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EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 11 to 14 March 2024

Requests to initiate new WHO reference material projects for biologicals

NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **15 February 2024** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technical Standards and Specifications (TSS). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Proposed new projects

1. WHO 5th proposed International Standard for Diphtheria Toxoid Adsorbed
2. WHO 2nd proposed International Standard for Vi Polysaccharide of S. Typhi
3. WHO 1st proposed International Standard for Group A Streptococcus Serum (human)
4. WHO 3rd proposed International Standard for Interleukin 2 (IL-2) Human rDNA derived
5. WHO 4th proposed International Standard for Endotoxin
6. WHO 1st proposed International Standard for Carcinoembryonic antigen
7. WHO proposed Reference Reagent for ADAMTS13 autoantibodies, plasma
8. WHO 1st proposed International Standard for anti-Junin virus antibodies, human
9. WHO 1st proposed International Standard for anti-Vaccinia virus antibodies, human

Running Title: **Diphtheria Toxoid Adsorbed (5th IS and BRP batch 5).**

Proposal (title)	5th WHO International Standard and Ph. Eur. Biological Reference Preparation batch 5 for Diphtheria Toxoid Adsorbed		
Proposer (name of Institution)	NIBSC	**Principal contact**	Laura Hassall
Rationale	Batches of the current World Health Organization International Standard (4th WHO IS) and European Pharmacopoeia (Ph. Eur.) Biological Reference Preparation (BRP batch 4) for Diphtheria Toxoid Adsorbed were established in 2009. The 4th WHO IS (with an assigned value of 213 IU/ml) and BRP batch 4 (with an assigned value of 97 IU/ampoule) have been used extensively for potency testing of diphtheria vaccines over the last 14 years and stocks of both materials are running low. There is therefore a requirement to replace these standards before stocks are depleted.		
Anticipated uses and users	Approximately 375 ampoules of the WHO IS (07/216) are sold each year. The intended users of these materials are vaccine manufacturers and control laboratories for the standardisation of *in vivo* potency assays for diphtheria vaccines.		
Source/type of materials	Two manufacturers have been approached to provide a sufficient quantity of purified diphtheria toxoid adsorbed with an aluminium adjuvant to allow us to prepare 2 candidate materials.		
Outline of proposed collaborative study	The collaborative study will be used to calibrate the proposed standard in IU units. Design of the study will be based on that used to calibrate the 4th IS and BRP batch 4 for Diphtheria Toxoid Adsorbed. Participants from laboratories representing multiple countries and regions will be included.		
Issues raised by the proposal	None		
Action required	ECBS to endorse proposal		
Proposer's project reference	SLP0023	**Date proposed:**	July 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			

Approval status of medicine or in vitro diagnostic method	Monovalent and combination vaccines containing diphtheria toxoid are licensed globally.
Number of products or methods	There are multiple manufacturers of diphtheria containing vaccines and more than 30 different products on the market.
Public health importance	Good childhood vaccination coverage and appropriate booster immunization of adults is essential to maintain protection against diphtheria in the population, and the supply of effective vaccines is dependent on confirmation of vaccine potency.
Global importance	Diphtheria vaccines are among the most widely used and successful human vaccines. They form an essential component of the primary immunization schedule of children, as well as being used for the reinforcement of immunity in adults and adolescents.
Global need from regulatory & scientific considerations	The supply of effective vaccines is dependent on confirmation of vaccine potency. The WHO and Ph. Eur. impose recommendations to confirm efficacy of every new batch of diphtheria vaccine manufactured. The specifications for these vaccines are therefore dependent on the use of the IS or material calibrated against it, with vaccine potency expressed in IU.
ECBS outcome	[BLANK]

Vi polysaccharide of S Typhi (2nd IS)

Proposal (title)	2nd International Standard for Vi Polysaccharide of S Typhi		
Proposer (name of Institution)	NIBSC	**Principal contact**	Sharon Tierney
Rationale	The 1st International Standard (IS) for Vi Polysaccharide (PS) of S Typhi (code 16/126) is a freeze-dried standard that contains 2.03±0.10 mg Vi PS as determined by quantitative nuclear magnetic resonance spectrometry (qNMR). The stock of the 1st IS is running low. There are currently approximately 280 ampoules of 16/126 left in stock and the rate of use is estimated to be 70 ampoules per year. The Working Standard for S Typhi Vi PS, which has previously been filled under the code 17/260, has been identified as a suitable replacement standard.		
Anticipated uses and users	The standard is primarily used by control laboratories to quantify the amount of Vi PS in final vaccine or bulk vaccine components. The most commonly used methods are: the Hestrin method, high-performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD) and rocket immuno-electrophoresis. Other methods include qNMR, the acridine orange dye binding method, rate nephelometry and ELISA.		
Source/type of materials	The Working Standard for Vi PS of S Typhi (NIBSC 17/260) has been identified as a replacement standard. If suitable, the ampoules will require relabelling as it has already been filled. There are ~850 of 17/260 which based on the current rate of use should last >10 years.		
Outline of proposed collaborative study	The collaborative study will be similar to that done for the 1st IS (Gao *et al.*, 2017, WHO/BS/2017.2310). Polysaccharide content will be assigned using qNMR and other methods will be included to assess suitability of use.		
Issues raised by the proposal	None.		
Action required	ECBS to endorse proposal.		
Proposer's project reference	SLP0031	**Date proposed:**	29th November 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			

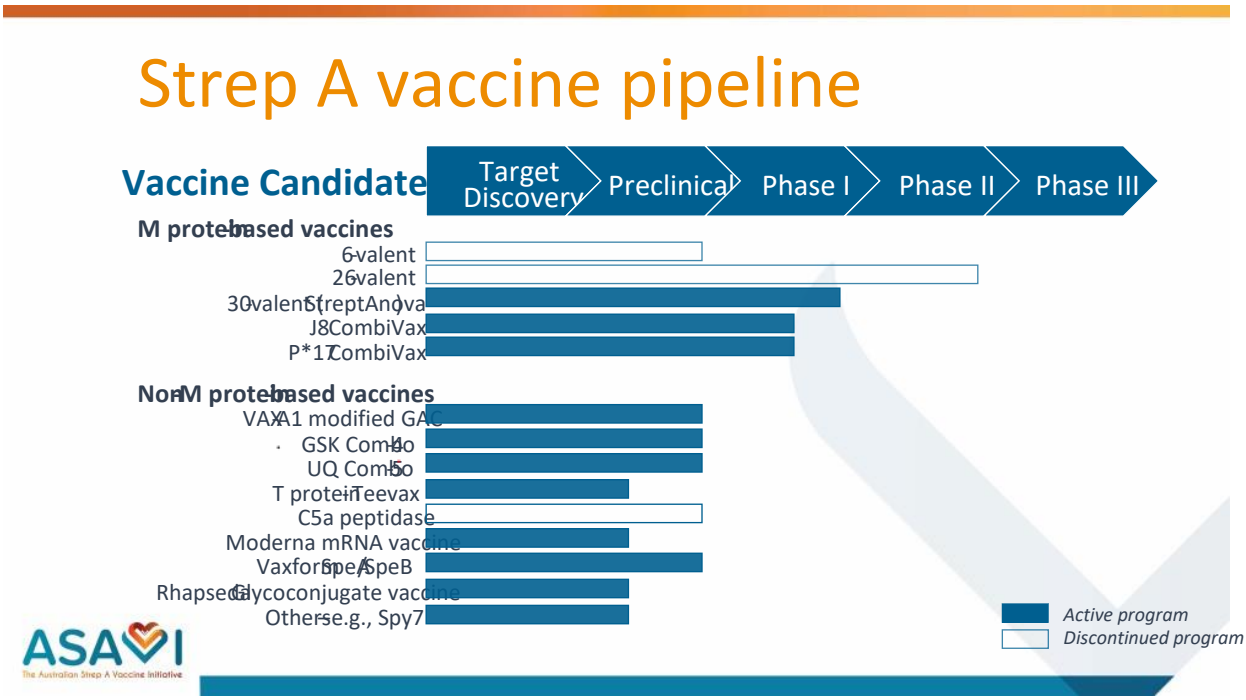
Approval status of medicine or in vitro diagnostic method	There are many licensed plain Vi PS vaccines which have been around since the 1990s. More recently, conjugate Vi PS vaccines have been developed as plain Vi PS vaccines are unable to provide immuno-protection for young children or infants. Two Vi PS - Tetanus Toxoid conjugate vaccines are licensed in India, and some Vi PS conjugate vaccines using a variety of carrier proteins are under development and some have entered clinical trials (Gao *et al.*, 2019).
Number of products or methods	Vaccine development in this area is active.
Public health importance	Vaccination is the most cost-effective preventative strategy to control typhoid, especially in areas where anti-microbial resistant S Typhi strains are endemic. Vaccination is recommended when travelling in areas where sanitation and food hygiene is poor.
Global importance	Typhoid fever is found throughout the world. High-risk areas include: The Indian subcontinent, Africa, south and southeast Asia, South America.
Global need from regulatory & scientific considerations	Vi PS-based typhoid vaccines rely on physicochemical and serological methods to provide an estimate of the polysaccharide content as a measure of vaccine potency and the IS for Vi PS is essential for manufacturers and NCLs to standardise Vi PS quantification.
ECBS outcome	[BLANK]

Proposal (title)	1st WHO International Standard for Group A Streptococcus Human Reference Serum		
Proposer (name of Institution)	MHRA / ASAVI	**Principal contact**	Dr. Fatme Mawas Dr Allie Shaw
Rationale	Group A Streptococcus (GAS) is a major global pathogen which causes a range of infections from mild pharyngitis to severe necrotising fasciitis, as well as autoimmune conditions such as rheumatic heart disease (RHD). Annually there are over 600 million cases of pharyngitis and approximately 33 million cases of RHD, the latter leading to a conservative estimate of 300,000 deaths globally. This places GAS among the top 10 causes of infectious disease mortality. Due to this high burden of disease, there is now a concerted global effort to develop the first licenced vaccines against GAS. Among them, the Australian Strep A Vaccine Initiative (ASAVI) is leading the effort to accelerate Strep A vaccine development. Several candidate vaccines are in pre-clinical or clinical trials (see Appendix 1) and contain a number of GAS antigens that have defined roles as determinants of pathogenesis. Detecting antibodies against these antigens is essential for vaccine development to demonstrate the magnitude and breadth of the antibody response and may also help to determine corelates of protection. NIBSC will produce a WHO International Standard human reference serum composed of pooled human serum containing antibodies to the different GAS antigens. The candidate standard will be assessed, and antibody titres determined, in an international collaborative study.		
Anticipated uses and users	This reference serum will be used to assess GAS vaccine immunogenicity in clinical trials and antibody levels in sero-epidemiological studies, and potentially will have a further use in diagnostic assays. It is anticipated that vaccine manufacturers will be a major user of this standard, along with centres for epidemiological surveillance and, potentially, diagnostic laboratories. A 10-plex immunoassay to measure antibodies against Strep A virulence and immune evasion factors (key vaccine targets), has been qualified and is being further validated by ASAVI and MSD, and will be used in this study to enable rapid screening of the candidate materials and to assess the stability of the candidate IS throughout the IS preparation process		
Source/type of materials	Pooled vaccinee sera from different clinical trials could be donated by vaccine manufacturers when availabe. Alternatively, convalescent sera with		

	high antibody titre to GAS antigens could be sourced and pooled to prepare the IS There will be sufficient material available to produce up to possibly 5,000 ampoules of the final product.		
Outline of proposed collaborative study	A collaborative study will be used to calibrate antibody titres against the target GAS antigens. Participants from laboratories representing multiple countries, and who are supporting GAS vaccine development, will be included. Paticipants will be asked to use their own in-house assay.		
Issues raised by the proposal	The candidate material is of human origin and needs to be tested to ensure it is non-infectious.		
Action required	ECBS to endorse proposal		
Proposer's project reference	TBC	**Date proposed:**	Dec 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	There are currently no licenced GAS vaccines, and no official antibody reference standards available for determining immune responses to vaccination. Consequently, there is an urgent need for a standardised assay system that can assure vaccine quality and comparability		
Number of products or methods	There are multiple candidate vaccines in pre-clinical and entering into clinical trials globally (see Appendix A below)		
Public health importance	The reference serum will be a critical standard for determining and comparing the antibody response to candidate GAS vaccines in clinical trials, to harmonise assays and for global epidemiological surveillance studies to determine the level of diseases and level of natural immunity to support implementation of vaccines with the long-term aim of helping to reduce the incidence of GAS infections globally. Currently correlate of protection are unknown and standards will be required for this analysis, as well as iterative vaccine design		
Global importance	Establishment and utilization of this standard will help to progress more rapidly the development of globally licenced GAS vaccines		
Global need from regulatory & scientific considerations	Standardisation of the measurement of antibody responses to GAS vaccines is essential to ensure the quality and efficacy of vaccine candidates, to help further progress the establishment of the first globally licenced products		
ECBS outcome			

Running Title: GAS Human Reference Serum (1st WHO IS)

Appendix A



Proposal (title)	Third WHO International Standard for Interleukin-2 (IL-2)		
Proposer (name of Institution)	NIBSC/MHRA	**Principal contact**	Kata Dix
Rationale	The 1st WHO International Standard (IS) for Interleukin-2 (IL-2) has been in use since 1986 (86/504) and was replaced by the 2nd IS (86/500) in 2013. These standards have been essential in the potency labelling of an IL-2-based clinical product (aldesleukin), used for the treatment of metastatic renal cell carcinoma and melanoma, and on its own or as part of a combination therapy in ongoing clinical studies in cancer immunotherapy, autoimmune disease therapy and as a potential therapeutic for amyotrophic lateral sclerosis. Over time, the usage of the IL-2 ISs have increased. While the 1st IS, filled at 4000 ampoules, depleted by 2013 (average sales around 130 ampoules/year), the 2nd IS is being dispatched at a rate of approximately 300 units per year, demonstrating the demand for this important reference material.		
Anticipated uses and users	The standard is used by manufacturers and developers of therapeutic products, reagents, and assays.		
Source/type of materials	While the 1st IS is a natural form of IL-2 derived from activated Jurkat-cells, the current (2nd IS) is full human sequence cytokine expressed in *E. coli*. We plan to use complete human sequence recombinant IL-2 in this replacement project.		
Outline of proposed collaborative study	The proposed collaborative study will be a multi-centre international study, aiming for global coverage and focusing on using bioassays for potency determination. Immunoassays will also be included. At least one candidate preparation and the current IS will be included in the study.		
Issues raised by the proposal	With less, than 700 ampoules remaining, sales of the current (2nd) IS for IL-2 will have to be restricted to ensure continued access until the replacement is available.		
Action required	ECBS to endorse proposal		
Proposer's project reference	SLP0028	**Date proposed:**	March 2024
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			

Approval status of medicine or in vitro diagnostic method	There is a licenced IL-2-based product (aldesleukin).
Number of products or methods	One product is licenced in the USA, Europe and Canada, further products are marketed elsewhere.
Public health importance	High – therapeutic product is available.
Global importance	Interleukin-2-based therapeutics are being developed worldwide.
Global need from regulatory & scientific considerations	There is an increasing need due to changing regulatory requirements in emerging markets.
ECBS outcome	

Proposal (title)	The 4th WHO International Standard for Endotoxin		
Proposer (name of Institution)	MHRA	**Principal contact**	Trusha Desai
Rationale	The 3rd WHO IS for Endotoxin, 10/178 was established in 2012 with a potency of 10,000 IU per vial, relative to the 2nd IS (94/580), based on the results of an international collaborative study. The collaborative study included 2 other candidates, derived from the same single bulk solution of endotoxin. To ensure a harmonised approach to endotoxin standardisation a portion of 10/178 was established as an EDQM BRP and the other candidates were established as USP reference standards. Stocks of the 3rd IS are nearing depletion and a replacement is required. This coincides with the depletion of USP and EDQM standards and a harmonised approach has once again been agreed. Using the same source material for successive WHO IS, and various national and pharmacopoeial standards, has ensured consistency and continuity of the IU, however as a result stocks of the source material are being depleted and every effort should be made to ensure the material is used efficiently. We therefore propose to decrease the potency of the 4th IS to approximately 5000 IU/vial. This will require less endotoxin bulk material, but should have no impact on the performance of the standard in assays. The decrease in potency will also ensure that sufficient source material is left for the next IS replacement exercise and to fulfil any additional requirements that may arise to maintain harmonisation, or in case the global usage of the material changes in the future.		
Anticipated uses and users	Pharmacopoeias, regulatory agency laboratories, pharmaceutical and pyrogen testing reagent manufacturers worldwide		
Source/type of materials	Lyophilised bulk endotoxin material derived from *Escherichia coli* (Braude strain) group O113:H10:K negative. The material was donated to MHRA/NIBSC by the US-FDA, and the same material has been the source of the 1st and 2nd WHO IS, previous lots of official USP Reference Standards (F and G Series), the FDA (Lots EC 1-6) as well as current lots available through EDQM, as referenced in the European Pharmacopoeia (EP), and in the International Pharmacopeia (Int.Ph.).		
Outline of proposed collaborative study	Potency estimates for a candidate lyophilised endotoxin preparation will be obtained in an international multi-centre collaborative study. Participants will include pharmacopoeia laboratories, regulatory agency laboratories,		

	pharmaceutical companies and manufacturers of reagents for pyrogen testing (various forms of Bacterial Endotoxin Tests and Monocyte Activation Test).		
Issues raised by the proposal	A wider consultation around the decrease in potency will take place after the publication of the proposal.		
Action required	ECBS to endorse proposal		
Proposer's project reference	TBC	**Date proposed:**	Dec 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	The proposed material is a replacement for a standard used in long-standing, as well as novel compendial assay methods for pyrogen detection.		
Number of products or methods	The proposed replacement IS will be used for the calibration of reference materials that are essential in three groups of compendial methods for pyrogen detection: the Rabbit Pyrogen Test (RPT), Bacterial Endotoxins Test (BET) and the Monocyte Activation Test (MAT). In addition, alternative methods are under intense development, in an effort to validate the replacement of reagents of animal origin in the BET, for which the continued availability of the Endotoxin IS is necessary.		
Public health importance	Pyrogen testing is essential to protect the public from potentially serious adverse events caused by contamination or inconsistent production, resulting in high pyrogen content, in parenteral medicines. Endotoxin is the most frequently occurring pyrogen contaminant in biologicals.		
Global importance	The WHO IS for Endotoxin is of global importance and is used to calibrate reference reagents for endotoxin testing worldwide.		
Global need from regulatory & scientific considerations	The WHO IS for endotoxin is required to ensure the endotoxin measurement in vaccine and therapeutic products is globally harmonised		
ECBS outcome			

Proposal (title)	The proposed 1st International Standard for Carcinoembryonic Antigen (CEA)		
Proposer (name of Institution)	NIBSC	**Principal contact**	Katherine Partridge
Rationale	CEA is a tumour associated antigen. Measurements of human CEA are an important component in the diagnosis of a number of cancers, including bowel, lung, breast and pancreatic cancer, and may also be be indicative of liver disease and some inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. In particular, CEA measurements can be used to monitor a patient's response to curative/palliative cancer treatment. Levels of human CEA are measured by in vitro immunoassay. A WHO International Reference Preparation, coded 73/601 was produced in the mid-1970s for the calibration of these immunoassays. Stocks of the 1st International Reference Preparation (IRP) for Carcinoembryonic Antigen (CEA) (73/601) are now running low and, at the current rate of dispatch (~150/year), will be exhausted by 2025. It is therefore important to begin the process of preparing a replacement.		
Anticipated uses and users	Users include manufacturers of CEA diagnostic tests and clinical, regulatory and quality control laboratories. The material is predominantly used to calibrate test kits measuring CEA in patient samples (mainly serum or plasma). Although the 1st IRP, 73/601, is calibrated in IU, the majority of diagnostic kits report in SI units of ng/ml.		
Source/type of materials	The 1st IRP was prepared from an extract of native CEA, purified from liver cancer tissue of one patient biopsy, formulated in 0.5% lactose. It is the intention to replace the IRP with a similar native material, sourced from an external supplier.		
Outline of proposed collaborative study	Participants in the collaborative study will be manufacturers and clinicial laboratories who perform CEA assays (~10-12). The study will have the following aims: 1) to confirm reactivity of the candidate preparation, and assess the relationship of this activity to the IRP 73/601 and existing local standards 2) to calibrate the candidate preparatation in terms of the 1st IRP for CEA, 73/601 3) to assess the stability of acceleratd thermal degradation (ATD) samples of the candidate preparation by immunoassay		

	4) to assess the commutability of the candidate preparation with the inclusion of serum and/or plasma samples Participants will be asked to report data to NIBSC for statistical analysis and a consensus will be reached on unitage.		
Issues raised by the proposal	None		
Action required	ECBS to endorse proposal		
Proposer's project reference	TBC	**Date proposed:**	December 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	Human CEA is measured using in vitro diagnostic tests, to indicate/assist in diagnosis of some cancers, such as bowel, lung, breast and pancreatic cancer. The measurements are also useful in the monitoring of a patient's response to curative/palliative cancer treatment. It can also be indicative of liver disease and some inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.		
Number of products or methods	>10 manufacturers of immunoassays for CEA		
Public health importance	CEA diagnostic kits are widely used to support the diagnoses of many cancers and other medical conditions. Inaccurate or unstandardised kits may results in incorrect diagnosis of these life-threatening conditions.		
Global importance	CEA products and kits are manufacturered and marketed worldwide. In order to maintain quality of care and availability of these diagnostic tests, there is a continued need for effective standardisation.		
Global need from regulatory & scientific considerations	There is a requirement to provide continuity with the provision of an International standard for CEA, to aid in standardisation and harmonisation of CEA immunoassays.		
ECBS outcome	[BLANK]		

Proposal (title)	Reference Reagent for ADAMTS13 autoantibodies, plasma		
Proposer (name of Institution)	MHRA	**Principal contact**	Helen Wilmot
Rationale	Thrombotic thrombocytopenic purpura (TTP) is a serious disease caused by a reduction in ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity. It can be either immune-mediated (iTTP), or congenital (cTTP). iTTP is more common than cTTP but is a rare disease (6 cases per million people). However, it has a fatality rate of 90% if left untreated. Death can occur within 24 hours after symptom onset, so fast and effective diagnosis is essential. iTTP is caused by autoantibodies against ADAMTS13, an enzyme that cleaves large multimers of von Willebrand Factor (VWF). A reduction in ADAMTS13 activity results in large VWF multimers, which can cause platelet-rich microthrombi to accumulate, particularly in the brain and heart. iTTP patients are treated with twice daily plasma exchange, with or without steroids and/or monoclonal antibodies. Once recovered, patients must be monitored throughout their life every 6 or 12 months in order to detect any onset of recurrence and therefore be administered pre-emptive treatment. An ADAMTS13 autoantibody standard would allow patient diagnosis monitoring to be standardised, allowing harmonisation between laboratories.		
Anticipated uses and users	An ADAMTS13 autoantibody standard would be used in clinical laboratories to aid diagnosis and monitoring of patients suffering from iTTP and for standardisation of diagnostic kits and assays for ADAMTS13 inhibitors.		
Source/type of materials	Pooled plasma from patients undergoing plasma exchange for iTTP		
Outline of proposed collaborative study	One or two candidate materials will be prepared from a pool of plasma containing ADAMTS13 autoantibodies. These will be distributed, alongside commutability samples, to 10-20 labs who carry out assays for iTTP diagnosis. Labs will either carry out functional assays for ADAMTS13 to measure a reduction in activity by the Bethesda method or use ELISA-based assays to detect anti-ADAMTS13 antibodies.		
Issues raised by the proposal	The high cost of the assays may limit laboratories' ability to take part and may limit the geographical spread of participants. The standard will either have an inhibitory value assigned to it, or work as a system suitability standard to help laboratories understand the sensitivities of		

	the assays. A feasibility study / collaborative study will help to determine which route is the most appropriate for the community.		
Action required	ECBS to endorse proposal		
Proposer's project reference	SLP00032	**Date proposed:**	March 2024
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	iTTP can be treated using plasma exchange, steroids, rituximab or caplacizumab (both are licensed). Patient monitoring utilises existing ADAMTS13 activity assays in a Bethesda method, or ADAMTS13 inhibitor assays (ELISA-based).		
Number of products or methods	There are several different functional assays for ADAMTS13 on the market, used in the Bethesda assay, and one ELISA-based assay.		
Public health importance	There have been observed discrepancies between methods used to diagnose iTTP and the Bethesda-type assay is inherently variable due to the different plasma pools used between laboratories. Standardisation of the assay and quantification of inhibitory activity is important for harmonisation between laboratories and to ensure correct treatment is given when required to avoid relapse.		
Global importance	Due to the fast progression from asymptomatic disease to death, fast and reliable diagnosis is required. Patients are monitored so that pre-emptive treatment can be administered, and a standard would allow the patient's inhibitory antibody levels to be quantified and tracked over time.		
Global need from regulatory & scientific considerations	The current Bethesda-type assay is very variable and is critical for diagnosis of iTTP rather than cTTP. This is an important distinction as treatment regimen differs according to diagnosis.		
ECBS outcome	No Comments		

Proposal (title)	First WHO International Standard for anti-Junín virus antibodies		
Proposer (name of Institution)	MHRA	**Principal contact**	Emma Bentley
Rationale	Junín virus is the etiologic agent of Argentine haemorrhagic fever, which has a 15-30% case fatality rate in untreated individuals. The virus is zoonotic, with a rodent reservoir, and circulation is so far restricted to Argentina. It falls within the *Arenaviridae* family, *Mammarenavirus* genus, and has been suggested as a prototype pathogen for the New World sub-division of the *Arenaviridae* family. The WHO R&D Blueprint is currently under review (expected to be published for public consultation early 2024) and it is expected that the pathogen prioritisation methodology will employ a viral family-centric approach, pinpointing representative viruses within a family as pathfinder viruses. This will likely focus efforts on Junín virus research and the development of vaccines, therapeutics and diagnostics. Currently, there is a live-attenuated vaccine licenced for limited use in Argentina, but this has not been approved in other regions. Several alternative vaccine platforms are in pre-clinical development and this includes a program funded by the Coalition for Epidemic Preparedness Innovations (CEPI). The availability of a WHO International Standard for Junín virus will support these efforts and compliment the prototype pathogen approach to pandemic preparedness.		
Anticipated uses and users	Standardisation of serological assays detecting anti-Junín virus antibodies (e.g. ELISA, neutralisation assays) used by: - Vaccine manufactures - National control / public health laboratories - Therapeutic antibody producers (e.g. mAb therapies) - Assay kit manufacturers - Research laboratories		
Source/type of materials	Donations of human serum/plasma will be sought, in partnership with CEPI, from convalescent individuals and vaccine recipients. Samples will be screened for Junín and other blood-borne viruses and in all cases undergo precautionary treatment steps for virus inactivation. This follows a safety pipeline developed for serological samples collected following other ACDP (Advisory Committee on Dangerous Pathogens) hazard group 4 virus exposures, to ensure the safety of materials prior to their onward distribution to laboratories as part of the WHO collaborative study.		

Outline of proposed collaborative study	The collaborative study will aim to involve 15-20 laboratories worldwide, performing a range of serological assays for the detection of anti-Junín virus antibodies. The range of laboratories will include representatives from national control laboratories, vaccine manufacturers, clinical and academic laboratories. To evaluate performance of the candidate standard and provide an assessment of commutability, the study panel will include convalescent and vaccinee serum/plasma samples of differing potencies. The aim will be to assess the suitability of the candidate preparation to serve as an International Standard, with an assigned unitage, for use in the harmonisation of Junín virus serological assays by: - Characterising the reactivity/specificity in different assay systems - Evaluating the potency/readout in a range of typical assays performed in different laboratories - Assess the extent to which the candidate is suitable to serve as a standard for a variety of different samples (i.e. commutability)		
Issues raised by the proposal	The identification and involvement of laboratories performing serological assays for Junín virus across all WHO regions may be challenging due to its circulation being restricted to Argentina.		
Action required	ECBS to endorse proposal		
Proposer's project reference		**Date proposed:**	March 2024
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	One vaccine, Candid#1, based on live-attenuated Junín virus has been licensed for limited use in at risk adult populations in Argentina since 1992. Wider adoption of the vaccine is unlikely due to concerns regarding the stability of the attenuation phenotype. As such, several other platforms are in the pre-clinical stages of investigation. Post-exposure therapeutic treatment options are limited to the transfusion of immune plasma and the off-label use of ribavirin and favipiravir, with studies on-going into the development of monoclonal antibodies for potential clinical use.		
Number of products or methods	In-house assays are available and have been published, these include ELISA, IFA and neutralisation assays.		
Public health importance	It is estimated that 5 million people are at risk of Argentine haemorrhagic fever, based on the endemic area covering 150,000 km^2. Although reported		

	cases have dropped in recent years to between 10-15 cases, the incidence of exposure remains unknown due to only severe cases being reported. There is also the risk of expansion of the geographic range. Further, diagnosis is complicated by a non-specific early clinical manifestation which may delay treatment prior to progression to the haemorrhagic phase.
Global importance	In recent years pandemic preparedness has focused on priority pathogens, identified through the WHO R&D Blueprint (updated in 2018, and currently under revision), which has been widely adopted or aligned to by other organisations such as CEPI. Recognising the uncertainty that these pathogens will be the causative agent of future outbreaks; the approach is shifting towards identifying prototype pathogens which can represent multiple viruses within a taxonomic group. For the *Arenaviridae* family, it is proposed to have a prototype representing the Old World and New World sub-division. Lassa virus has been identified to represent the Old World, for which a WHO IS for antibody was established in 2021 (WHO/BS/2021.2406). The availability of a WHO IS for anti-Junín virus antibodies, as the New World prototype, will compliment this.
Global need from regulatory & scientific considerations	Standardised and calibrated assays will be vital for the accurate evaluation and development towards regulatory approval of treatments, including vaccines and therapies, and for the development of licensed serological diagnostics. This includes efforts towards defining correlates of protection.
ECBS outcome	[BLANK]

Proposal (title)	First WHO International Standard for anti-Vaccinia virus antibodies		
Proposer (name of Institution)	MHRA	**Principal contact**	Giada Mattiuzzo
Rationale	Vaccinia virus (VACV)-based vaccines have been used since the late 18th century, to control and eventually eradicate smallpox, a highly contagious and often deadly disease caused by variola virus. VACV is a member of the *Poxviridae* family, *Orthopoxvirus* genus which encompasses 12 species, including variola, cowpox and monkeypox (Mpox) virus. These viruses have a linear DNA genome of around 170-250 Kb and due to their antigenic similarity may confer cross-reactive immunity. Indeed, VACV vaccination confer cross protection not only for smallpox but has been employed to control the Mpox outbreak in 2022, as preclinical studies have shown that VACV vaccines provide a level of immunity and are efficacious in reducing severity of illness. There are three licensed vaccines which are based on VACV. ACAM2000 vaccine is approved in the USA and is generated from the New York City Board of Health (NYCBH) strain by propagation on calf skin, single clone selection and growth in Vero cells; IMVANEX (also licensed as JYNNEOS and IMVAMUNE) vaccine, is a highly modified Vaccinia Ankara strain produced by Bavarian Nordic which has been extensively passages in chicken embryo fibroblast cells; in Japan, LC16 KMB vaccine is derived from the Lister strain and passage in rabbit kidney cells. Availability of serological assays to quantify and compare the immune responses elicit by VACV vaccines will help evaluate current and new vaccines, support sero-epidemiology studies and understand the antibodies cross-reactivity between Orthopoxviruses. The availability of an International Standard to VACV antibodies will help support the development and harmonization of these assays and will complement the planned IS for Mpox antibodies for the detection of antibodies against specific Orthopoxviruses species.		
Anticipated uses and users	National reference/control laboratories, vaccines and kit manufacturers, research groups. The standard will be used for the development and calibration of assays detecting anti-VACV antibodies to investigate immune responses following vaccination and in sero-epidemiological studies.		
Source/type of materials	A candidate for the 1st International Standards (IS) for anti-Vaccina virus was produced from a pool of defibrinated human plasma in 2005. The preparation was freeze-dried at the South Mimms Laboratories of the MHRA using a well-established procedure (NIBSC code 05/124). Approximately 2600 ampoules are available for distribution. The candidate material was evaluated in a collaborative study in 2010 (WHO/BS/10.2134) but the limited number of assays used in the study (6 PRNT and 3 ELISA) and different performance of the material in those assays when compared with an anti-		

	smallpox serum preparation did not support the establishment as an International Standard and the ECBS required a further analysis of the data.		
Outline of proposed collaborative study	We will add the candidate material 05/124, to the organized Collaborative study for the establishment of the First WHO IS for Mpox antibodies 10-15 laboratories worldwide will analyse the neutralising activity of the preparations against VACV or Mpox. Some of the binding antibody assays are expected to be able to discriminate between anti-VACV and anti-Mpox responses.		
Issues raised by the proposal	Cross-reactivity between other Orthopoxviruses complicates the interpretation of the results. Limited number of “clinical samples” to evaluate the commutability of the candidate material		
Action required	ECBS to endorse proposal		
Proposer's project reference		**Date proposed:**	March 2024
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	Three vaccines approved in different regions. New Orthopoxvirus vaccines are under development (e.g. mRNA based) One antiviral medication approved in several countries: tecovirimat (TPOXX). Treatment for smallpox, cowpox and Mpox, although only safety clinical trials, no efficacy, have been conducted.		
Number of products or methods	No commercial assays are available for the detection of antibodies to VACV, but there are several methods published in the literature for binding and neutralising antibody detection.		
Public health importance	The use of the VACV vaccines has been influential in the management of the Mpox outbreak in 2022. Reference reagents are needed to support the development of assays to determine a previous vaccination from a Mpox infection and the evaluation of new vaccines.		
Global importance	VACV vaccines played an essential role in the successful eradication of smallpox, caused by the variola virus. Its cross-reactivity has enabled its application in controlling other Orthopoxvirus infections, like the PHEIC caused by Mpox virus in 2022. Albeit the Mpox PHEIC was declared over in November 2022, Orthopoxviruses infections represent still a significant burden to countries where these are endemic, such as African countries.		

Global need from regulatory & scientific considerations	During the WHO R&D consultation on Mpox held on 2nd-3rd June 2022 the following research priorities were identified: Characterization of immune responses to identify biomarkers that could predict protection – the availability of a WHO International Standard is critical for the comparison of vaccine and immunological studies to identify potential surrogate of protection. To achieve this, there is the need to have distinct standards for different Orthopoxvirus species.
ECBS outcome	[BLANK]

Running Title: **VACV antibody standard**