (Table 8, Figure 5b). The mAb cocktail samples L12 had the highest titre of anti-GP antibodies, while the mAb cocktail sample L10 had titre comparable to the convalescent plasma samples. Sample L5 was the most potent among the convalescent plasma/serum samples, followed by L6, L2 and the International Standard candidate L4. Sample L1 and L8 were the lowest titre samples.

Harmonisation of the results by the monoclonal antibody cocktails

The two monoclonal antibody mixes #1 (L12) and #2 (L10) were formulated to include binding antibodies to anti-GP and anti-NP, as well as neutralising antibodies (Appendix 2). Both mAb cocktails were the most potent samples, with antibody titres approximately 10-fold higher than the convalescent plasma samples. Some binding assays failed to detect one or both of the mAb mix samples; lab 6 using a double antigen bridging assays directed against GP, and lab 9 using a commercially available anti-NP assay could not detect anti-LASV antibodies in either of the two mixes.

Reduction of the inter-laboratory variation by expressing the geometric mean titre of each sample relative to the mAb cocktails was measured by %GCV, Lab GM:Med <2 and UQ/LQ and reported in Table 14 for the neutralisation assays and Table 15 for the binding assays. In almost all the cases the mAbs were able to harmonise the data. For the neutralisation assays, the candidate International Standard achieved the greatest reduction in inter-laboratory variation except for sample L5, mid neutralizer, high anti-GP binder, which has a lower %GCV when reported relative to sample L10 and L12. Also the two mAb cocktails harmonised each other better than the sample L4 (Table 14). For the binding antibody assays, the majority of the samples achieved better harmonisation between laboratories when expressed relative to the candidate International Standard with a few exceptions; the low sample L1 had a lower %GCV and smaller interquartile range when expressed relative to sample L12, mAb mix#1; similar results for the mid titre sample L6 when expressed relative to sample L10, mAb mix#2. Sample L7, high titre from Sierra Leone and L9, working standard had a lower UQ/UL ratio when expressed relative to sample L12 and sample L9, also had also a lower %GCV when expressed relative to sample L10.

Stability study of the candidate WHO International Standard

The stability of the candidate International Standard is being assessed by an accelerated thermal degradation study. Ampoules of the candidate WHO IS, NIBSC code 20/202 (sample L4) were stored at different temperatures -20 (baseline), +4, +20, +37 and +45°C for 1 month, 3 months, 6 months and 12 months. The freeze-dried preparations retrieved at 1 month, 3 and 6 months were reconstituted as per instructions for use (Appendix 5) and tested concurrently in duplicate in 4 independent assays by LASV-GP pseudotyped VSV(Luc) neutralisation assays as described in the Materials and Methods. Relative potencies to the -20°C baseline and 95% confidence limits were calculated by parallel line analysis using the software CombiStats™. Real time data on the degradation samples are reported as relative to the baseline -20 °C, and showed minimal loss of potency up to a month at 37°C, and up to 6 months at ambient temperature (20°C) (Figure 5). The long-term stability of the candidate WHO IS was estimated by the Arrhenius model (Table 16). The predicted loss in potency for 20/202, when stored at -20°C, was 0.17% per year. The

results obtained from both the real time data and the predicted stability suggested that the preparation 20/202 is adequately stable to serve as WHO IS for anti-LASV immunoglobulin, and can be shipped at ambient temperature.

Stability studies were not conducted for each individual member of the Reference Panel due to the increased workload and the COVID-19 pandemic. The convalescent plasma pooled to generate the Reference Panel samples were processed similar to the candidate International Standard, and each panel member formulated using the same freeze-dry conditions. This is also supported by similar product reviews (Table 3 and 4).

Discussion and conclusion

The purpose of this collaborative study was to evaluate a pool of convalescent plasma from Lassa fever recovered patients as a candidate International Standard. The aim was to assess whether the candidate material is able to harmonise the results from serological assays detecting anti-LASV antibodies. Also, as part of the study, a candidate International Reference Panel for anti-LASV antibody was characterised; the panel will facilitate the development and evaluation of serological assays. Seventeen participants from seven countries returned thirty-six datasets of results; these included live and pseudotyped virus neutralisation assays, ELISA, immunofluorescence assay and surface plasmon resonance (Table 2). The majority of the methods for detection of binding antibodies detected IgG (n=14), but five laboratories used IgM-specific assays (Table 13). The two main antigens used in the binding antibodies methods were glycoprotein (GP) and nucleoprotein (NP) or a mix of both. There were two commercially available assays in this study: Blackbox LASV IgG NP ELISA and ReLASV pan ELISA kits (for anti-GP, anti-NP or combo GP/NP) from Zolgen laboratories. From an online search, only two other commercially available kits were found from MyBioSource (antigen used is not disclosed) and MAGPIX from Luminex which uses a combination of NP and GP. To the best of our knowledge the assays used in this study covered the targets used in commercially available assays.

The candidate International Standard, sample L4, was detected in all neutralising assays and binding assays for IgG. All five assays detecting anti-LASV IgM scored the candidate International Standard negative. The two high antibody titre samples in the candidate Reference Panel were also negative for IgM and the results from the other panel members were inconsistent between assays. This is not surprising as the samples were collected at least 1 year post-disease and IgM levels would have declined/disappeared.

Expressing the neutralising and binding antibody titres relative to the candidate International Standard reduced inter-laboratory variation for all the positive samples as measured by a reduction in %GCV, narrowing of the inter-quartile range and an increased percentage of laboratories within two-fold range of the sample median (Table 7, 10, 11, 12 and Figure 2 and 4). We also looked at whether a cocktail of monoclonal antibodies could harmonise the titres between laboratories. For both neutralising assays (Table 14) and binding assays (Table 15) expressing the potency of the samples relative to either of the mix of mAbs could reduced inter-laboratory variation. In the majority of the cases, the harmonisation achieved with the candidate International Standard L4, pool of convalescent plasma, was greater than using the mAb cocktails; also, the mAb preparations were scored negative by two laboratories (lab 6 and 9a); nevertheless, these results suggest that a carefully designed mAb cocktail could act as a reference

material and they could play an important role in the rapid response to outbreaks when sourcing convalescent plasma/serum is challenging.

The majority of the participants used the Josiah isolate for live virus or GP and NP proteins derived from the LASV Josiah isolate (lineage IV). A few laboratories used different isolates, such as lineages II (101LV18) and III (GA391) in live virus neutralisation assay, lineage V (AV) in a pseudotyped virus neutralisation assay and lineage II, III (GA391), V (AV) in the binding assays. Despite the low amount of samples, there was no evidence of a bias between lineages. This observation is also supported by recent data on serological cross-reactivity between LASV lineages [19]. Therefore, the candidate IS may be used to calibrate across these lineages.

The ranking of the members of the candidate Reference Panel was similar but not identical between laboratories (Figure 1 and 3). The high titre neutralising antibodies sample L7 (20/244) was detected between the highest samples in all assays, but sample L5 (20/204) had the highest anti-GP binding antibody whilst mid titre neutralising antibodies; sample L2 (20/228) was between the highest titre samples for neutralising antibody, but was mid-high for binding antibodies; conversely sample L6 (20/226) had a mid-titre neutralising antibody but was among the highest for binding antibodies to both GP and NP. The low titre samples L1 (20/246), L3 (20/222) and L8 (20/248) were consistently the lowest samples in all the assays. These data clearly illustrate that the antibody composition in the samples is very distinct, and three unitages are required to differentiate the antibody titre in the samples; one for neutralising activity, one for anti-GP binding activity and one for anti-NP binding activity. While such unitage is arbitrarily assigned, for ease in transition between current titre reporting and International Unit, we propose values which are closer to the consensus titre obtained by the participating laboratories for sample L4, candidate International Standard: 100 IU/mL for neutralising activity, 1000 IU/mL for binding antibody activity against GP, and 1000 IU/mL for binding antibody activity for anti-NP.

Sample L9 (NIBSC code LASV-3) is a working standard which was made available with CEPI support to vaccine developers for setting up serological assays for LASV, following a feasibility study in February 2020 (Appendix 3). It has been included in this collaborative study to permit accurate calibration against the candidate International Standard thus allowing laboratories to back calibrate data obtained using LASV-3 as an internal control and report their results in IU/mL. The potency of LASV-3 was calculated as the geometric mean antibody titre across all assays and expressed as relative to the candidate International Standard sample L4; LASV-3 titre is calculated to be 43 IU/mL (95% confidence limits 33-57) for neutralising antibody activity, 940 IU/mL (95% confidence limits 642-1418) for anti-GP binding IgG activity and 1370 IU/mL (95% confidence limits 655-2856) for anti-NP binding IgG activity .

Proposal

It is proposed that the pool of convalescent plasma from LASV recovered patients, sample L4, NIBSC code 20/202, is established as the WHO International Standard for anti-LASV immunoglobulin G. It is proposed the assigned unitage of 25 IU/ampoule for neutralising antibody, 250 IU/ampoule for anti-GP binding IgG, 250 IU/ampoule for anti-NP binding IgG. Instructions for Use of the proposed WHO International Standard are presented in Appendix 5. Approximately 3100 ampoules (0.25mL/ ampoule) are available for distribution. Based on the

stability study results, we proposed that the International Standard is kept at -20°C for long term storage, but can be shipped at ambient temperature.

It is proposed that a panel of freeze-dried pools of convalescent plasma from LASV recovered patients consisting of sample code 20/204 (sample L5, high GP, mid NP and neutralising antibodies titre), 20/222 (sample L3, low, Nigeria), 20/226 (sample L6, mid, Nigeria), 20/228 (sample L2, high, Nigeria), 20/244 (sample L7, high, Sierra Leone), 20/246 (L1, mid/low, Sierra Leone) and 20/248 (sample L8, very low, Sierra Leone) are established as the WHO International Reference Panel for anti-LASV immunoglobulin G. No unitage in IU/mL will be proposed for the Reference Panel members, however, in the Instructions for Use of the proposed WHO International Reference Panel the geometric mean of the antibody titres from this collaborative study will be included as representative data to provide guidance in the use of the panel (Appendix 6).

Approximately 450 reference panels (0.25mL/ ampoule) are available for distribution. Similar to the International Standard, we proposed that the Reference Panel is kept at -20°C for long term storage, but can be shipped at ambient temperature.

Comments from participants:

Twelve laboratories returned comments. Ten participants agreed with the proposal (lab 1, 4, 5, 7, 8, 9, 11, 14, 15, 16) and two of them returned correction on the data reported.

The other comments were:

Lab 2: if the unitage of the International Standard is to be assigned based on the consensus obtained from the titres in the assays used in this study, then anti-N binding activity should be 660 IU/mL (165 IU/ampoule);

Lab 6: “We are concerned about the use monoclonal antibodies to harmonise the results. The data may tell you about the specificity of the Mab. However, the Mab will have a very narrow specificity and therefore may not inform on the performance of the various tests”

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Tables

Table 1. Collaborative study samples

Samples were shipped under NIBSC dispatch reference CS686

CS code	NIBSC code	description	Volume (mL)	appearance
L1	20/246	RP member, mid/low (SL)	0.25	f/d
L2	20/228	RP member, high (N)	0.25	f/d
L3	20/222	RP member, low (N)	0.25	f/d
L4	20/202	candidate WHO IS	0.25	f/d
L5	20/204	RP member, high binding, mid neut (N)	0.25	f/d
L6	20/226	RP, member, mid (N)	0.25	f/d
L7	20/244	RP member, high (SL)	0.25	f/d
L8	20/248	RP member, v. low (SL)	0.25	f/d
L9	LASV-3	CEPI Working Standard	0.05	liquid
L10	-	mAb mixture 2	0.1	liquid
L11	-	Negative serum (SL)	0.1	liquid
L12	-	mAb mixture 1	0.1	liquid

RP: reference panel; IS: International Standard; SL: Sierra Leone; N: Nigeria; mAb: monoclonal antibody; f/d: freeze-dried

Table 2. Assay methods

Lab code	method	target	strain	output
1	PV-Neut (LVV-Luc)	GP	Josiah (IV)	NT ₅₀
2a	PV-Neut (LVV-Luc)	GP	Josiah (IV)	NT ₅₀
2b	PV-Neut (VSV-Luc)	GP	Josiah (IV)	NT ₅₀
2c	PV-Neut (VSV-Luc)	GP	AV (V)	NT ₅₀
3a	Commercial ELISA (ReLASV Pan-Lassa-pfGP IgG (Zalgen Labs))	pfGP- IgG	pan	inver dil factor >CO
3b	Commercial ELISA (ReLASV Pan-Lassa-NP IgG (Zalgen Labs))	NP-IgG	pan	inver dil factor >CO
3c	Commercial ELISA (ReLASV Pan-Lassa-Combo IgG (Zalgen Labs))	pfGP/NP-IgG	pan	inver dil factor >CO
3d	Commercial ELISA (ReLASV Pan-Lassa-pfGP IgM (Zalgen Labs))	pfGP- IgM	pan	inver dil factor >CO
3e	Commercial ELISA (ReLASV Pan-Lassa-NP IgM (Zalgen Labs))	NP-IgM	pan	inver dil factor >CO
3f	Commercial ELISA (ReLASV Pan-Lassa-Combo IgM (Zalgen Labs))	pfGP/NP-IgM	pan	inver dil factor >CO
4a	EIA (in house)	NP-IgG	AV (V)	inver dil factor >CO
4b	Neut (FRNT)	-	GA391(III)	NT ₅₀
4c	IFA	NP and GP-IgG	AV (V)	inver dil factor >CO
5a	Neut (FRNT)	-	Josiah (IV)	NT ₅₀
5b	PV-Neut (VSV-Luc)	GP	Josiah (IV)	NT ₅₀
6	EIA-DABA	GP	GA391(III)	inver dil factor >CO
7	EIA (in house)	pfGP-IgG	Josiah (IV)	inver dil factor >CO
8a	Neut	-	Josiah(IV)	Endpoint titer
8b	IFA	whole virus-IgG	Josiah(IV)	inver dil factor >CO
8c	IFA	whole virus-IgM	Josiah(IV)	inver dil factor >CO
9a	Commercial ELISA (Blackbox LASV IgG NP ELISA)	NP-IgG	AV (V)	inver dil factor >CO
9b	Neut (FRNT)	-	101LV18 (II)	NT ₅₀
9c	Neut (FRNT)	-	Josiah (IV)	NT ₅₀
10	Commercial ELISA (ReLASV Lineage IV linked GP IgG ELISA (Zalgen Labs))	pfGP-IgG	Lineage IV	inver dil factor >CO

11a	EIA (in house)	GP-IgG	lineage II	inver dil factor >CO
11b	EIA (in house)	GP-IgG	Lineage IV	inver dil factor >CO
11c	PV-neut assay (rVSV-GFP)	GP	Josiah (IV)	NT ₅₀
12	PV-neut assay (rVSV-GFP)	GP	Josiah (IV)	(PR)NT ₅₀
13	Neut (FRNT)	-	Josiah (IV)	NT ₅₀
14a	EIA (in house)	whole virus-IgG	Josiah (IV)	inver dil factor >CO
14b	EIA (in house)	whole virus -IgM	Josiah (IV)	inver dil factor >CO
14c	Neut (FRNT)	-	Josiah (IV)	NT ₅₀
15a	Neut (CPE)	-	Josiah (IV)	Endpoint titre
15b	PV-Neut assay (rVSV)	GP	Josiah (IV)	Endpoint titre
16a	EIA (in house)	GP-IgG	Josiah (IV)	inver dil factor >CO
16b	Surface Plasmon Resonance	GP	Josiah (IV)	resonance unit

EIA: enzymatic linked immunoassay; **FRNT:** foci reduction neutralisation assay; **GFP:** green fluorescent protein; **GP:** glycoprotein; **pGP:** stabilised pre-fusion protein; **HIV:** human immunodeficiency virus; **IFA:** Indirect fluorescence assay; **inver dil factor >CO:** inverse of the dilution factor with a result above cut-off; **Neut:** neutralisation assay; **Luc:** luciferase; **LVV:** lentiviral vector; **NT₅₀**= 50% neutralisation titre; **PV:** pseudotyped virus; **VSV:** vesicular stomatitis virus; **rVSV:** replication competent VSV.

Table 3. Candidate International Standard for anti-LASV antibody formulation review

Microbiological test for bacterial and mould/yeast colony count returned negative

Sample name	L4, candidate IS
Product code	20/202
No. containers filled	3508
Mean fill mass (g)	0.2684 (n=127)
CV of fill mass (%)	1.06
Mean residual moisture (%)	1.1 (n=12)
CV of residual moisture (%)	26.67
Mean oxygen head space (%)	0.28 (n=12)
CV of oxygen space (%)	50.42

n = number of samples tested

Table 4. Candidate Reference Panel members for anti-LASV antibody formulation review

Microbiological test for bacterial and mould/yeast colony count returned negative

Sample name	L5, high binding, mid neutralising	L3, low	L6, mid	L2, High	L7, High	L1, mid/low	L8, very low
Origin	Nigeria	Nigeria	Nigeria	Nigeria	Sierra Leone	Sierra Leone	Sierra Leone
Product code	20/204	20/222	20/226	20/228	20/244	20/246	20/248
No. containers filled	597	728	561	543	581	543	584
Mean fill mass (g) n=41-70	0.2657	0.2659	0.2674	0.2655	0.2680	0.2671	0.2666
CV of fill mass (%)	1.25	1.07	1.54	1.39	1.00	1.32	1.35
Mean residual moisture (%) n=12	1.1	0.858	1.1	1.57	0.318	1.506	1.013
CV of residual moisture (%)	15.28	13.57	23.49	19.08	21.73	68.23	12.88
Mean oxygen head space (%) n=12	0.36	0.43	0.13	0.29	0.38	0.15	0.77
CV of oxygen space (%)	34.53	25.79	71.36	47.24	42.77	77.79	19.27
Virology report	negative	negative	negative	negative	HBsAg positive	HIV Ab, HBsAg positive	HBsAg positive

n = number of samples tested; HIV: human deficiency virus; Ab; antibody; HBsAg; hepatitis B virus surface antigen.

Table 5. Geometric mean of LASV neutralising antibodies, as reported by the participants

Type	Isolate/ Lineage	Lab	L1, mid/low SL	L2, High N	L3, low N	L4, IS N	L5, mid* N	L6, mid N	L7, High SL	L8, v.low SL	L9, WS SL	L10, mAb Mix 2	L11, neg SL	L12, mAb Mix 1
Live-PRNT	GA391 (III)	4b	<10	40	<10	16	<10	40	16	<10	<10	>40	<10	>40
Live-FRNT	Josiah (IV)	5a	42	204	44	260	88	90	243	43	100	736	<20	1472
Live-CPE	Josiah (IV)	8a	<10	48	10	12	<40	<10	<10	<10	<10	80	<10	190
Live-FRNT	101LV18 (II)	9b	9	73	4	89	34	42	103	13	17	262	-	309
Live-FRNT	Josiah (IV)	9c	8	62	-	69	21	26	94	8	29	277	-	290
Live-FRNT	Josiah (IV)	13	25	194	25	243	14	168	275	<10	-	235	<10	250
Live-FRNT	Josiah (IV)	14c	<20	20	<20	32	20	20	20	<20	20	127	<20	80
Live-CPE	Josiah (IV)	15a	10	68	<8	45	13	10	45	<8	12	246	<8	256
GM	Live neut		15	68	15	57	24	39	71	17	26	227	-	278
PV-LVV	Josiah (IV)	1	27	101	30	87	56	49	88	22	53	1003	<10	791
PV-LVV	Josiah (IV)	2a	191	903	272	581	394	473	965	120	284	1374	<20	1279
PV-VSV	Josiah (IV)	2b	176	746	152	1106	262	189	646	91	364	6722	<20	5521
PV-VSV	AV (V)	2c	39	336	51	308	247	157	360	57	126	5172	<20	6461
PV-VSV	Josiah (IV)	5b	30	127	8	166	84	107	272	15	97	2065	-	2156
PV-rVSV	Josiah (IV)	15b	12	45	<8	43	18	21	76	<8	18	202	<8	181
PV-rVSV (GFP)	Josiah (IV)	11c	63	243	39	228	124	161	337	48	215	3118	-	2955
PV-rVSV (GFP)	Josiah (IV)	12	<10	14	<10	14	<10	19	14	<10	<10	239	<10	205
GM	PV- neut		50	160	51	158	115	92	193	47	114	1401	-	1307
GM	All		31	104	31	95	56	31	121	33	61	600	-	635

IS: international standard candidate, SL: Sierra Leone; N: Nigeria; v.: very; WS: working standard; mAb: monoclonal antibody cocktail; neg: negative; * mid neutralising antibodies, but high binding antibody titre; PRNT: plaque reduction neutralisation assay; FRNT: foci reduction neutralisation assay; CPE: cytopathic effect detection assay; PV: pseudotyped virus-based neutralisation assay; LVV: lentiviral (HIV) vector; VSV: vesicular stomatitis virus; GM: geometric mean. Laboratories reported their titre as 50% neutralisation titre except laboratories 8a, 15a and 15b (shaded) which reported the endpoint titre.

Table 6. Geometric mean of LASV neutralising antibodies expressed relative to the candidate IS, Sample L4

Type	Isolate/ Lineage	Lab	L1, mid/low	L2, High	L3, low	L5, mid*	L6, mid	L7, High	L8, v.low	L9, WS	L10, mAb	L11, neg	L12, mAb
			SL	N	N	N	N	SL	SL	SL	Mix 2	SL	Mix 1
Live-PRNT	GA391 (III)	4b	-	2.00	-	-	2.00	1.00	-	-	2.52	-	2.52
Live-FRNT	Josiah (IV)	5a	0.19	0.78	0.17	0.34	0.35	0.93	0.17	0.38	2.83	-	5.65
Live-CPE	Josiah (IV)	8a	-	4.00	0.84	-	-	-	-	-	6.73	-	16.00
Live-FRNT	101LV18 (II)	9b	0.10	0.82	0.04	0.38	0.47	1.16	0.15	0.19	2.95	-	3.48
Live-FRNT	Josiah (IV)	9c	0.17	0.90	-	0.30	0.37	1.37	0.17	0.42	4.02	-	4.21
Live-FRNT	Josiah (IV)	13	0.09	0.80	0.10	0.05	0.69	1.13	-	-	0.97	-	1.03
Live-FRNT	Josiah (IV)	14c	-	0.63	-	0.50	0.63	0.63	-	0.63	4.00	-	2.52
Live-CPE	Josiah (IV)	15a	0.22	1.50	-	0.28	0.23	1.00	-	0.26	5.45	-	5.66
GM	Live neut		0.15	1.17	0.15	0.26	0.53	1.01	0.16	0.35	3.24	-	3.86
PV-LVV	Josiah (IV)	1	0.31	1.17	0.34	0.64	0.57	1.01	0.26	0.61	11.57	-	9.13
PV-LVV	Josiah (IV)	2a	0.33	1.55	0.47	0.68	0.81	1.66	0.21	0.49	2.36	-	2.20
PV-VSV	Josiah (IV)	2b	0.16	0.67	0.14	0.24	0.17	0.58	0.08	0.33	6.08	-	4.99
PV-VSV	AV (V)	2c	0.13	1.09	0.17	0.80	0.51	1.17	0.19	0.41	16.78	-	20.96
PV-VSV	Josiah (IV)	5b	0.18	0.76	0.05	0.50	0.64	1.63	0.09	0.59	12.40	-	12.95
PV-rVSV	Josiah (IV)	15b	0.29	1.06	-	0.42	0.50	1.79	-	0.42	4.73	-	4.24
PV-rVSV (GFP)	Josiah (IV)	11c	0.28	1.06	0.17	0.54	0.71	1.48	0.21	0.94	13.69	-	12.98
PV-rVSV (GFP)	Josiah (IV)	12	-	0.96	-	-	1.36	0.98	-	-	16.72	-	14.33
GM	PV-neut		0.23	1.01	0.17	0.51	0.58	1.22	0.16	0.51	8.85	-	8.25
GM	all		0.19	1.09	0.17	0.37	0.56	1.12	0.16	0.43	5.35	-	5.64

SL: Sierra Leone; N: Nigeria; v.: very; WS: working standard; mAb: monoclonal antibody cocktail; neg: negative; * mid titre neutralising antibodies, but high binding antibody titre; PRNT: plaque reduction neutralisation assay; FRNT: foci reduction neutralisation assay; CPE: cytopathic effect detection assay; MN: microneutralization assay; PV: pseudotyped virus-based neutralisation assay; LVV: lentiviral (HIV) vector; VSV: vesicular stomatitis virus; GM: geometric mean.

Table 7. Inter-laboratory variation in the neutralisation assays

		L1, mid/low, SL	L2, high, N	L3, low, N	L4, WHO IS	L5, high bind, mid neut, N	L6, mid, N	L7, high, SL	L8, v.low, SL	L9, WS	L10, mAbs, mix 2	L11, neg	L12, mAbs, mix 1
%GCV	Reported	189	228	264	285	231	198	276	150	233	303	n/a	297
	Relative to L4	53	60	162	-	99	85	38	47	54	126	n/a	134
GM:Med <2	Reported	42%	44%	50%	31%	31%	33%	27%	44%	33%	40%	n/a	47%
	Relative to L4	92%	94%	50%	-	92%	73%	100%	89%	83%	50%	n/a	44%
UQ/LQ	Reported	3.97	4.53	3.93	6.24	6.18	6.80	5.19	3.79	7.41	7.11	n/a	7.88
	Relative to L4	1.85	1.56	2.62	-	1.78	1.68	1.44	1.39	1.60	4.03	n/a	4.03

GCV: Inter-Lab geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM within 2-fold of sample median; UQ/LQ: Ratio of upper quartile to lower quartile; n/a: could not be calculated as less than three data points.

Table 8 . Geometric mean of the binding anti-LASV IgG methods, as reported by the participants

	Isolate/ Lineage	Lab	L1, mid/low SL	L2, High N	L3, low N	L4, IS N	L5, mid* N	L6, mid N	L7, High SL	L8, v.low SL	L9, WS SL	L10, mAb Mix 2	L11, neg SL	L12, mAb Mix 1
EIA	GP/pan	3a	100	100	-	100	300	-	100	-	100	2700	-	16849
	GP/III	6	7	55	25	67	100	67	67	8	21	-	-	neat
	GP/pan	7	317	1008	252	1008	1600	504	1270	252	1008	>12800	-	>12800
	GP/IV	10	400	400	200	504	800	400	800	200	400	6400	-	16127
	GP/II	11a	1524	9644	3254	8909	16278	10848	7072	1110	7389	199403	-	473102
	GP/IV	11b	2914	13378	4473	12150	18951	12598	16979	2458	11986	343548	-	730511
	GP/IV	14a	-	400	-	400	400	1600	400	-	400	6400	-	6400
	GP/IV	16a	300	2700	900	2700	2700	2700	2700	300	900	900	-	>24300
	GM (GP)		272	863	506	891	1375	1345	984	263	636	13770		10556
EIA	NP/pan	3b	100	900	208	433	300	900	624	100	900	8100	-	11682
	NP/V	4a	>6400	>6400	>2540	>6400	>6400	>6400	>6400	>2540	>4032	>6400	-	>6400
	NP/V	9a	400	635	252	1008	800	1600	1270	200	2016	-	-	-
	GM(NP)		200	756	229	660	490	1200	890	141	1347	8100		11682
IFA	NP/ V	4c	<10	160	40	63.5	63.5	>403.2	80	<10	>640	>640	-	>640
	GP/NP/pan	3c	100	300	100	300	900	900	900	100	900	8100	-	24300
	Whole virus/IV	8b	28	320	160	640	640	640	640	-	320	3620	-	3620
	GM (IFA)		53	249	86	230	331	759	359	100	537	5415		9380

IS: international standard candidate, SL: Sierra Leone; N: Nigeria; v.: very; WS: working standard; mAb: monoclonal antibody cocktail; neg: negative; * mid titre neutralising antibodies, but high binding antibody titre; GM: geometric mean; GP: glycoprotein; NP: nucleoprotein; EIA: enzymatic linked immunoassay; IFA: immunofluorescence assay. Values “>” were excluded from the calculation of the geometric mean.

Table 9 . Geometric mean of the binding anti-LASV IgG methods, expressed relative to the candidate IS

	Isolate/ Lineage	Lab	L1, mid/low SL	L2, High N	L3, low N	L5, mid* N	L6, mid N	L7, High SL	L8, v.low SL	L9, WS SL	L10, mAb Mix 2	L11, neg SL	L12, mAb Mix 1
EIA	GP/pan	3a	1.18	0.77	-	2.96	-	1.71	-	1.87	27.66	-	183.63
	GP/III	6	0.19	1.22	0.47	2.02	1.51	n/a	n/a	n/a	n/a	-	n/a
	GP/pan	7	0.38	1.07	0.27	1.64	0.62	1.33	0.25	1.13	34.85	-	np
	GP/IV	10	0.56	0.85	0.35	1.78	0.82	1.69	0.3	0.86	13.37	-	93.38
	GP/II	11a	0.16	1.04	0.38	1.73	1.08	0.9	0.11	0.86	22.57	-	52.96
	GP/IV	11b	0.23	1.02	0.34	1.4	0.8	1.12	0.17	0.75	22.76	-	56.4
	GP/IV	14a	-	np	-	0.99	1.39	np	-	0.86	np	-	np
	GP/IV	16a	0.19	0.87	0.29	0.92	0.8	1.19	0.13	0.64	n/a	-	68.9
	GM (GP)		0.32	0.97	0.34	1.58	0.96	1.29	0.18	0.94	23.13		81.19
	NP/pan	3b	0.31	1.17	0.38	1.06	2.11	1.26	0.23	1.64	27.01	-	29.93
	NP/V	4a	0.61	1.21	0.4	1.16	1.54	1.81	0.33	0.97	np	-	np
	NP/V	9a	0.34	0.92	0.21	1.31	0.93	1.46	0.16	1.61	-	-	-
	GM(NP)		0.40	1.09	0.31	1.17	1.45	1.49	0.23	1.37	27.01	-	29.93
IFA	NP/ V	4c	-	2.52	0.63	1.00	n/a	1.26	-	n/a	n/a	-	n/a
	GP/NP/pan	3c	0.31	0.84	0.30	1.49	1.33	1.28	0.20	1.30	28.99	-	-
	Whole virus/IV	8b	0.04	0.50	0.25	1.00	1.00	1.00	-	0.50	5.66	-	5.66
	GM (IFA)		0.31	0.84	0.30	1.49	1.33	1.28	0.20	1.30	28.99		-

IS: international standard candidate, SL: Sierra Leone; N: Nigeria; v.: very; WS: working standard; mAb: monoclonal antibody cocktail; neg: negative; *mid titre neutralising antibodies, but high binding antibody titre; GM: geometric mean; GP: glycoprotein; NP: nucleoprotein; EIA: enzymatic linked immunoassay; IFA: immunofluorescence assay; np: non-parallel as described in the statistical methods; n/a: not enough data.

Potency values have been calculated by parallel line model from the raw data provided by the participants. Lab 4c and 8b (red) were calculated as ratio of the value provided by the participants, and not included in the statistical analysis. Value for sample L10 are provided excluding Lab 16 as outlier.

Table 10. Inter-laboratory variation in the EIA methods for anti-GP binding antibodies

		L1, mid/low, SL	L2, high, N	L3, low, N	L4, WHO IS	L5, high bind, mid neut, N	L6, mid, N	L7, high, SL	L8, v.low, SL	L9, WS	L10, mAbs, mix 2	L11, neg	L12, mAbs, mix 1
%GCV	Reported	628	641	597	583	549	558	597	614	707	1001	-	8587
	Relative to L4	107	17	22	-	46	38	28	52	41	42	-	66
GM:Med <2	Reported	43%	38%	17%	38%	25%	29%	25%	50%	50%	33%	-	43%
	Relative to L4	71%	100%	100%	-	100%	100%	100%	100%	86%	100%	-	80%
UQ/LQ	Reported	4.51	13.12	11.14	12.87	11.37	12.05	12.14	3.78	5.86	25.19	-	10.55
	Relative to L4	2.46	1.23	1.22	-	1.43	1.53	1.40	1.93	1.23	1.23	-	1.66

GCV: Inter-Lab geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM within 2-fold of sample median;

UQ/LQ: Ratio of upper quartile to lower quartile;n/a: could not be calculated as less than three data points

Table 11. Inter-laboratory variation in the EIA methods for anti-NP binding antibodies

		L1, mid/low, SL	L2, high, N	L3, low, N	L4, WHO IS	L5, high bind, mid neut, N	L6, mid, N	L7, high, SL	L8, v.low, SL	L9, WS	L10, mAbs, mix 2	L11, neg	L12, mAbs, mix 1
%GCV	Reported	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	n/a
	Relative to L4	44	16	45	-	11	51	20	46	34	n/a	-	n/a
GM:Med <2	Reported	50%	100%	100%	100%	100%	100%	100%	100%	100%	100%	-	100%
	Relative to L4	100%	100%	100%	-	100%	100%	100%	100%	100%	100%	-	100%
UQ/LQ	Reported	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	n/a
	Relative to L4	1.40	1.14	1.39	-	1.11	1.51	1.20	1.46	1.30	1.00	-	1.00

GCV: Inter-Lab geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM within 2-fold of sample median;

UQ/LQ: Ratio of upper quartile to lower quartile; n/a: could not be calculated as less than three data points

Table 12. Inter-laboratory variation in the binding antibodies methods

		L1, mid/low, SL	L2, high, N	L3, low, N	L4, WHO IS	L5, high bind, mid neut, N	L6, mid, N	L7, high, SL	L8, v.low, SL	L9, WS	L10, mAbs, mix 2	L11, neg	L12, mAbs, mix 1
%GCV	Reported	n/a	47	102	226	325	n/a	271	n/a	n/a	n/a	-	n/a
	Relative to L4	n/a	n/a	n/a	-	n/a	n/a	n/a	n/a	n/a	n/a	-	n/a
GM:Med <2	Reported	50%	100%	67%	33%	67%	100%	67%	100%	100%	100%	-	50%
	Relative to L4	100%	100%	100%	-	100%	100%	100%	100%	100%	100%	-	100%
UQ/LQ	Reported	n/a	1.41	2.00	3.19	3.78	n/a	3.35	n/a	n/a	n/a	-	n/a
	Relative to L4	n/a	n/a	n/a	-	n/a	n/a	n/a	n/a	n/a	n/a	-	n/a

GCV: Inter-Lab geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM within 2-fold of sample median; UQ/LQ: Ratio of upper quartile to lower quartile. Laboratories 4c and 8a were excluded.

Table 13. Geometric mean of anti-LASV IgM titres as reported by the participants

		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12
Lab	Lineage target	mid/low (SL)	high (N)	low (N)	WHO IS	high bind, mid neut (N)	mid (N)	high (SL)	v. low (SL)	CEPI WS	mAB mix 2	neg (SL)	mAB mix 1
3d	GP, pan	100	-	100	-	300	100	-	100	-	8100	-	8100
3e	NP, pan	-	-	-	-	-	100.0	-	-	100.0	72900	-	24300
3f	GP/NP pan	100	-	-	-	432.7	100	-	-	100	24300	-	24300
8c	whole virus, IV	-	-	-	-	-	-	-		-	3620	-	1810
14a	whole virus, IV	-	-	-	-	-	-	-	-	400	6400	-	6400

Table 14. Inter-laboratory variation in the neutralising antibody titres when expressed relative to the mAb cocktails

		L1, mid/low, SL	L2, high, N	L3, low, N	L4, WHO IS	L5, high bind, mid neut, N	L6, mid, N	L7, high, SL	L8, v.low, SL	L9, WS	L10, mAb, mix 2	L11, neg	L12, mAb, mix 1
%GCV	reported	189	228	264	285	231	198	276	150	233	303	n/a	297
	vs L4	53	60	162	-	99	85	38	47	54	126	n/a	134
	vs L10	125	143	258	126	82	168	143	128	83	-	n/a	39
	vs L12	130	129	238	134	96	174	135	124	100	39	n/a	-
Lab GM:Med<2	reported	42%	44%	50%	31%	31%	33%	27%	44%	33%	40%	n/a	47%
	vs L4	92%	94%	50%	-	92%	73%	100%	89%	83%	50%	n/a	44%
	vs L10	58%	38%	30%	50%	69%	60%	47%	44%	67%	-	n/a	94%
	vs L12	58%	56%	40%	44%	85%	53%	60%	67%	75%	94%	n/a	-
UQ/LQ	reported	3.97	4.53	3.93	6.24	6.18	6.8	5.19	3.79	7.41	7.11	n/a	7.88
	vs L4	1.85	1.56	2.62	-	1.78	1.68	1.44	1.39	1.6	4.03	n/a	4.03
	vs L10	2.56	3.41	7.11	4.03	2.5	3.15	3.79	3.72	2.20	-	n/a	1.23
	vs L12	1.69	2.22	4.06	4.03	2.07	3.54	3.15	2.14	1.92	1.23	n/a	-

highlighted in green is the best result for each parameter for each sample: lower %GCV, higher % lab GM:Med<2 and lower UQ/LQ ratio. GCV: Inter-Lab geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM within 2-fold of sample median; UQ/LQ: Ratio of upper quartile to lower quartile

Table 15. Inter-laboratory variation in the binding antibody titres when expressed relative to the mAb cocktails

		L1, mid/low, SL	L2, high, N	L3, low, N	L4, WHO IS	L5, high bind, mid neut, N	L6, mid, N	L7, high, SL	L8, v.low, SL	L9, WS	L10, mAb, mix 2	L11, neg	L12, mAb, mix 1
%GCV	reported	458	411	404	425	442	341	417	400	457	604	n/a	3838
	vs L4	82	17	27	-	39	45	24	45	42	36	n/a	75
	vs L10	104	37	40	36	55	35	53	76	35	-	-	90
	vs L12	59	98	58	75	43	107	58	63	86	90	-	-
Lab GM:Med<2	reported	36%	38%	45%	38%	31%	55%	46%	44%	42%	56%	n/a	50%
	vs L4	91%	100%	100%	-	92%	100%	100%	100%	100%	86%	n/a	71%
	vs L10	71%	100%	100%	86%	86%	100%	83%	80%	100%	-	-	57%
	vs L12	86%	71%	83%	71%	100%	83%	86%	83%	86%	57%	-	-
UQ/LQ	reported	4	3.36	3.76	3.36	5.33	3.66	3.17	3	3.17	2.24	n/a	3.27
	vs L4	2.22	1.3	1.3	-	1.53	1.78	1.35	1.59	1.68	1.25	n/a	1.58
	vs L10	2.16	1.43	1.19	1.25	1.79	1.34	1.31	1.20	1.59	-	-	1.92
	vs L12	1.78	1.74	1.53	1.58	1.64	1.59	1.27	1.45	1.52	1.92	-	-

highlighted in green is the best result for each parameter for each sample: lower %GCV, higher % lab GM:Med<2 and lower UQ/LQ ratio. GCV: Inter-Lab geometric coefficient of variation; GM:Med <2: Percentage of labs with a Lab GM within 2-fold of sample median; UQ/LQ: Ratio of upper quartile to lower quartile. Excluding Lab 16 as outlier.

Table 16. Predicted stability of the candidate WHO IS for anti-LASV immunoglobulin, NIBSC code 20/202.

Temp (°C)	K	S.E.	% loss (per year)	95% UCL
-20	0.0017	0.00062	0.17	0.479
4	0.0313	0.00648	3.082	6.171
20	0.16749	0.01959	15.421	23.313
37	0.82331	0.02845	56.102	61.923

k: degradation rate constant; S.E.:standard error of k; UCL: upper confidence level

Figures

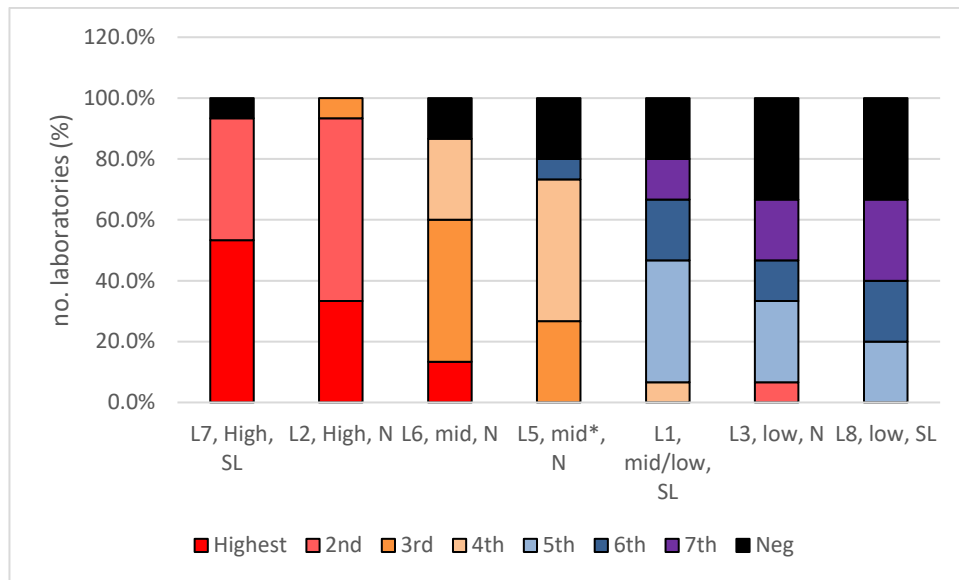


Figure 1. Ranking of the neutralising antibody titre in LASV Reference Panel members.

The geometric mean of the neutralising antibody titer as reported by the participants was used to rank the candidate Reference panel members from the highest neutralising activity to the lowest in each assay. For each panel member the column in the graph represents the proportion, expressed as percentage, of the laboratories which ranked the sample in that order. Lab 14c was excluded from the analysis as it scored 3 out of 7 panel member as negative and the remaining four had the same antibody titer.

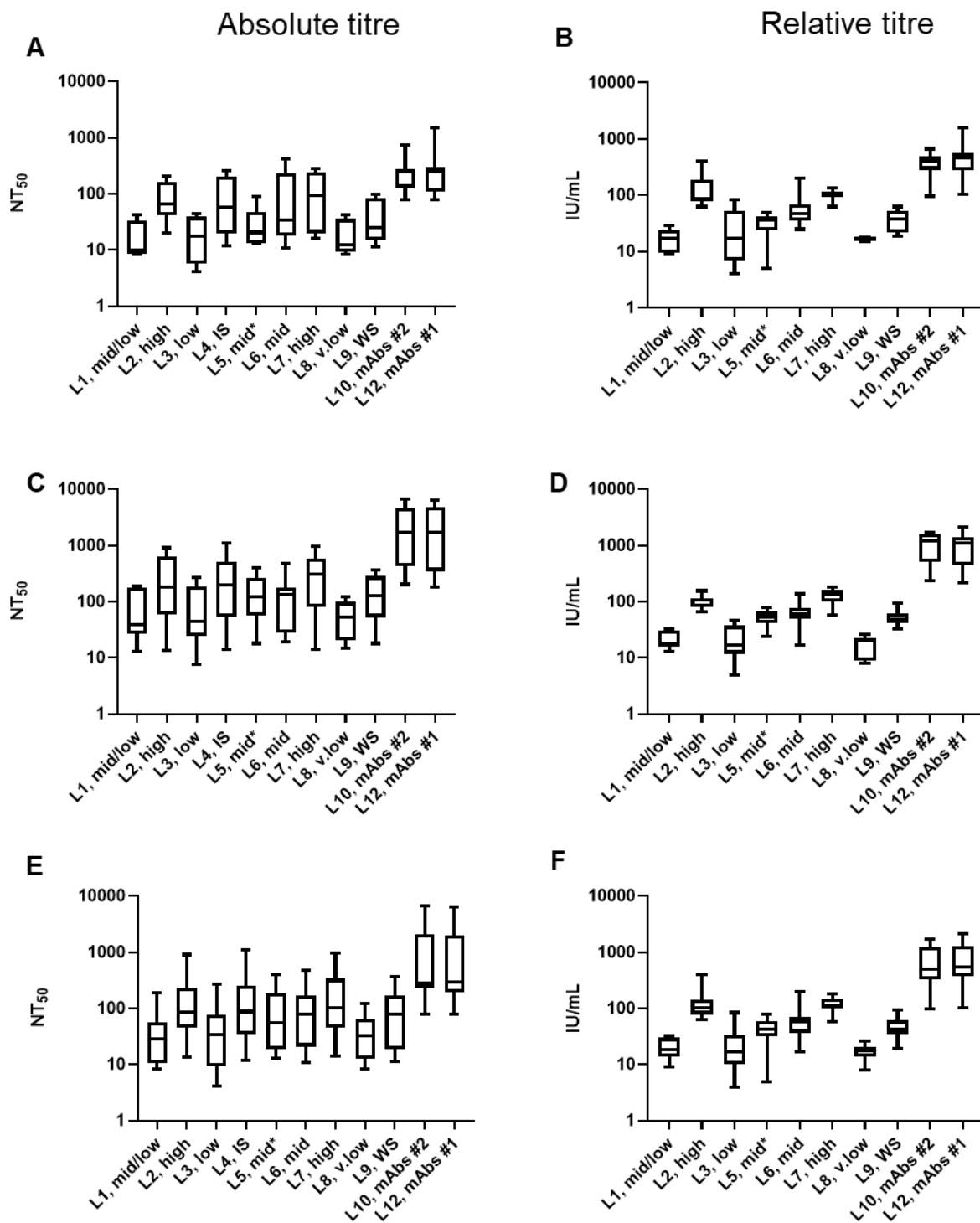


Figure 2. Harmonisation of LASV antibody titres in all of the neutralisation assays when reported as relative to the candidate International Standard. Neutralisation titres reported by participants for the live virus neutralisation assay (A), the pseudotyped-based neutralisation assays (C) and all the neutralisation assays (E). On the right panels, the antibody potencies expressed as relative to the candidate International Standard, sample L4 with an arbitrary assigned unitage of 100 International Units per mL: B) live virus neutralisation assays, D) pseudotyped virus neutralisation assays, and F) all neutralisation assays combined. The range of the values for each samples from each laboratory is represented as a box; the black line within the box marks the median; the boundary of the box indicate minimum (lower bar) and maximum (upper bar) value.

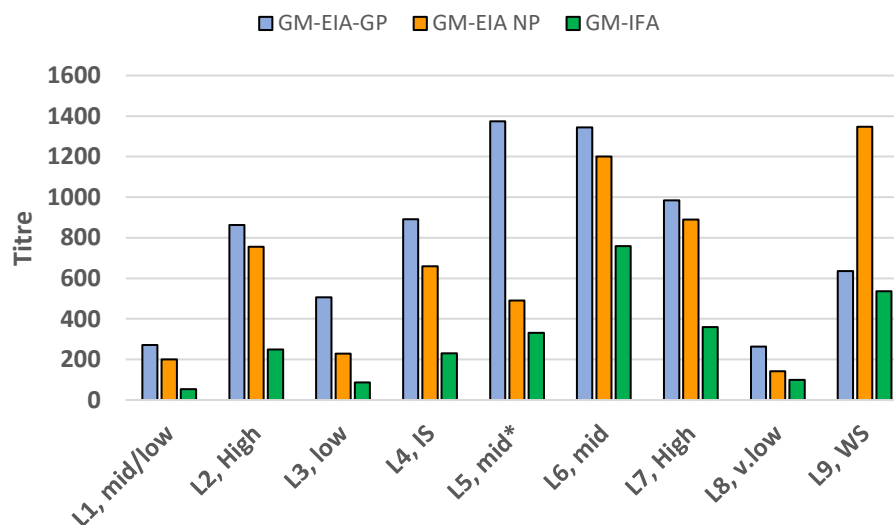
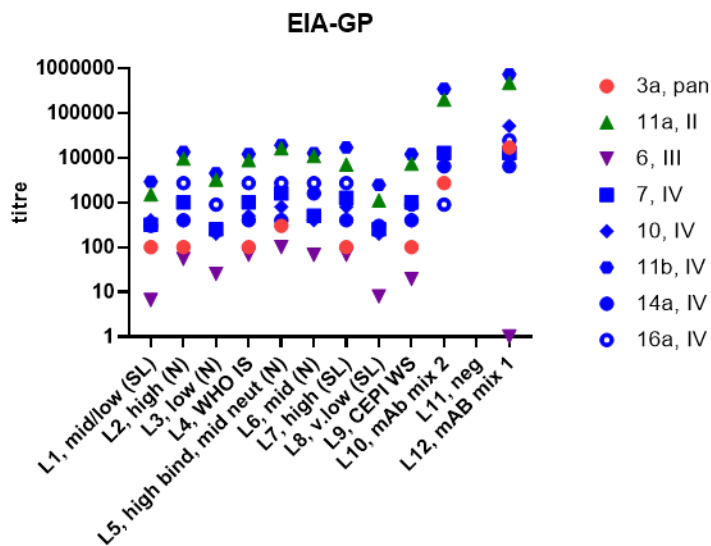


Figure 3. LASV binding antibody titres for the convalescent plasma samples. Geometric mean of the antibody titre as reported by the participants in anti-GP EIA (blue), anti-NP EIA (orange) or against both in IFA (green).

Absolute titres



Relative titres

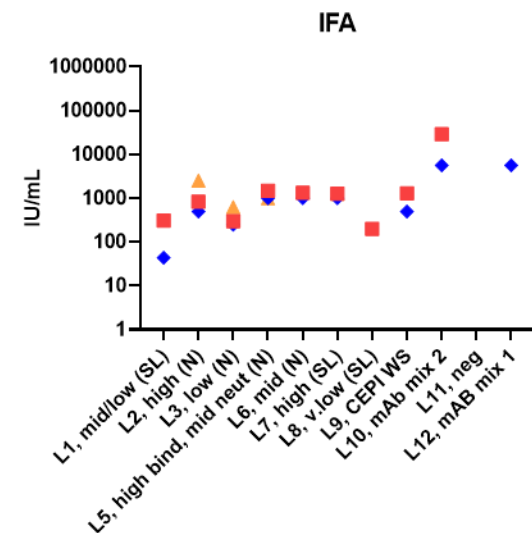
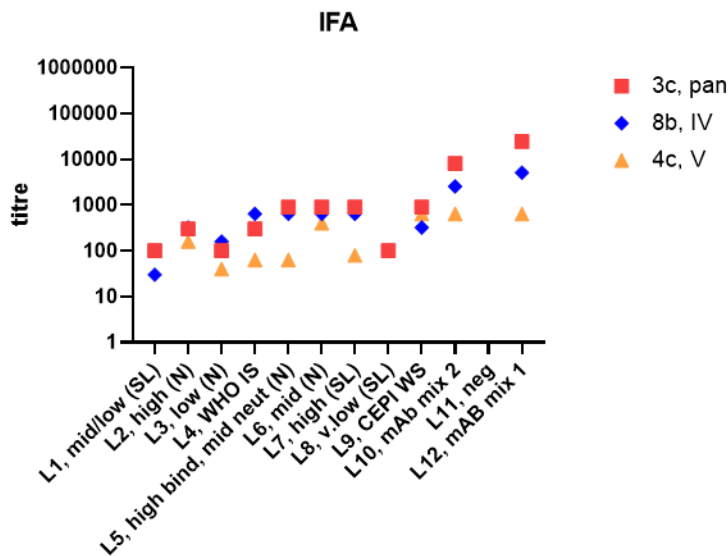
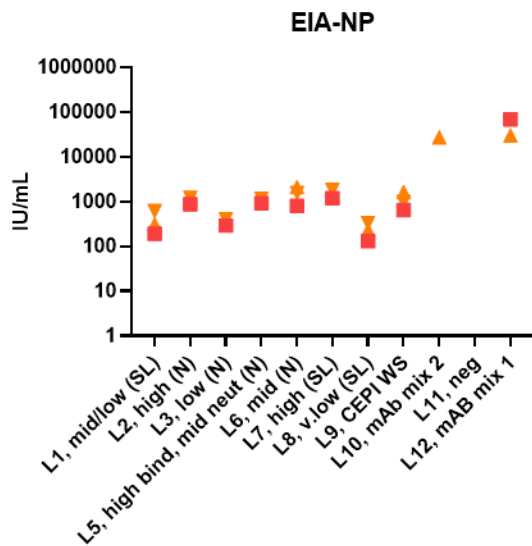
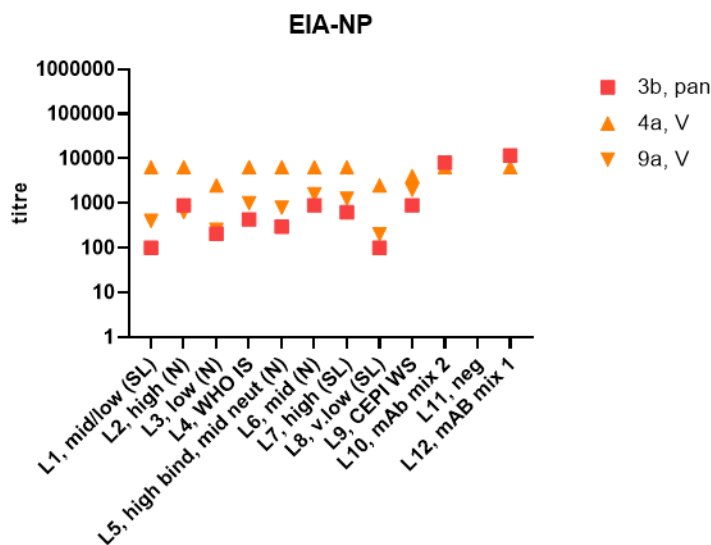
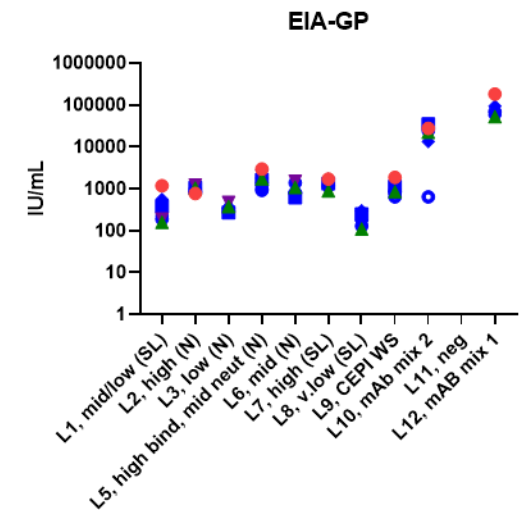


Figure 4. Harmonisation of LASV antibody titres in the binding assays when reported as relative to the candidate International Standard. Binding antibody titres reported by participants in their enzymatic immunoassays (EIA) for anti-GP (**top row**), anti-NP (**middle row**) and in the immunofluorescence assays (IFA, **bottom row**). On the right panels, the antibody potencies expressed as relative to the candidate International Standard, sample L4 with an arbitrary assigned unitage of 1000 International Units per mL. In each graph different marker colour correspond to different lineage: pan (red), II (green), II (purple), IV (blue) and V (orange).

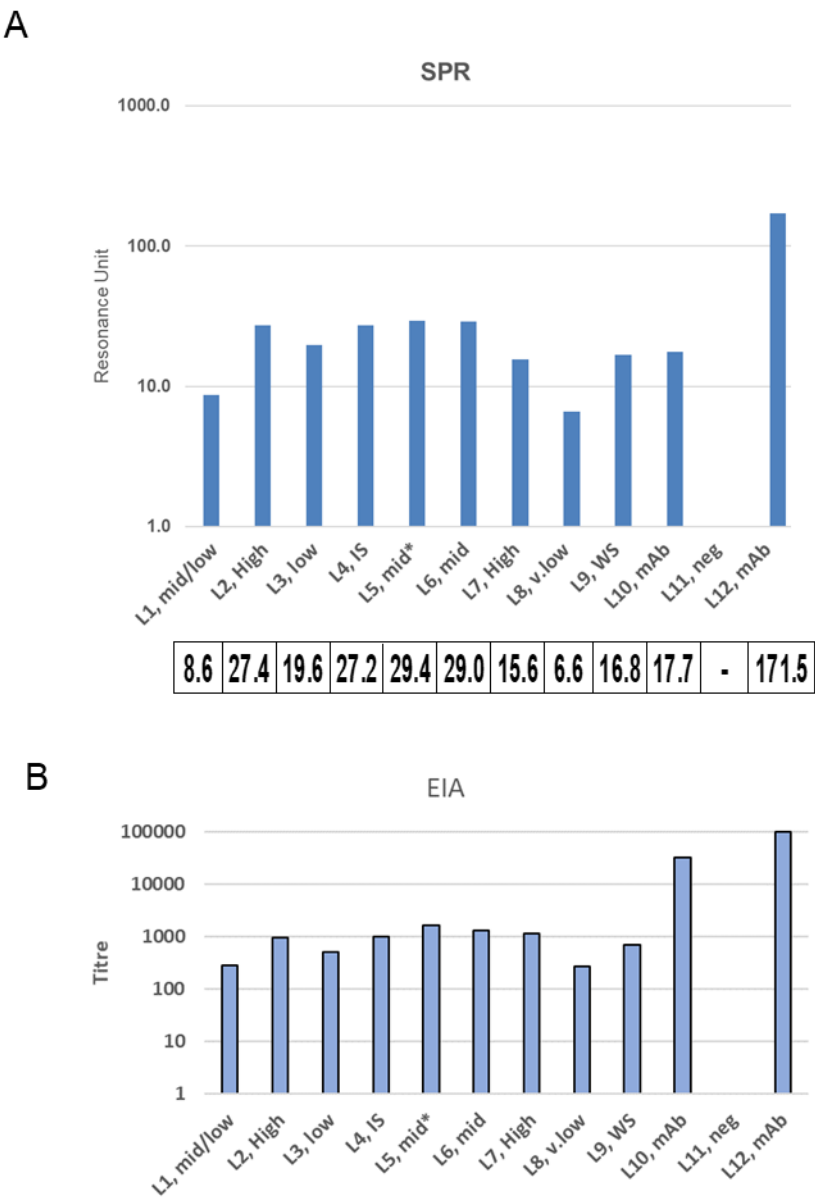


Figure 5. Surface plasmon resonance for anti-LASV GP antibody. A) Geometric mean from three independent experiments of the anti-LASV GP antibody titre as reported by lab 16b. The resonance units GM for each sample are reported underneath the graph. B) The geometric mean of the anti-GP antibody by EIA from Table 8 for comparison.

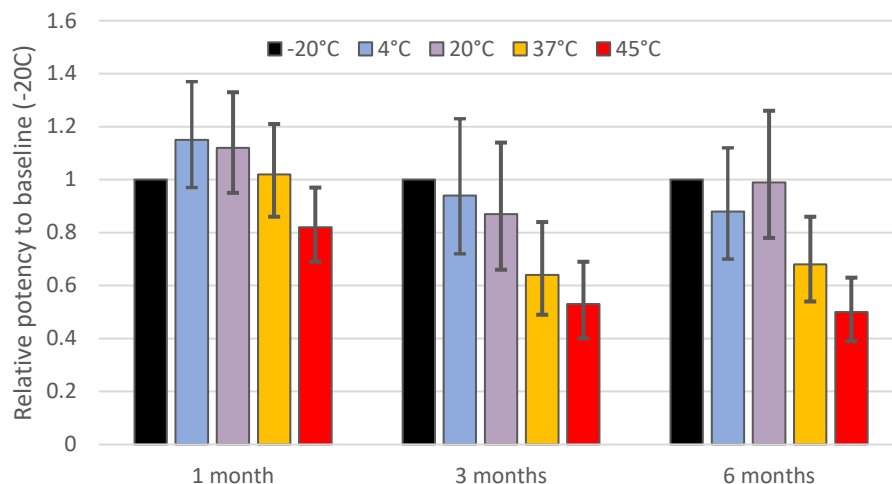


Figure 6. Thermal degradation assessment of the candidate International Standard for anti- LASV immunoglobulin. Freeze-dried ampoules of sample L4, NIBSC code 20/202 were stored at five different temperatures (-20, 4, 20, 37 and 45°C). At each time point, three vials were retrieved and reconstituted according to the instruction for use. Each vial was assessed by LASV GP pseudotyped-VSV(Luc) neutralisation assay. Data are reported relative to the the baseline storage temperature of -20°C and are derived from 4 independent experiments run in duplicate. Error bars represent the upper and lower 95% confidence limit.

Appendix 1

Collaborative study participants

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

Participant	Organisation	Country
Sylvain Baize	UBIVE Pasteur Institute	France
Delphine Pannetier	INSERM Jean Mérieux BSL4 Laboratory	France
Stephan Günther, Ndapewa Ithete	Bernhard-Nocht Institute for Tropical Medicine	Germany
Thomas Strecker, Sarah Fehling	Philipps University Marburg	Germany
Alessandro Manenti, Inesa Hyseni Emanuele Montomoli	VisMederi	Italy
Concetta Castilletti, Francesca Colavita, Daniele Lapa	National Institute for Infectious Diseases "L. Spallanzani"	Italy
M. Shimojima, T. Kurosu, T. Yoshikawa, Y. Takamatsu	National Institute for Infectious Diseases	Japan
Olivier Engler, Denise Siegrist, Sylvia Rothenberger, Roland Züst	Spiez laboratory	Switzerland
Steve Dicks, Samreen Ijaz, Hilary Bower, Richard Tedder	Public Health England and Public Health Rapid Support Team	United Kingdom
Emma Bentley, Samuel Richardson	National Institute for Biological Standards and Control	United Kingdom
Jonathan Hare	IAVI	United Kingdom
Marian Killip, Ulrike Arnold, David Jackson	High Containment Microbiology, Public Health England	United Kingdom
Jean D. Boyer, Eduardo Barranco, Idania Marrero	Inovio Pharmaceuticals	California, U.S.A
Matthew Boisen, Doug Simpson, Whitney Phinney	Zalgen Labs LLC	Colorado, U.S.A.
Rafael A. De La Barrera	Walter Reed army Institute of Research	Maryland, USA
Surender Khurana	US Food and Drug administration	Maryland, USA
Michael Egan, Victor Leyva-Grado	Auro Vaccines LLC	New York, U.S.A.

Appendix 2. Anti-Lassa fever virus antibody cocktails

The monoclonal antibody cocktails were provided by Zalgen Labs, LLC, Germantown, MD, USA in PBS containing 0.1% bovine serum albumin (BSA, Sigma Lot: SLCD4720), pH 7.4 at the concentration of 0.25 mg/mL each as per table below. The mAb were purified by affinity chromatography and purity was assessed by SDS-PAGE and found to be greater than 90%. The individual mAb have been described in Robinson, J., Hastie, K., Cross, R. et al. Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits. *Nat Commun* 7, 11544 (2016).
<https://doi.org/10.1038/ncomms11544>

LASV mAb cocktail mixture 1

NP binding	34.11C IgM	Lot: 111720-A	0.25 mg/mL
NP binding	34.11C IgG	Lot: 062919-A	0.25 mg/mL
GP binding	3.3B IgM	Lot: 101420-A	0.25 mg/mL
GP binding	3.3B IgG	Lot: 081319-A	0.25 mg/mL
GP binding	22.5D IgG	Lot: 021317-B	0.25 mg/mL
GP neutralising	12.1F IgG	Lot: 042220-B	0.25 mg/mL
GP neutralising	37.7H IgG	Lot: 122319-A	0.25 mg/mL

LASV mAb cocktail mixture 2

NP binding	34.11C IgM	Lot: 111720-A	0.25 mg/mL
NP binding	34.11C IgG	Lot: 062919-A	0.25 mg/mL
GP binding	3.3B IgM	Lot: 101420-A	0.25 mg/mL
GP neutralising	12.1F IgG	Lot: 042220-B	0.25 mg/mL

Appendix 3 Feasibility study report for the establishment of a working standard for anti-Lassa virus antibodies



Establishment of a working standard for anti-Lassa virus antibodies

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Summary

Lassa fever virus (LASV) is the aetiological agent of Lassa fever transmitted to humans by infected *Mastomys spp.* rats, as well as person-to-person contact with contaminated body fluids. The virus is endemic in western Africa where it causes recurrent outbreaks. LASV has been identified by the WHO R&D Blueprint as one of the top ten priority pathogens for outbreak potential. Vaccines and treatments are in development and reliable assays are needed for their evaluation. The availability of an antibody standard would facilitate the standardisation of LASV serological assays. In this report we have evaluated pools of sera from Lassa fever patients as a candidate reference reagent for the calibration of the anti-Lassa antibody assays. The study involved CEPI-partner vaccine developers, national regulatory laboratories and a diagnostic manufacturer, testing in a blinded manner the panel of candidate serological preparations using LASV assays established in their laboratories. Nineteen sets of data, for ELISA and neutralisation assay-based methods, were returned and statistically analysed. Sample 3, a pool of sera from Lassa convalescent individuals from Nigeria, produced the highest reduction in the variation between neutralisation assays between laboratories; it also increased harmonisation of the ELISA antibody titers for both IgG and IgM. It was proposed and accepted by the participants that sample 3 will be established as the working standard for anti-Lassa virus antibodies with an assigned potency of 1000 unit/mL and should be used to calibrate serological assays for the detection of anti-LASV antibodies. The remaining samples in the study will be distributed as an evaluation panel containing high (LASV-17), mid (LASV-2, 5 and 13) and low (LASV-11 and 19) titre antibody. The use of the working standard and the evaluation panel will contribute to the standardisation of assay for anti-LASV antibodies.

Introduction

Lassa virus (LASV) is endemic in several West African countries, causing seasonal outbreaks. While it has been reported about 80% of infections may be asymptomatic, it can result in severe haemorrhagic fever [1]. The case fatality rate (CFR) in these severe cases, has been reported to be up to 60-70% in previous outbreaks within Nigeria [2] and Sierra Leone [3]. The disease incidence has been increasing each year, with more than 800 confirmed cases within Nigeria in 2019 with a CFR of 21% [4]. There is a high level of sequence diversity amongst LASV strains, which have been grouped phylogenetically into 7 lineages [5-7]. The different lineages are spatially segregated, with lineage II and III strains circulating within Nigeria and lineage IV within Sierra Leone, as well as Guinea and Liberia.

As identified in the WHO R&D Blueprint, there is a lack of, and therefore a need, to develop effective treatments and vaccines against LASV [8, 9]. Analysis of vaccine potency and efficacy will include the requirement of assays to measure the antibody response. The availability of an antibody reference reagent for these assays early in this process is of value, allowing assay harmonisation by calibration to the reference which is given an arbitrary unitage. It assists better definition of parameters such as analytical sensitivity of tests or clinical parameters such as protective levels of antibody. The reference reagent will enable the comparison of Lassa serological data reported between laboratories and at different stages of vaccine efficacy clinical trials. Currently, the Coalition for Epidemic Preparedness Innovations (CEPI) is funding the development of six vaccines against LASV [10].

The highest order reference reagent for a biological substance is a WHO International Standard (IS), established by the WHO Expert Committee on Biological Standardisation (ECBS) and assigned a potency in International Units (IU). Typically, the process of establishment takes 2-3 years and requires the donation of volumes of sera to last 5-10 years [11]. In October 2018, the WHO ECBS endorsed the preparation of the International Standard for anti-Lassa virus antibodies [12]; in the meantime to support the Lassa fever vaccine development, a candidate Working Standard for Lassa virus antibody has been evaluated in this study. This will allow the calibration and standardisation of Lassa serological assays in the period until a WHO IS becomes available. The Working Standard, assigned a potency in units (U) per mL, will provide a valuable means of harmonisation for serological data reported and eventual traceability to an IU.

This study involved testing pools of convalescent sera collected from either Sierra Leone or Nigeria with different anti-LASV antibody titres, including a negative control, in serological assays that are in routine use in participants' laboratories. In addition to establishing one of these samples as the Working Standard, the remaining samples will be made available as a Reference Panel. The Reference Panel can be used to facilitate the characterisation of factors which may contribute to assay variability, such as antigenic variation, with samples collected from different regions where different LASV strains predominate and have a range of antibody titres.

The aims of this study are to:

- assess the suitability of different antibody preparations to serve as the working standard with an assigned unit per mL for use in the harmonisation of Lassa serology assays;

- characterise the antibody preparations in terms of reactivity/specificity in different assay systems, to serve as a reference panel for anti-Lassa virus antibodies;
- assess each preparation's potency i.e. readout in a range of typical assays performed in different laboratories;
- recommend to the CEPI Programme Advisory Committee (PAC) for Lassa the antibody preparations found to be suitable to serve as the standard. The working standard and the reference panel will then be made available to the vaccine developer for calibration and evaluation of their assays.

Materials and Methods

Participants in the study included four vaccine developers, two national control laboratories and a diagnostics manufacturer. Seven laboratories returned results and they are listed in Table 1. Laboratories are referred to by a number code allocated at random which is not reflected in the order of listing in Table 1.

Study samples

Samples are listed in Table 2. They were provided to the participants blinded and labelled "CS646 Sample X" where X is one of the following numbers; 2, 3, 5, 7, 11, 13, 17 or 19. Each screw cap tube was filled under aseptic conditions with a 50 µL aliquot and stored at -20°C. Samples were shipped to the participants on dry ice. Dispatch of the material commenced in September 2019 with the last shipment sent at the end of October 2019.

The source material was kindly donated by Prof. Robert Garry (University of Tulane, Viral Hemorrhagic Fever Consortium) and comprised pools of sera obtained from patients infected with Lassa virus, collected more than a year after discharge from hospital.

The samples are:

- five pools of convalescent sera from Sierra Leone with different anti-LASV antibody titres
- a pool of anti-LASV antibody-negative sera from Sierra Leone
- two pools of convalescent sera from Nigeria with different anti-LASV antibody titres

Prior to receipt at NIBSC, to fulfil local health and safety requirements, the source material was tested for presence of LASV RNA using two PCR methods with three sets of primers within containment level (CL) 4 facilities at Public Health England (Colindale, UK). As one of the PCR methods produced a very low signal (Appendix 2), samples were blind passaged on a permissive cell line within the CL4 facilities for a month. No cytopathic effect or increase in PCR signal was detected over three serial passages. Samples were certified as containing no detectable live Lassa virus (certificate available on request, an example is provided in Appendix 2) and shipped to NIBSC.

At NIBSC all samples were tested for known human blood-borne pathogens; some of the samples tested positive in the anti-HIV antibody test and for Hepatitis B surface antigens (HBsAg). All samples have been treated using a validated solvent detergent treatment to

inactivate possible enveloped virus contamination [13, 14]. Samples positive for the LASV-GP RNA (Table 2) were analysed for presence of LASV sequences, by Nextera DNA Flex library (cDNA prepared using random primers) and analysed on an MiSeq Illumina platform. All samples were found negative.

Study protocol and assay methods

The study protocol is given in Appendix 1. Briefly, the participants were requested to test the study samples using their established method(s) for the detection of antibodies against LASV. Participants were asked to perform three independent assays. For each assay, participants were requested to make at least 2 independent serial dilutions of the study samples.

Assays used by the participants are summarized in Table 3. Where laboratories performed multiple assay methods, laboratory codes are followed by a letter indicating the different methods e.g. lab 1a, 1b. Methods used by the participants were ELISA or neutralisation assay formats.

Statistical methods

For the neutralisation assays, the geometric mean (GM) of the potency of each sample was calculated from the endpoint titres or 50% reduction neutralisation titres (NT₅₀) provided by the participants.

Quantitative ELISA data were analysed using a sigmoid curve model with log transformed responses for IgG or a parallel line model for the IgM. Calculations were performed using the European Directorate for the Quality of Medicines & Healthcare (EDQM) software CombiStats™[15]. 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 0.80 – 1.25. 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 geometric means for each analyte.

Variability between laboratories has been expressed using geometric coefficients of variation ($GCV = [10s-1] \times 100\%$ where s is the standard deviation of the log₁₀ transformed estimates). Further assessment of agreement in geometric mean results for each pair of laboratories was performed by calculating Lin's concordance correlation coefficient with log transformed data, although these values are only based on a small number of samples. Calculations were performed using the R package DescTools [16].

Results

A total of 19 sets of data were returned (Table 3) by 15th December 2019, with 13 methods testing by quantitative ELISA, either detecting IgG or IgM, and 6 by neutralisation assays.

Enzyme immunoassays

Laboratory 2, 5 and 6 performed in-house ELISA, using whole LASV as coating antigen (lab 2a, b and 5a, b) or the recombinant pre-fusion LASV GP from Zalgen Labs (lab 6). For all the in-

house ELISAs, antibody titres were calculated as the inverse of the highest dilution factor which produced a positive result. The remaining four laboratories tested the samples using one of the Zalgen ReLASV pan-Lassa kits, either targeting pre-fusion GP or NP. For all of them, an internal control was run in parallel to the samples. Following manufacturer's instructions, the titres were calculated in either µg/mL (for IgG) or U/mL (for IgM), based on the value of the internal control (Figures 1 and 3, and Table 4).

- **Anti-LASV IgG**

The IgG titres for Lassa virus were calculated or provided by the participants as per the protocol in their laboratories (Figure 1, Table 4).

All the laboratories identified sample 3 and 17 as the highest titre samples (Figure 1 and 2, Table 4). For the two anti-NP IgG ELISA (lab 3b and 4a) sample 3 had the highest titre. Sample 17 from Sierra Leone was the highest for all the assays for IgG, except for lab 5b, which used an irradiated LASV lineage II, and identified sample 3 from Nigeria as the highest. However, no lineage bias can be inferred as the remaining samples ranked identically with the lab's other assay, 5a, which uses the Josiah strain (lineage IV) LASV (Figure 2).

The negative sample 7, was correctly identified as negative by all laboratories except by lab 6; however, it was the lowest titre sample in their assay and this false positive result may reflect an improperly set cut-off for the assay. The lowest titre sample in the majority (7 out of 9) of assays was sample 19, despite being provided as mid-titre for anti-GP antibodies. Sample 19 was found to be negative by lab 2b and 3b. Lab 3b used the same commercial assay for the detection of anti-LASV NP IgG as lab 4a, which detected sample 19 as positive, but used a higher initial dilution of 1/100 whereas lab 4a used a 1/50 starting dilution. Sample 11 was also a low titre sample in the majority of assays, and found negative by lab 2b. Samples 2, 5 and 13 provided as mid-titres (2 and 13) or low titre (5), were ranked differently in the assays, even by the same commercial assay performed by three laboratories (1a, 3a, 4c, Figure 2 and Table 4) suggesting an operator-based difference.

- **Anti-LASV IgM**

Four sets of data were returned for the detection of anti-LASV IgM. The in-house capture ELISA with the whole virus used as coating antigen (lab 2a), scored positive the two high titre samples 3 and 17, and sample 11 which was scored low in the IgG assays (Figure 3 and Table 4). Lab 4d, using a Zalgen pan-Lassa pre-fusion GP IgM kit, was able to detect all the samples and correctly identified sample 7 as negative. In this case, sample 11 was the lowest titre sample, followed by the IgG high titres samples 3 and 17. Sample 19 which was the lowest titre sample for most of the IgG assays had the highest IgM titre against pre-fusion GP. Lab 3c and 4b used the same Zalgen pan-Lassa NP IgM assay; both scored sample 2 and 7 negative. Sample 11 had the highest anti-NP antibody titre by Lab 4b and second highest by lab 3c. Sample 3 was scored high NP titre by both. Only sample 19 was discordant between the two labs with lab 3c identifying it as low titre and failing to detect it in one of the three independent experiments, while lab 4b identified sample 19 to have the second highest anti-NP IgM antibody titre.

Neutralisation assays

Five laboratories tested the samples in six neutralisation assay against LASV (Figure 4, Table 5). In all cases, a pseudotyped system was used instead of the wildtype LASV, which requires BSL4 facilities. Lab 2c used a replication competent Mopeia virus pseudotyped with LASV GP. This system has been previously compared with the live LASV neutralisation assay in use in the same lab and showed very good correlation (personal communication with the participant). The assay was only performed once, and only identified as positive the samples with high antibody titre (sample 3 and 17). This is not unusual and a lower sensitivity of assays using replication competent virus has been observed in previous studies [14, 17, 18]. Lab 1b and 7 used a HIV-based pseudotyped system while lab 3d,e and 5c used a VSV-based system. The reporter genes for the non-replicative pseudotyped-based neutralisation assays were either enhanced green fluorescent protein (EGFP) or luciferase. No apparent trends can be drawn based on system or reporter gene used. Anti-LASV neutralising antibody titres were calculated by each lab as 50% neutralisation titre (NT₅₀), while lab 7 expressed the titre as the inverse of the highest dilution factor which gave at least 50% reduction in infectivity compared to virus only. Samples ranked similarly to the anti-LASV IgG assay, with an increased concordance between the labs in their ranking. Sample 17 had the highest neutralising titre in all assays, except by lab 7 which ranked it second to sample 3; this was the sample with the second highest titre by all other labs (Table 5). Similar to the ELISA IgG titres, sample 19 and 11 had the lowest neutralising antibody titre, while 2, 5 and 13 had mid titres. Sample 7 was negative or below the first dilution of all the assays. Lab 3e reported the only results using a pseudotype incorporating a lineage II GP. By comparison to the lab's other assay, 3d, which incorporated a lineage IV GP, the ranking of the high titre samples 17 and 3 was the same. However, sample 11 from Nigeria, which ranked lowest with the lineage IV GP, was ranked higher with the lineage II GP than samples 2 and 19 from Sierra Leone.

Harmonisation of the antibody titres by a candidate standard

The ELISA raw data provided by the participants was processed by parallel line analysis and the potencies calculated as relative to one of the two high titre samples (3 or 17), which have been assigned an arbitrary value of 1000 units/mL (Table 4 and Figures 5, 6). This resulted in a tighter distribution of the IgG titres reported for each sample (Figure 1 vs Figure 5). The false positive result for sample 7, reported by lab 6, had a misleading high titre when expressed as inverse of the highest positive dilution factor (Figure 1) but it is clearly a borderline result when expressed in units/mL (Figure 5, Table 4). The interlaboratory variation expressed as geometric coefficient of variation (%GCV) is also reduced (Table 6). Sample 17 as a calibrant reduces the variation between laboratories the most and increased harmonisation of the potencies.

The same was also observed for the anti-LASV IgM, by reporting the sample potencies relative to either sample 3 (Figure 6A) or sample 17 (Figure 6B). Expressing the titre of anti-LASV IgM relative to either of the high titres samples reduced inter-laboratory variation (Table 6) however, sample 17 did not produce data suitable for parallel line analysis and three sets of data had to be excluded (nd, Table 4 and 6). Sample 3 harmonised the IgM data the best (Figure 6, Table 6) and similar results were obtained when sample 11 was used as calibrant (data not shown).

The data from the neutralisation assays was not suitable for consistent parallel line analysis. Therefore, for each independent experiment, the neutralising antibody titres were expressed as relative to the potency of either sample 3 or sample 17 with an assigned value of 1000 units/mL,

and the geometric mean calculated (Table 5, Figure 7). Both of these high titre samples were able to harmonise the results, with a reduction of inter-laboratory variation (Table 6), with sample 3 achieving the greatest level of harmonisation.

For the IgG results, the high %GCV in Table 6 is due to the different assay readouts. Using the same unitage greatly improves the comparability of the results. To better analyse the role of a standard, the %GCV were divided between in-house and commercial assays (Table 7). The in-house assays were harmonised by either of the two high titres samples, with sample 17 achieving the greatest reduction in %GCV. The commercial kits had, as expected, only a low level of variability between them owing to performance by different operators. Thus unsurprisingly, normalizing the results to a common standard did not improve the %GCV. The two sets of data obtained by the Zalgen kit for anti-LASV NP IgG were very close and expressing results relative to a standard gave a slight increase in the level of variability (Table 8), albeit the %GCV were still low. A caveat to these data is the minimal, not statistically significant number of data sets ($n=2$). There is a better level of harmonisation for the three Zalgen anti-LASV pre-fusion GP IgG kits (Table 8).

Assay Concordance

The level of concordance between laboratories has been calculated after harmonisation to sample 3 or sample 17 (Figure 8). Concordance coefficients can range from -1 (perfect inverse correlation) to 1 (perfect matching); thus, values closer to 1 represent a similar pattern between two labs and a value closer to 0 suggests no pattern. This analysis does not include labs performing ELISA detecting IgM (2a, 3c, 4b and 4d), due there not being enough data points available. For the same reason, lab 2b which performed an in-house ELISA detecting IgG and lab 2c which performed a neutralisation assay using pseudotyped Mopeia virus, are excluded from the analysis.

Most labs have concordance values greater than 0.5 and there is a good level of agreement between labs reporting neutralisation data (1b, 3d, 3e, 5c and 7), with slightly better concordance using sample 3 (Figure 8a). Interestingly, there are poor levels of concordance for labs 3a and 4c which used the same Zalgen pan-Lassa pfGP IgG assay, however the same assay performed by lab 1a produces a stronger level of concordance. While this small dataset requires a cautious interpretation, the strongest level of concordance between the different ELISA platforms and neutralisation results appears to be with the in-house IgG ELISA assays using either whole virus (lab 5a and 5b) or the recombinant pfGP (lab 6) as coating antigen. The level of concordance between the labs reporting ELISA data is stronger when using sample 17 (Figure 8b).

Discussion and conclusion

The purpose of this study was to evaluate pools of convalescent sera from Lassa fever recovered patients as a candidate Working Standard to harmonise serological data produced by laboratories. Seven laboratories took part in the study and tested a panel of serological preparations containing anti-LASV antibodies. The potencies reported by the participants varied greatly among assays, but this mainly reflected the different method readouts (Table 4 and 5). As shown in Figure 1a and Table 4, reporting the ELISA IgG titres as the inverse of the highest

positive dilution, produced numerically greater values than expressing them as $\mu\text{g/mL}$ using a commercially available kit, which could be misleading. Expressing the titres in units/mL (Figure 5) relative to samples 3 and 17, harmonised the results between laboratories and improved the inter-laboratory variation (Table 4 and 6). The same result was seen for the four sets of data using an anti-LASV IgM ELISA (Figure 3 vs 6 and Table 6) and for the neutralisation assays (Figure 4 vs 7 and Table 6). The high titre sample 17 from Sierra Leone showed better harmonisation of the ELISA data than sample 3, while the high titre sample 3 from Nigeria achieved a greater reduction in the inter-laboratory variation in the neutralisation assays (Table 6). In both of the high titre samples 3 and 17, anti-LASV IgM were detected, however, expressing the potencies for IgM to sample 17 caused a loss of data (Figure 6 and Table 4). Based on these data, sample 3 seems the best candidate to act as a Working Standard for Lassa virus antibodies.

Only three samples were consistently detected in all four IgM methods used: the two high titre sample 3 and 17, and the low IgG sample 11. It is not surprising that most of the samples do not possess high IgM titres as they have been collected more than 1-year post infection. Lab 4 detected IgM in all the samples, except the negative control 7 with a starting dilution of 1:50, while the other laboratories 2a and 3c started at 1:100. Laboratory 3 tested the study panel samples once in the same assay as lab 4d, again with an initial dilution 1:100, and had no positive results and decided not to repeat the assay (personal communication). These results highlight that when using the same commercially available assay this can lead to a difference in the results, not only quantitatively but also qualitatively (positive/negative).

The scope of this study is not the evaluation of the assays, but to test that the candidate standard will perform satisfactorily in different serological methods and will achieve harmonisation of the results between laboratories. Nevertheless, we looked at the concordance between ELISA and neutralisation methods (Figure 8) and we found that the neutralisation assays produced higher concordant results than the ELISAs. The difference in the neutralising antibody titres was no more than 15-fold in the results reported by the participants and expression of the data relative to sample 3 reduced it to less than 6-fold (Table 5). For the in-house ELISA the original titres were more than 100-fold different, and the use of a standard reduced this to about 10-fold, in line with the neutralisation data.

The lack of harmonisation of the results obtained by commercial assays could be explained by a low inter-laboratory variation in the original data (Table 7 and 8) and low potencies (Table 4). The greatest difference in the amount of antibodies expressed as $\mu\text{g/mL}$ was only 5-times. Normalisation of the titres to sample 3 produced a difference of about 10-fold for sample 19; there is less difference for the remaining samples, but it is still higher than the original data. However, this is similar to the normalised data from the neutralisation assays and the in-house ELISAs.

Cross reactivity of different Lassa lineages cannot be discussed based on the results from this study; most of the ELISA were developed as pan-reactive and indeed they detected with similar potencies samples from Nigeria and Sierra Leone. Only one neutralisation assay used a LASV GP from lineage II, while all the remaining assay used the lineage IV Josiah strain. A separate

study with a larger number of samples from different geographical areas would be needed to investigate the cross reactivity of the assays.

Proposal

Pending consultation with all the participants, we propose that sample 3 (code: LASV-3) from Nigeria will be established as the Working Standard for anti-Lassa virus antibodies for both ELISA and neutralisation assays with an arbitrary value assigned of 1000 unit/mL. The working standard will be included in the upcoming Collaborative Study for the establishment of the 1st WHO International Standard for anti-Lassa virus antibody and calibrated as part of that study to the International Standard in International Units/mL.

A panel of samples is also established and available for distribution to CEPI partners which includes a high titre sample from Sierra Leone (LASV-17), a low titre sample from Sierra Leone (LASV-19), a low IgG, high IgM sample from Nigeria (LASV-11) and three mid-range titre samples from Sierra Leone (LASV-2, LASV-5, LASV-13). A Lassa negative sample from Sierra Leone will also be included in the panel. Sample 7 has been depleted, but similar material has been provided by Prof Robert Garry and will be prepared as sample 7 (LASV-Negative).

There are 160 working standards and 260 reference panels available for distribution, free of charge (shipping costs will still apply). Requests should be directed to either

Giada.Mattiuzzo@nibsc.org or Emma.Bentley@nibsc.org.

Comments from participants:

Lab 7 The correlation results, especially, between the various Neutralization test performed by different partners are very interesting. Considering the variability of this platform I believe that this is an important achievement

Acknowledgments

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 Donald S Grant (Kenema Government Hospital, Kenema, Sierra Leone) Christian T. Happi (Redeemer's University, Ede, Nigeria), the Viral Hemorrhagic Fever Consortium and the African Center of Excellence for Genomics of Infectious Disease for the collection and testing of the serum samples. We gratefully acknowledge the important contributions of the feasibility study participants and members of the CEPI Lassa virus Task Force. We would also like to thank NIBSC Standards Production and Development staff for distribution of materials.

Tables and Figures**Table 1. Study participants**

(in alphabetical order by country)

Name	Organisation	Country
Sylvain Baize	Institut Pasteur (THEMIS partner)	France
Nicole Armbruster	CureVac AG	Germany
Alessandro Manenti, Emanuele Montomoli	VisMederi (CureVac partner)	Italy
Emma Bentley	National Institute for Biological Standards and Control	United Kingdom
Jean D. Boyer, Veronique Schulten	Inovio Pharmaceuticals	California, U.S.A
Matthew Boisen, Doug Simpson	Zalgen Labs LLC	Colorado, U.S.A.
Rong Xu	Profectus Biosciences Inc.	New York, U.S.A.

Table 2. Study samples

Pools of sera from Nigeria or Sierra Leone provided as frozen, liquid 50 µL aliquots. All samples have been solvent detergent treated at NIBSC. Anti-LASV antibodies titre yield (low, mid, high) were determined by the donor organization using Zalgen ELISA kits.

CS646 sample	Description	Infectious LASV*	LASV GP RNA**	HCV RNA***	Anti-HIV Ab***	HBVsAg ***
2	SL mid pool #1	Neg	+	Neg	+	+
3	Nigeria high	Neg	+	Neg	Neg	+
5	Sierra Leone low	Neg	+	Neg	Neg	+
7	SL Negative control	Neg	Neg	Neg	Neg	Neg
11	Nigeria low	Neg	+	Neg	+	+
13	SL mid pool#2	Neg	Neg	Neg	Neg	+
17	SL high	Neg	+	Neg	Neg	+
19	SL GP mid, NP Low	Neg	+	Neg	Neg	Neg

SL: Sierra Leone; GP: glycoprotein; NP: nucleoprotein; HCV: Hepatitis C virus; HIV: human immunodeficiency virus; Ab: antibodies; HBVsAg: Hepatitis B virus surface antigen; *tested by serial passages at PHE, Colindale, UK; ** Altona RealStar Lassa 2.0 real-time PCR; ***tested by Blood Virology group at NIBSC.

Table 3. Laboratory codes and assay methods

Lab	Assay method	Target/strain	output
1a	ELISA - Zalgen ReLASV pan-Lassa prefusion GP	GP (IgG)/ pan	µg/mL
1b	Neutralisation: HIV core pseudotyped, EGFP reporter gene	GP / Josiah (IV)	NT ₅₀
2a	ELISA- in house	Whole virus (IgM)/ Josiah (IV)*	Inverse dilution factor
2b	ELISA- in house	Whole virus (IgG)/ Josiah (IV) *	Inverse dilution factor
2c	Neutralisation: Mopeia pseudotyped, plaque reduction	GP/Josiah (IV)	NT ₅₀
3a	ELISA- Zalgen ReLASV pan-Lassa prefusion-GP	GP (IgG)/ pan	µg/mL
3b	ELISA- Zalgen ReLASV pan-Lassa NP	NP (IgG)/pan	µg/mL
3c	ELISA- Zalgen ReLASV pan-Lassa NP	NP (IgM)/pan	U/mL
3d	Neutralisation: VSV core pseudotyped, Luciferase reporter gene	GP / Josiah (IV)	NT ₅₀
3e	Neutralisation: VSV core pseudotyped, Luciferase reporter gene	GP / (II)	NT ₅₀
4a	ELISA- Zalgen ReLASV pan-Lassa NP	NP (IgG)/ pan	µg/mL
4b	ELISA- Zalgen ReLASV pan-Lassa NP	NP (IgM)/pan	U/mL
4c	ELISA- Zalgen ReLASV pan-Lassa prefusion-GP	GP (IgG)/ pan	µg/mL
4d	ELISA- Zalgen ReLASV pan-Lassa prefusion-GP	GP (IgM)/ pan	U/mL
5a	ELISA- in house	Whole virus (IgG)/ Josiah (IV)	Inverse dilution factor
5b	ELISA- in house	Whole virus (IgG)/(II)	Inverse dilution factor

5c	Neutralisation: VSV core pseudotyped, EGFP reporter gene	GP / Josiah (IV)	NT ₅₀
6	ELISA- in house	Pre-fusionGP (IgG)	Inverse dilution factor
7	Neutralisation: HIV core pseudotyped, luciferase reporter gene	GP / Josiah (IV)	Inverse dilution factor

*known to cross react with other lineages; NT₅₀: 50% neutralisation titre; GP: glycoprotein; NP: nucleoprotein.

These labs used the same commercial ELISA kit: 1a,3a and 4c, 3b and 4a, 3c and 4b.

Table 4. Geometric means of the anti-LASV antibody titres detected by ELISA.

Raw data provided by the participants were analysed as per protocol in use in their laboratory (top table) or by parallel line analysis using sample 3 or sample 17 as standard with an arbitrary value of 1000 U/mL. (-) negative; nd: not determined, not suitable for parallel lines analysis; *assigned value.

	as analysed by the participants																
	in house ELISA					Commercial ELISA						TOTAL IgG	IgM ELISA				
	2b	5a	5b	6	GM	1a	3a	3b	4a	4c	GM	GM	2a	3c	4b	4d	GM
2, mid SL	100	1298	624	10687	965	2.04	3.42	1.16	1.43	1.73	1.82	13.18	-	-	-	4.59	4.59
3, high N	400	8100	3894	18275	3897	8.14	4.66	12.05	8.52	2.52	6.29	46.34	1600	2.71	3.6	2.72	12.35
5, mid SL	400	1298	624	3655	1043	3.32	4.16	1.53	2.08	1.98	2.44	15.92	-	0.63	1.81	4.76	1.76
7, neg	-	-	-	428	428	-	-	-	-	-	-	428	-	-	-	-	-
11, low N	-	208	144	2137	400	1.22	3.96	1.76	2.18	1.66	1.99	7.43	1600	2.58	5.72	2.01	14.76
13, mid SL	400	1872	900	6250	1433	3.01	3.01	1.93	2.7	1.67	2.39	17.2	-	0.72	2.59	5.46	2.17
17, high SL	635	8100	2700	31250	4564	8.83	6.66	6.12	7.8	3.68	6.35	45.35	252	0.84	2.36	3.45	6.44
19, mid/low SL	-	433	300	2137	652	0.52	1.24	-	1.17	0.95	0.92	6.29	-	0.63	3.71	5.85	2.39
	U/mL (relative to sample 3)																
2, mid SL	250	160	130	370	210	250	700	90	210	730	300	260	-	nd	nd	1510	
3, high N	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*
5, mid SL	1000	170	130	480	320	420	830	120	270	750	380	350	-	330	360	1440	560
7, neg	-	-	-	26	26	-	-	-	-	-	-	26	-	-	-	-	-
11, low N	nd	20	30	260	60	180	810	140	290	710	330	170	1000	970	1020	780	940
13, mid SL	1000	270	160	420	360	440	630	160	340	720	400	390	-	340	500	175	670
17, high SL	1590	950	680	1110	1030	1070	1500	630	900	1290	1030	1030	160	nd	220	1190	350
19, mid/low SL	nd	70	50	110	70	70	270	40	170	490	150	110	-	nd	490	1710	920
	U/mL (relative to sample 17)																
2, mid SL	160	160	200	370	210	230	470	140	230	560	290	250	-	-	-	1270	1270
3, high N	630	1050	1470	900	970	930	660	1600	1110	770	970	970	6350	nd	4570	840	2900
5, mid SL	630	180	190	430	310	390	550	180	300	580	370	340	-	nd	1450	1210	1320
7, neg	-	-	-	23	23	-	-	-	-	-	-	23	-	-	-	-	-
11, low N	-	30	40	230	60	170	540	220	320	550	320	170	6350	nd	3710	520	2310
13, mid SL	630	280	230	380	350	410	420	260	370	550	390	370	-	nd	2000	1690	1840
17, high SL	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*	1000*
19, mid/low SL	-	70	80	110	90	60	180	70	190	380	140	120	-	nd	1210	1660	1420

Table 5. Geometric means of the neutralisation titres as reported by the participants or relative to a candidate Standard.

Anti-LASV neutralising antibody titre as reported by the participants (top table) or expressed as relative to sample 3 or sample 17, which have been given the arbitrary value of 1000 U/mL. * assigned value; ** reported in 3/3 experiments a value below the lowest dilution factor (<10).

	NT ₅₀						
	1b	2c	3d	3e	5c	7	GM
2, mid SL	54	-	59	15	96	12	35
3, high N	154	20	188	164	326	71	114
5, mid SL	66	-	43	79	111	20	55
7, neg	-	-	-	-	-	**	-
11, low N	-	-	10	20	93	9	21
13, mid SL	63	-	77	42	103	36	60
17, high SL	193	20	308	300	381	65	144
19, mid/low SL	-	-	26	10	45	7	17
	U/mL (relative to sample 3)						
2, mid SL	320	-	320	90	290	180	220
3, high N	1000*	1000*	1000*	1000*	1000*	1000*	1000*
5, mid SL	430	-	230	480	340	300	340
7, neg	-	-	-	-	-	-	-
11, low N	-	-	50	-	290	150	130
13, mid SL	410	-	410	260	310	590	380
17, high SL	1260	1000	1640	1640	1180	1190	1290
19, mid/low SL	-	-	140	60	130	110	100
	U/mL (relative to sample 17)						
2, mid SL	250	-	190	60	250	150	160
3, high N	800	1000	610	610	850	840	770
5, mid SL	340	-	140	290	290	250	250
7, neg	-	-	-	-	-	-	-
11, low N		-	30	-	250	130	100
13, mid SL	320	-	250	160	270	500	280
17, high SL	1000*	1000*	1000*	1000*	1000*	1000*	1000*
19, mid/low SL	-	-	90	40	110	90	70

Table 6. Reduction in the inter-laboratory variance by expressing the anti-LASV antibody titres as relative to the candidate Standard.

The percentage of the geometric coefficient of variation (%GCV) was calculated either on the values reported by the participants or the potencies expressed as relative to sample 3 or sample 17 (Table 4). A reduction in GCV greater than 10% is highlighted in green, while an increase is highlighted in red. nd: not determined

	GCV (%) ELISA IgG			GCV (%) ELISA IgM			GCV (%) Neutralisation Assay		
	as reported	vs sample 3	vs sample 17	as reported	vs sample 3	vs sample 17	as reported	vs sample 3	vs sample 17
2, mid SL	3291	103	65	-	-	-	158	70	90
3, high N	3422	0	38	2224	129	196	79	0	25
5, mid SL	2502	126	68	175	0	nd	104	34	42
7, neg	-	-	-	-	-	-	-	-	-
11, low N	1671	269	207	2247	13	274	193	145	192
13, mid SL	3051	89	40	179	136	nd	56	37	61
17, high SL	3631	38	0	1138	196	0	215	22	0
19, mid/low SL	3456	135	89	224	nd	nd	148	46	62

Table 7. Difference in harmonisation of the data between in-house IgG ELISA and commercial kit.

The geometric mean titres and the relative %GCV obtained by lab 2b, 5a, 5b and 6 using an in-house ELISA and expressing the potencies as the inverse of the highest positive dilution factor were analysed separately from lab 1a, 3a, 3b, 4a, 4c, which uses a commercial kit and expressed the potencies as µg/mL. A reduction in GCV greater than 10% is highlighted in green, while an increase is highlighted in red

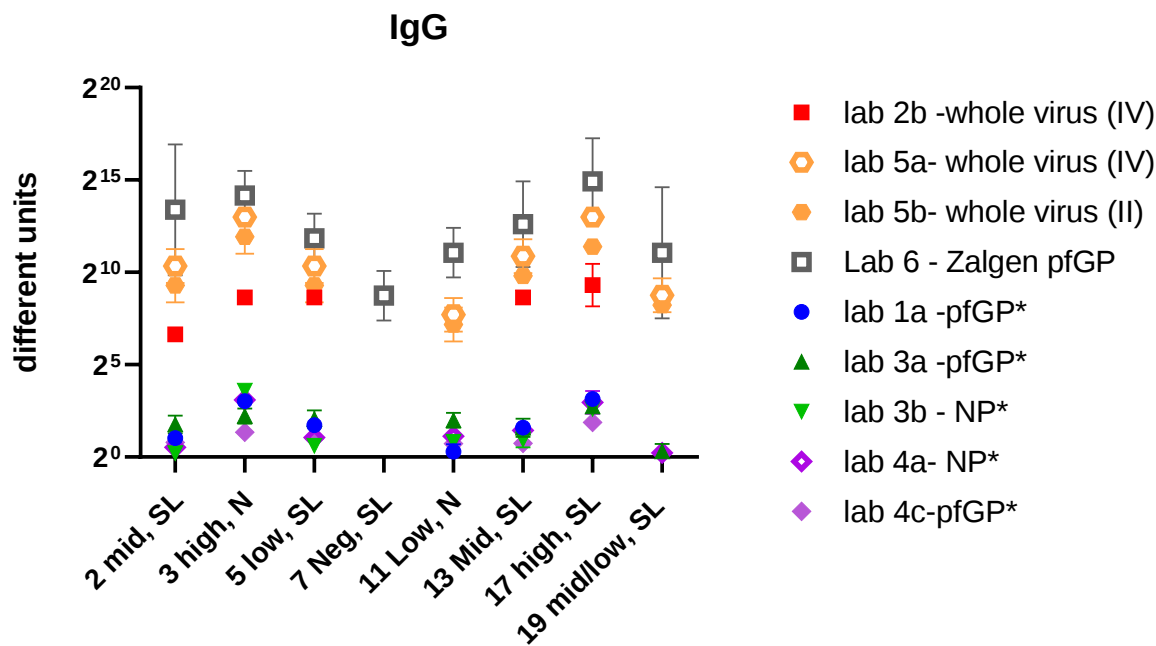
	GCV (%) in house assay				GCV (%) commercial kit		
	as reported	vs sample 3	vs sample 17		as reported	vs sample 3	vs sample 17
2, mid SL	590	59	47	2, mid SL	51	141	78
3, high N	417	0	42	3, high N	85	0	40
5, mid SL	163	159	87	5, mid SL	50	122	61
7, neg				7, neg			
11, low N	332	275	218	11, low N	55	119	69
13, mid SL	221	120	55	13, mid SL	31	80	32
17, high SL	422	42	0	17, high SL	40	40	0
19, mid/low SL	184	44	22	19, mid/low SL	50	170	113

Table 8. Inter-laboratories difference in anti-LASV IgG ELISA using commercial kits. The variation in the geometric mean IgG potencies for each sample has been analysed based on the commercial kit target, nucleoprotein (NP) or pre-fusion glycoprotein (pfGP).

	GCV (%) NP kits				GCV (%) pfGP kits		
	as reported	vs sample 3	vs sample 17		as reported	vs sample 3	vs sample 17
2, mid SL	16	81	45	2, mid SL	43	84	60
3, high N	28	76	30	3, high N	80		18
5, mid SL	24		41	5, mid SL	46	44	24
7, neg				7, neg			
11, low N	16	63	28	11, low N	84	129	97
13, mid SL	27	65	29	13, mid SL	41	29	19
17, high SL	19	30		17, high SL	56	18	
19, mid/low SL	nd	164	104	19, mid/low SL	57	174	145

Figures

A



B

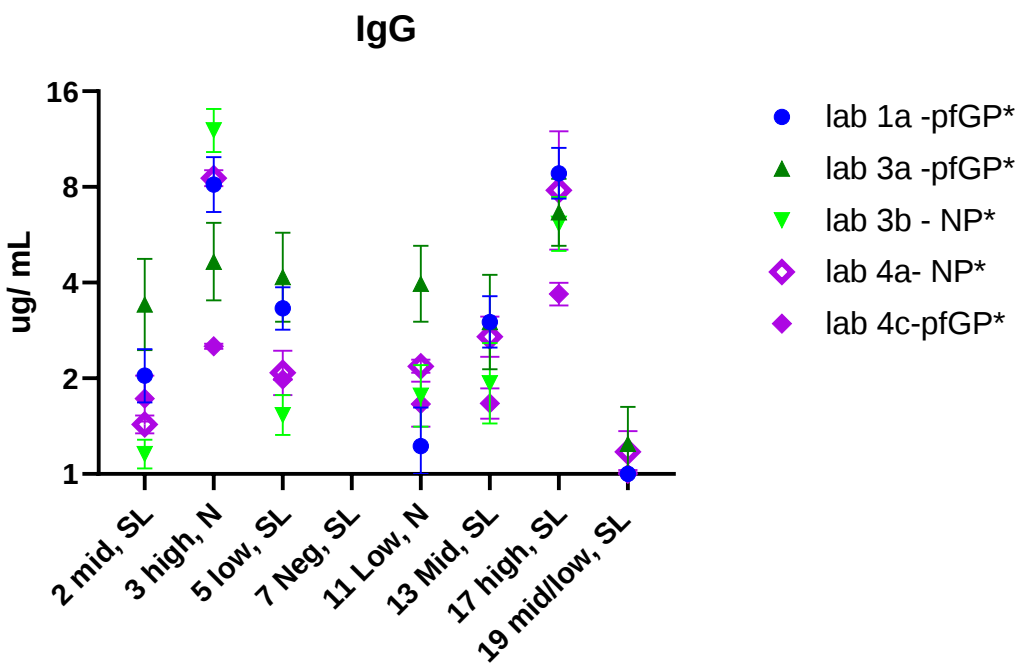


Figure 1. Anti-LASV IgG titres by ELISA. A) The potencies for each sample are expressed as the geometric mean of three independent experiments ($\pm$ standard deviation). Values were calculated using the method of analysis in use in each laboratory or provided in the instructions for use with commercial kits. For laboratories 2b, 5a, 5b and 6, the titre is the inverse of the highest dilution factor which still produced a positive result; **B)** the titres obtained using a commercial kit were calculated by curve fitting analysis using an internal control with a known potency expressed in $\mu\text{g/mL}$.

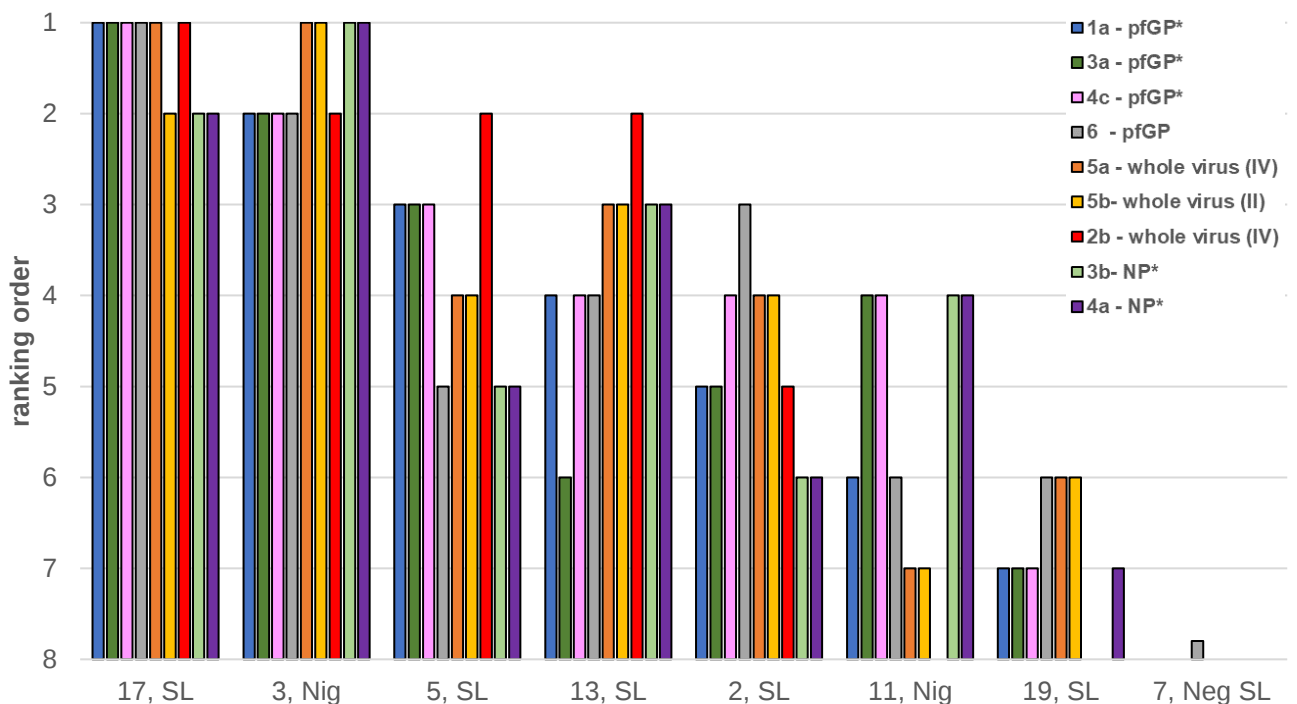


Figure 2. Sample ranking based on anti-LASV IgG titres. For each ELISA, samples were scored from the highest geometric mean titre, as calculated from the returned data, to the lowest, with 1 being the highest titre and 8 being the lowest.

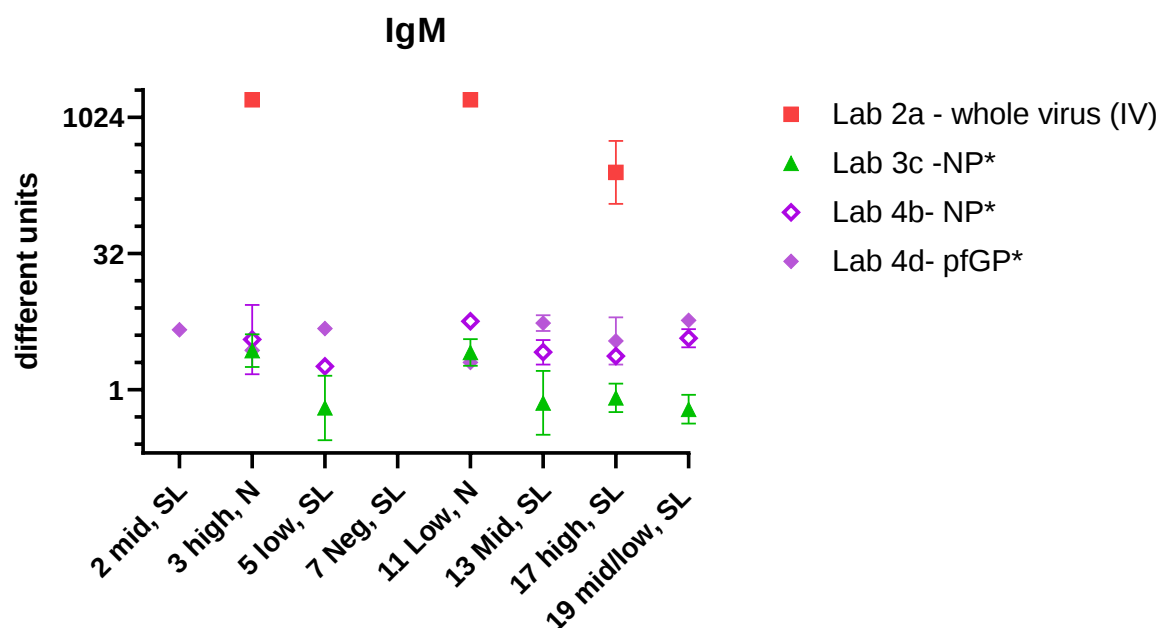


Figure 3. Anti-LASV IgM titres by ELISA. The IgM potencies for each sample are expressed as geometric mean of three independent experiments ($\pm$ standard deviation). Values were calculated using the method of analysis in use in each laboratory or provided in the instructions for use with commercial kits. For laboratory 2a the titre is the inverse of the highest dilution factor which still produced a positive result; the titres obtained for laboratories 3c, 4b, 4d using a commercial kit were calculated by curve fitting analysis using an internal control with known potency expressed in units/mL.

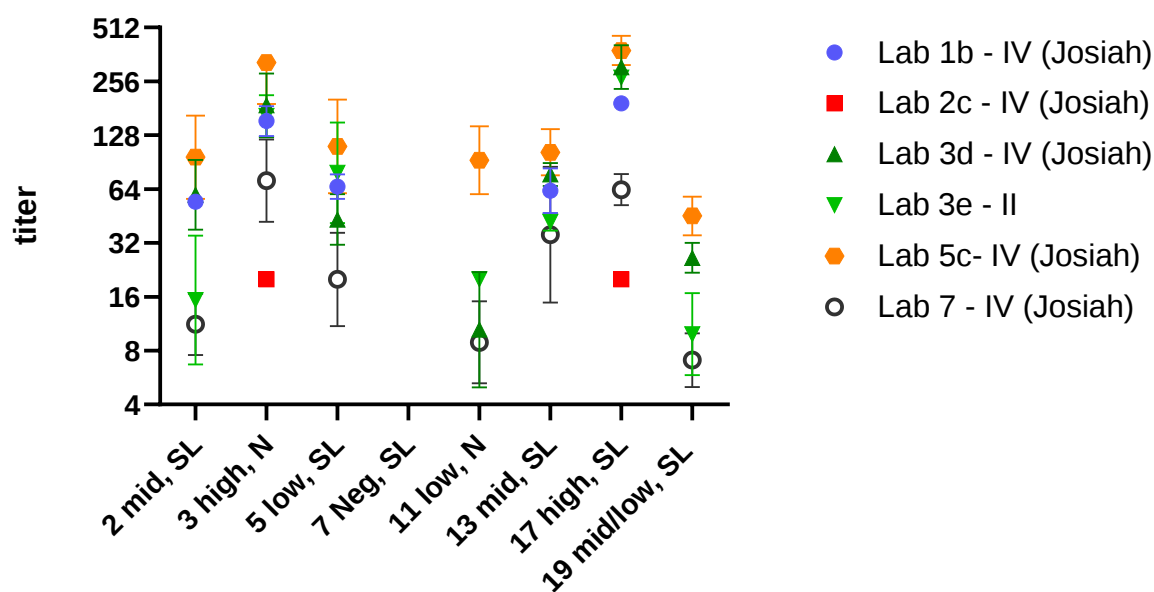
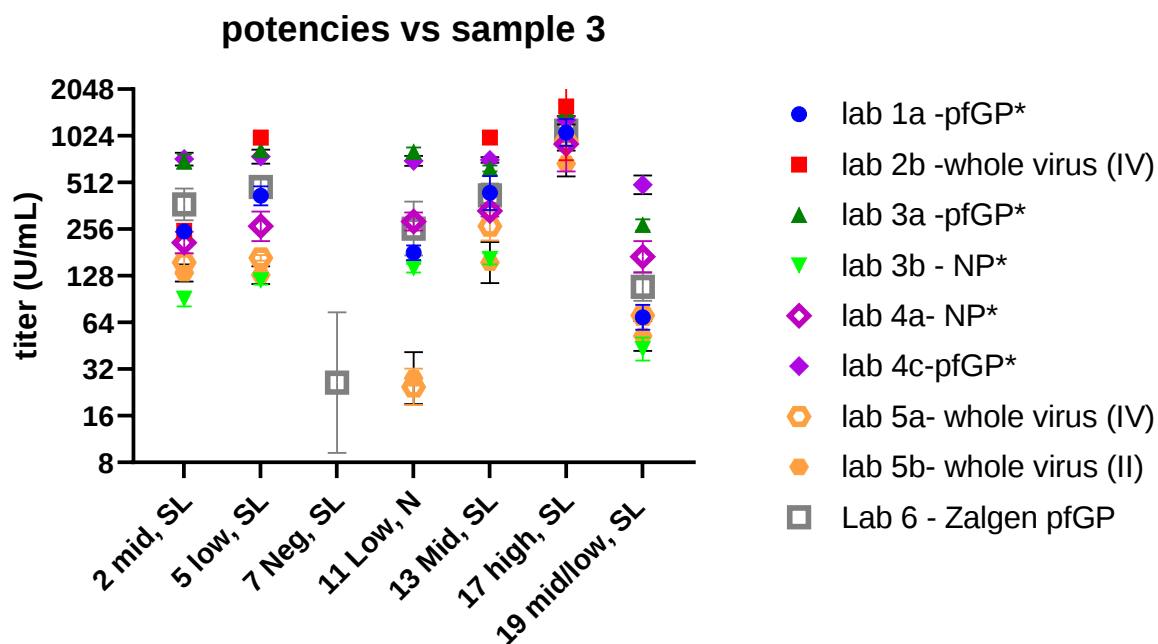


Figure 4. Neutralisation titres as reported by the participants. Geometric mean titres of neutralising antibodies for each sample were calculated from three independent experiments performed by the participants $\pm$ standard deviation. Laboratory 2 performed only one independent assay.

A.



B.

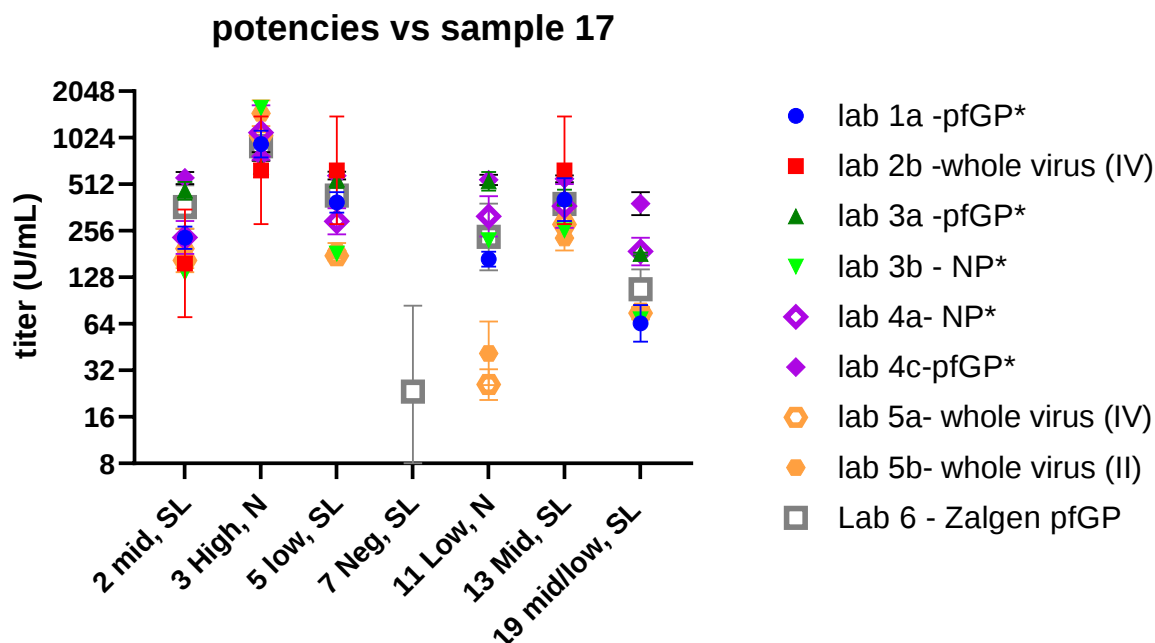
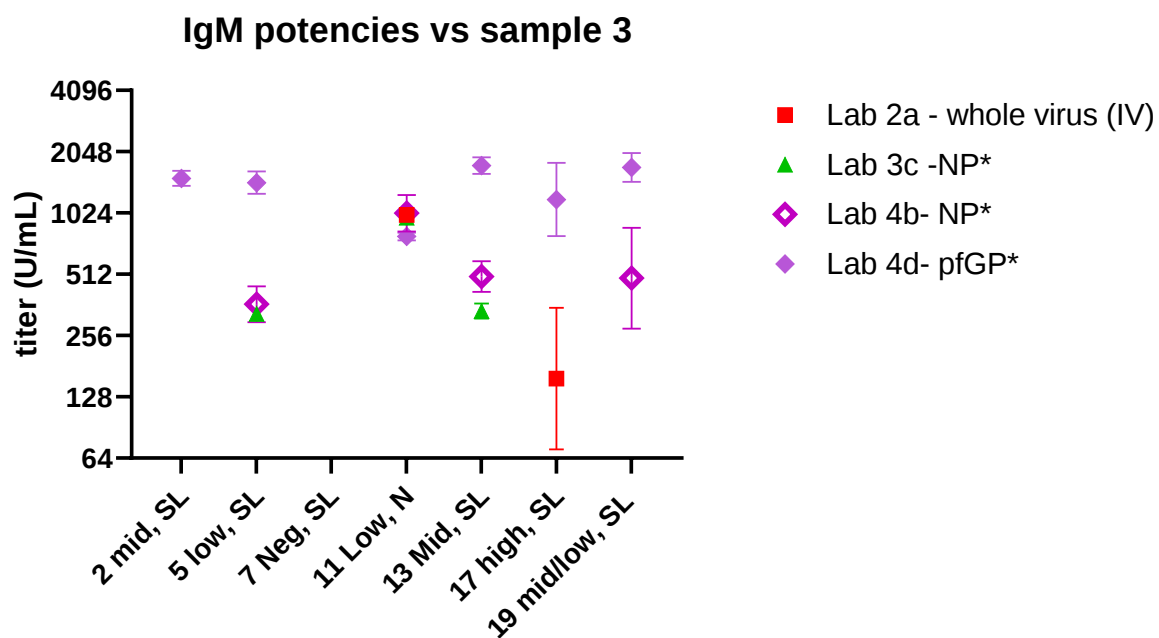
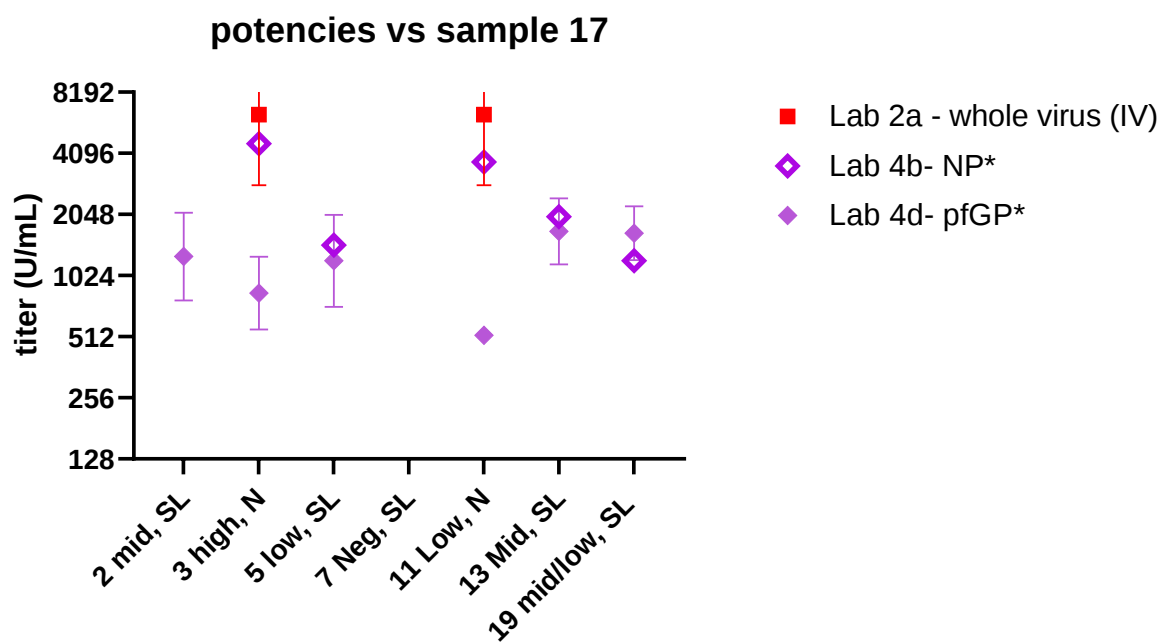


Figure 5. Harmonisation of the anti-LASV IgG titres when reported as relative to the candidate standard. The raw optical density (O.D.) data reported for the IgG ELISA was used to calculate samples' potencies relative to either sample 3 **(A)** or sample 17 **(B)** by parallel line analysis. Values are indicated in units/mL assuming an arbitrary potency for the candidate standard of 1000 U/mL. Each point represents the geometric mean of three independent experiments $\pm$ standard deviation.



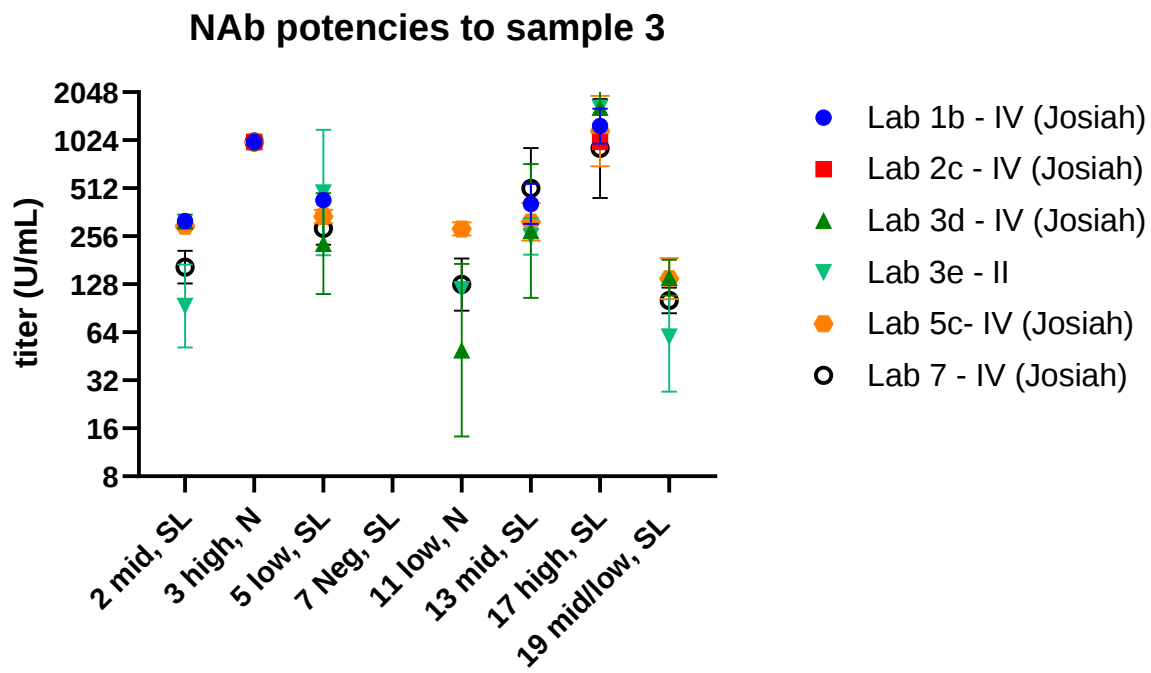
A.



B.

Figure 6. Harmonisation of the anti-LASV IgM titres when reported as relative to the candidate standard. The raw optical density (O.D.) data for the IgM ELISA was used to calculate samples' potencies relative to either sample 3 (**A**) or sample 17 (**B**) by parallel line analysis. Values are indicated in units/mL assuming an arbitrary potency for the candidate standard of 1000 U/mL. Each point represents the geometric mean of three independent experiments $\pm$ standard deviation.

A



B

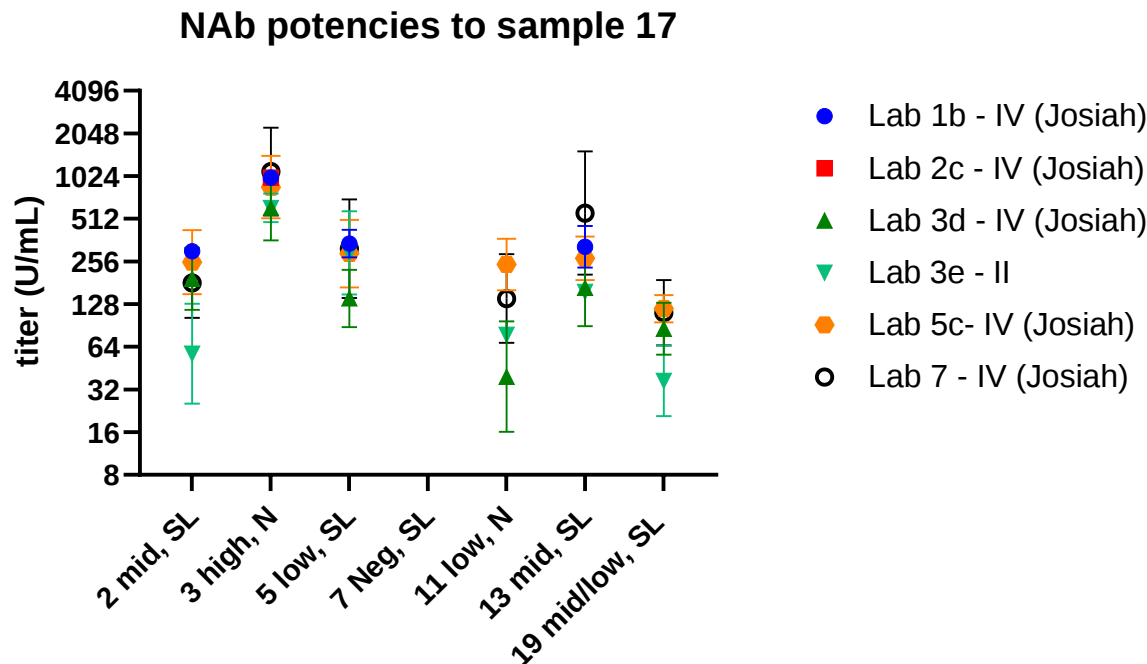


Figure 7. Harmonisation of the anti-LASV neutralising antibodies through calibration to a candidate standard. The geometric mean of neutralisation titres for each sample relative to either sample 3 (A) or sample 17 (B) assuming an arbitrary value of 1000 unit/mL (Table 5).

A

		ELISA								NEUT				
	Lab	1a	3a	3b	4a	4c	5a	5b	6	1b	3d	3e	5c	7
ELISA	1a													
	3a	0.44												com pfGP
	3b	0.64	0.21											com NP
	4a	0.77	0.36	0.54										ih WV
	4c	0.26	0.79	0.12	0.24									ih pfGP
	5a	0.64	0.16	0.64	0.42	0.12								HIV PV
	5b	0.56	0.14	0.71	0.37	0.10	0.96							VSV PV (II)
	6	0.84	0.56	0.49	0.80	0.34	0.49	0.42						VSV PV (IV)
Neut	1b	0.93	0.42	0.52	0.78	0.29	0.66	0.56	0.93					
	3d	0.50	0.24	0.52	0.57	0.19	0.87	0.75	0.66	0.85				
	3e	0.50	0.34	0.75	0.66	0.21	0.73	0.68	0.67	0.79	0.75			
	5c	0.66	0.50	0.55	0.92	0.32	0.47	0.42	0.92	0.89	0.70	0.74		
	7	0.59	0.36	0.64	0.81	0.24	0.69	0.59	0.81	0.91	0.85	0.86	0.88	

B

		ELISA									NEUT				
	Lab	1a	3a	3b	4a	4c	5a	5b	6	1b	3d	3e	5c	7	
ELISA	1a														
	3a	0.53												com pfGP	
	3b	0.85	0.40											com NP	
	4a	0.74	0.57	0.77										ih WV	
	4c	0.25	0.57	0.21	0.34									ih pfGP	
	5a	0.67	0.15	0.66	0.43	0.11								HIV PV	
	5b	0.75	0.21	0.77	0.53	0.14	0.97							VSV PV (II)	
	6	0.84	0.70	0.73	0.82	0.36	0.52	0.61						VSV PV (IV)	
Neut	1b	0.93	0.40	0.77	0.71	0.21	0.75	0.82	0.87						
	3d	0.48	0.11	0.57	0.36	0.09	0.97	0.92	0.56	0.72					
	3e	0.47	0.23	0.72	0.42	0.11	0.73	0.75	0.52	0.64	0.68				
	5c	0.66	0.57	0.85	0.88	0.27	0.51	0.63	0.91	0.86	0.57	0.59			
	7	0.59	0.34	0.85	0.72	0.19	0.76	0.80	0.78	0.91	0.75	0.73	0.86		

Figure 8. Level of concordance between laboratory methods. Concordance coefficients were calculated using the potencies either relative to sample 3 **(A)** or sample 17 **(B)**. Darker shades of green indicate high concordance between laboratories. com: commercial; pfGP: pre-fusion glycoprotein; NP: nucleoprotein; ih: in-house; WV: whole virus; HIV: human immunodeficiency virus; PV: pseudotyped virus; VSV: vesicular stomatitis virus; II: lineage II; IV: lineage IV.

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Appendix 4. Collaborative study Protocol

Protocol for the WHO collaborative study to establish the first International Standard for anti-Lassa fever virus antibody

This multi-center International collaborative study aims to evaluate candidate preparations to serve as first WHO International Standard for anti-Lassa fever virus (LASV) antibody and is organized by NIBSC in collaboration with the World Health Organization (WHO). The study has been facilitated by the Coalition for Epidemic Preparedness Innovations (CEPI) which sponsored the sourcing and formulation of the candidate material.

International Standards (IS) are recognized as the highest order of reference materials for biological substances and they are assigned potencies in International Units (IU). International Standards are 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-LASV antibodies will facilitate the standardisation of Lassa fever serological assays used for detection of anti-LASV antibodies to establish infection, epidemiology and vaccine responses. The establishment of such a standard will follow published WHO guidelines and be submitted for formal endorsement 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 different antibody preparations to serve as the International Standard with an assigned unitage per ampoule for use in the harmonisation of Lassa fever serology assays.
- characterise the antibody preparations in terms of reactivity/specificity in different assay systems.
- assess each preparation's potency i.e. readout in a range of typical assays performed in different laboratories.
- 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.

Materials

Coded study samples

The study samples should be stored at -20°C or below. The study samples shall not be administered to humans or animals in the human food chain.

All samples will be provided coded and blinded. The samples are labelled "CS686 Sample Lx" where x is a number from 1 to 12 (e.g. L1, L2, etc.). The coded samples may include negative samples as well as reactive samples. Laboratories will receive at least 4 sets of study samples

which should allow for 3 independent assays (plus 1 spare) by one method. Laboratories with more than one method or which require more than 0.1 mL of material per method will receive additional sample sets to allow 3 independent tests per method. Sample L9 only contains a volume of 0.05 mL, however, due to limited stock it was not possible to provide all the participants with enough vials for each assay. Please include sample L9 in at least one assay, preferably one which is quantitative.

Plasma/serum obtained from convalescent patients

The source materials are pools of serum or plasma samples obtained from patients infected with Lassa virus, collected more than one-year post-discharge from hospital. Source material was kindly donated by Dr Stephan Günther, Bernhard Nocht Institute for Tropical Medicine, Germany, in collaboration with Dr Ephraim Ogbaini-Emovon, Irrua Specialist Teaching Hospital, Nigeria, and by Dr Robert Garry, University of Tulane, on behalf of the Viral Hemorrhagic Fever Consortium and the African Center of Excellence for Genomics of Infectious Disease.

All the samples have been tested using the Altona RealStar Lassa Real-Time RT-PCR 2.0 (comprising GPC and L gene targets) at Public Health England, Colindale, UK and no Lassa virus RNA was detected. At NIBSC all samples were also tested for known human blood-borne pathogens HIV, HCV and HBV. Sample L1 was found positive for anti-HIV-1 antibodies, samples L1, L7, L8 and L9 were positive for HBV surface antigen. All samples have been treated using a validated solvent-detergent treatment to inactivate possible enveloped viral contamination (Appendix 1) and therefore provided as not infectious.

The samples are:

- pools of Lassa fever convalescent serum samples from Nigeria;
- pools of Lassa fever convalescent plasma from Nigeria or Sierra Leone;
- negative control serum from LASV-negative donors from Sierra Leone;
- pool of human monoclonal anti-LASV antibodies, formulated in PBS containing 0.1% bovine serum albumin (Sigma, lot SLCD4720), pH 7.4

Preparations are either freeze-dried, filled in 0.25 mL aliquots into 2.5 mL ampoules (sample L1-L8) or liquid frozen and filled in 0.05 mL (sample L9) or 0.1 mL (samples L10-L12) aliquots into screw cap tubes.

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 LASV. 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 for detection of antibodies against LASV
- Reconstitute freeze-dried samples according to the Instructions for Use (IFU) supplied with the sample shipment. Use a freshly thawed/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 used
- For each independent test, prepare a series of dilutions from each coded sample, using the sample matrix specific to their 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.
- Use the Excel reporting sheet to record for each dilution the raw assay readout (e.g. absorbance O.D./RLU/plaques/GFP%, etc.). Provide the result (endpoint titre/IC50 etc.) as per analysis in your laboratory. Our statistician will use the raw data readouts to perform statistical analysis.
- Include the cut-off value indicating sero-reactivity for each assay and state whether each sample dilution tested is considered positive or negative according to their criteria (it is of interest for us to know whether the samples are considered 'positive' in each assay)
- 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.
- Record in the Excel reporting sheet any deviations from the assay protocol.

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.

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-LASV 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 NIBSC study organiser.

NIBSC, 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, NIBSC 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 12 weeks from receipt of study materials. If it is not practical to return results within 12 weeks, please inform Giada Mattiuzzo.

All completed results spreadsheets should be returned electronically to:

Dr Giada Mattiuzzo
Senior Scientist
Emerging Viruses Group
Division of Virology
National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Hertfordshire
EN6 3QG
UK

Tel. +44(0)1707 641283

Giada.Mattiuzzo@nibsc.org

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.

Appendix 5. Instruction for use for First WHO International Standard for anti-LASV immunoglobulin G (human)

WHO International Standard
First WHO International Standard for anti-LASV immunoglobulin G

NIBSC code: 20/202
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

The First WHO International standard for anti-Lassa fever virus (LASV) immunoglobulin G (IgG) is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from seven individual recovered from Lassa fever. 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-LASV neutralising antibodies and anti-glycoprotein (GP) and nucleoprotein (NP) binding IgG. The preparation does not contain a detectable level of anti-LASV IgM. The preparation has been solvent detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with 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 anti-LASV immunoglobulin G is 25 IU/ampoule for neutralising antibody activity; 250 IU/ampoule for anti-GP and anti-NP binding IgG. After reconstitution of the lyophilised cake in 0.25 mL of distilled water or other matrix, the final concentration will be 100 IU/mL for neutralising activity, 1000 IU/mL for anti-GP binding IgG and 1000 IU/mL for anti-NP binding IgG.

4. CONTENTS

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

5. STORAGE

The International Standard 20/202 should be stored at -20°C or below upon receipt.

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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufacturer's instructions provided with the ampoule breaker.

7. USE OF MATERIAL

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

This material should be reconstituted in 0.25 mL sterile distilled water. Following addition of water, the ampoule may be left at ambient

temperature for approximately 30 minutes until dissolved and then mixed thoroughly, avoiding the generation of excessive foam.

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.

9. REFERENCES

- 1) Mattiuzzo et al. Establishment of the first WHO International Standard and Reference Panel for anti-Lassa Fever virus antibody. 2021, WHO Expert Committee on Biological Standardization. WHO/BS/2021.2406
- 2) Dichtelmüller et al. Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion. 2009;49:1931-43

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 Ephraim Ogbaini-Emovon (Institute for Lassa Fever Research and Control, Imua Specialist Teaching Hospital, Edo State, Nigeria), Donald S Grant (Kenema Government Hospital, Kenema, Sierra Leone), Christian T. Happi (Redeemer's University, Ede, Nigeria), the Viral Hemorrhagic Fever Consortium and the African Center of Excellence for Genomics of Infectious Disease for the collection and testing of the serum samples. We gratefully acknowledge the important contributions of the collaborative study participants. We would also like to thank NIBSC Standards Production and Development staff for the formulation and distribution of materials.

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

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/jo/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



Medicines & Healthcare products
Regulatory Agency



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

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

15. LIABILITY AND LOSS

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

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom
* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight:
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

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_biologstandardsrev2004.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 6. Instruction for use for the WHO International Reference Panel for anti-LASV immunoglobulins (human)

WHO Reference Panel WHO International Reference Panel for anti-LASV immunoglobulins (human) Instructions for use (Version 1, Dated 06/07/2021)

1. INTENDED USE

The WHO International Reference Panel of anti-Lassa fever virus (LASV) immunoglobulin consist of the equivalent of 0.25 mL plasma samples obtained from individuals recovered from Lassa fever from Nigeria (N) or Sierra Leone (SL). The panel was evaluated in a WHO International collaborative study (1). For performance in difference assays of each panel member refer to the report. Individual panel members are NIBSC code 20/228 (high, N), 20/244 (high, SL), 20/204 (high binding, mid neut, N), 20/226 (mid, N), 20/246 (mid/low, SL), 20/222 (low, N) and 20/248 (very low, SL). It is intended that the panel is used in the development and assessment of assays used in the detection and quantification of anti-LASV antibody. The preparation has been solvent-detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

This preparation is not for administration to humans.

The preparation contains material of human origin, and has been tested and found negative for HCV RNA. Sample 20/246 tested positive for anti-HIV antibodies, and samples 20/244, 20/246, 20/248 also positive for HBsAg. As the preparation has been solvent-detergent treated is still deemed not infectious for shipping. As with 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

No unitage has been assigned to the panel members. Representative anti-LASV antibody titres have been provided in section 7 calculated from the consensus titre from the collaborative study; this is for guidance only, and different assay will produce different results.

4. CONTENTS

Country of origin of biological material: Nigeria and Sierra Leone.

Each ampoule contains the freeze-dried equivalent of 0.25 mL of human plasmat

5. STORAGE

The WHO International reference panel should be stored at -20C or below upon receipt.t

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open

the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker

7. USE OF MATERIAL

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

This material should be reconstituted in 0.25 mL distilled water. Following addition of water, the ampoules should be left at ambient temperature for approximately 30 minutes until dissolved and then mixed thoroughly, avoiding the generation of excessive foam.

Anti-LASV antibody titres were calculated as geometric mean of the potencies obtained from the collaborative study participants calibrated against the WHO International Standard for anti-LASV immunoglobulin G (NIBSC code 20/202) and expressed in International Unit (IU)/mL.

	20/228	20/244	20/204	20/226	20/246	20/222	20/248	
neutralising Ab	109		112	37	56	19	17	16
anti-GP IgG	970	1290		1580	960	320	340	180
anti-NP IgG	1090	1490		1170	1450	400	310	230

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.

Please complete with stability information for this product (CE Marked Materials only)

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

9. REFERENCES

- 1) Mattiuzzo et al. Establishment of the first WHO International Standard and Reference Panel for anti-Lassa Fever virus antibody . 2021 WHO Expert Committee on Biological Standardization .WHO/BS/2021.2406
- 2) Dichtelmüller et al. Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion. 2009;49:1931–43

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<http://www.who.int/biologicals/en/>

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Derivation of International Units:

http://www.nibsc.org/standardisation/international_standards.aspx

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<http://www.nibsc.org/products/ordering.aspx>

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http://www.nibsc.org/terms_and_conditions.aspx

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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

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):	Material of human origin
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin

Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

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

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16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*:

United Kingdom

* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.

Net weight: 0.25 g

Toxicity Statement: Non-toxic

Veterinary certificate or other statement if applicable.

Attached: No