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EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 16 to 19 October 2023

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 **2 October 2023** 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 7th International Standard for FVIII and Von Willebrand factor

2. WHO 1st International Standard for Plasma Anti-D levels by Automated haemagglutination

3. WHO 3rd International Standard for Protein C, Plasma

4. WHO 1st International Standard for anti-Hepatitis E Serum human and reference panel

5. WHO International Standards for nOPV type 1,2,3

6. First WHO International Reference Panel for adventitious virus detection in biological products using high-throughput sequencing technologies

7. WHO 6th International Standard for Thromboplastin, Human, Recombinant, Pain

Proposal (title)	The 7th International Standard for Blood Coagulation Factor VIII and Von Willebrand Factor in Plasma		
Proposer (name of Institution)	MHRA -NIBSC	**Principal contact**	Helen Wilmot
Rationale	The FVIIII and VWF Plasma standard is required to harmonise laboratory testing and diagnosis of FVIII deficient and von Willebrand Disease patients, and for assignment of FVIII potency to Virus Inactivated Fresh Frozen Plasma (VIFFP) products. Stocks deplete quite quickly (up to 1000 ampoules per year) so the project for replacement needs to be initiated. Around 20,000 ampoules will be produced.		
Anticipated uses and users	Used for patient monitoring and diagnosis. Used by manufacturers of plasma calibrators, diagnostic and clinical laboratories, plasma purification sites and manufacturers of VIFFP		
Source/type of materials	Normal plasma from the transfusion service.		
Outline of proposed collaborative study	The final product will be value assigned relative to the 6th IS for FVIII/VWF Plasma by international collaborative study. Units will be assigned for FVIII activity and antigen, and for VWF (by VWF:RCo, VWF:GPIbR , VWF:GPIbM, VWF Collagen binding, VWF propeptide and antigen). 20-30 laboratories will be invited to take part for each analyte; with a smaller number for those tests which are less commonly performed and more difficult to recruit participants for (e.g. VWF propeptide) The current International Reference Reagent for GPIbM and GPIbR was established by adopting the previously assigned value for VWF:RCo. This was done to reduce assay discrepancy and a shift in unitage that would have occurred if the values had been established using the convention of plasma pools. The relationship between these 3 analytes will be explored in the collaborative study, with an overall aim of establishing IU for the GpIbM and GPIbR analytes.		
Issues raised by the proposal	None		
Action required	ECBS to endorse proposal		
Proposer's project reference	SLP0013	**Date proposed:**	October 2023

CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)	
Approval status of medicine or in vitro diagnostic method	There are at least 2 manufacturers of VIFFP and many licensed FVIII/VWF treatments are available
Number of products or methods	Two methods are used to measure FVIII activity – clotting and chromogenic. Within each method, there are several different reagents or kits. There are several different VWF analytes and each will be assigned a value on the IS.
Public health importance	This standard is used to monitor treatment of patients and to potency assign FVIII values to VIFFP. Lack of standardisation could lead to uncontrolled bleeding in patients.
Global importance	This standard is critical for the global harmonisation of FVIII and VWF measurement in FVIII deficient and von Willebrand Disease patients for effective diagnosis and treatment monitoring.
Global need from regulatory & scientific considerations	This standard will ensure the continuity of the IU for VIFFP manufacturers and clinical laboratories world-wide.
ECBS outcome	[BLANK]

Proposal (title)	1st WHO International Standard for quantification of plasma anti-D levels		
Proposer (name of Institution)	MHRA	**Principal contact**	Dina Vara
Rationale	Rho(D) immunoglobulin is used to prevent sensitisation in mothers who are RhD-negative, transfusion of RhD-positive blood to RhD-negative recipients, and treat idiopathic thrombocytopenic purpura (ITP) in people who are Rh-positive. A mother is at risk of RhD alloimmunisation when the maternal RhD-negative RBC is exposed to foetal RhD-positive resulting in Haemolytic Disease of the Foetus and Newborn (HDFN). The measurement of anti-D levels in plasma is carried out for determining and monitoring antibody levels during and after pregnancy; decisions for clinical treatment are made based on assay results, and hence, there is a requirement for accurate standardisation. Interlaboratory variability of up to ±100% of the mean was not uncommon when using non-standardised methods. The Directors of the UK National Transfusion Centres decided that a national working standard for anti-D; for use in automated assay techniques, was required for routine use in hospitals. A British Standard (73/517) was produced and CE marked in 2005 under the directive on in vitro diagnostic to comply with UK Guidelines for Blood Transfusion Services. Since the introduction of a standard and the use of standardised protocols, there has been a significant reduction in interlaboratory variability to around 20%. The requirement for UKCA marking has identified a need to revisit this standard; redefining an repurposing 73/517 as an International Standard would support testing of plasma anti-D and provide harmonisation across laboratories globally.		
Anticipated uses and users	Establishing 73/517 as the 1st International Standard would allow to standardise plasma anti-D levels by serology methods across globally in hospitals, transfusion laboratories, and in the NHS.		
Source/type of materials	The candidate standard was prepared from 23 L of pooled citrated plasma containing incomplete (IgG) anti- D from donors in early, mid, and late stages of immunisation. The material was distributed into glass ampoules and lyophilised and stored at −20°C. Analyses showed that each ampoule contained 0.58% residual moisture and 0.14% oxygen.		
Outline of proposed collaborative study	73/517 has been evaluated in a collaborative study assayed against the International Standard for Anti-D Incomplete Blood Typing Serum (64/19) on the AutoAnalyser by five UK clinical laboratories. The preparation was assigned a potency of 11.5 IU/ampoule for Anti-D (Rh0) antibodies. In 2019, three UK		

	transfusion centres assessed the stability of anti-D in lyophilised preparation and found no loss in potency and 73/517 would continue to be sufficiently stable for least another 10 years. The standard will be re-assessed in an international collaborative study in laboratories that determine anti-D levels in plasma to prevent sensitisation in RhD-negative individuals.		
Issues raised by the proposal	The standard may not be suitable for all methods other than automated haemagglutination. Nonetheless, a potency will be assigned either in IU or titre levels for plasma anti-D to allow global harmonisation. Producing a commutable standard may be challenging.		
Action required	ECBS to endorse proposal		
Proposer's project reference	TBC	**Date proposed:**	03/07/2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	Automated methods and manual methods are used globally		
Number of products or methods	Plasma anti-D levels are determined using automated haemagglutination and serology methods to detect titers of the anti-D antibodies most commonly using gel strips or test tubes.		
Public health importance	The CE marked British Standard 73/517 supports the measurement of anti-D in plasma using the autoanalyzer, primarily to the blood services (47%), NHS (29%), Government funded testing activities (2%), and the remaining 22% classified as commercial.		
Global importance	Before developing anti-D prophylaxis, HFDN was responsible for foetal loss in 1% of all pregnancies. The incidences decreased to 0.1% with the introduction of antepartum RhD immunoprophylaxis in 1970. Although there is sufficient RhD immunoprophylaxis, it is still approximated that between 1 to 3 of a thousand women who are Rh negative still develop alloimmunisation presently.		
Global need from regulatory & scientific considerations	An plasma anti-D IS would allow global harmonisation of unitage for anti-D IgG measured in the serum of RhD negative patients to prevent Rhesus alloimmunisation.		
ECBS outcome	[BLANK]		

Proposal (title)	The 3rd International Standard Protein C, plasma		
Proposer (name of Institution)	NIBSC/MHRA	**Principal contact**	John Hogwood
Rationale	The Protein C standard is required for the laboratory diagnostics of protein C in patients as part of a screen should they present with thrombophilia, and for the measurement of protein C in the product 'virus inactivated fresh frozen plasma'. Whilst there are sufficient stocks for 3 + number of years (based on current use), the current reference is over 20 years old and measurement/technology has advanced/changed in this period. Establishment of a new reference by a multicentre study is required.		
Anticipated uses and users	Expected use is about 300 ampoules per year, with main users being diagnostic manufacturers (to assign value to their calibrators) and occasional use by therapeutic manufacturers for protein C determination in their products		
Source/type of materials	Human plasma sourced from UK's national Blood and Transfusion Service.		
Outline of proposed collaborative study	A multi-centre study, with about 20+ laboratories, will be carried out to assign value to the two analytes associated with the material – functional and antigen.		
Issues raised by the proposal	None – replacement of existing reference material		
Action required	ECBS to endorse proposal		
Proposer's project reference		**Date proposed:**	16-Jun-23
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	This is a plasma standard which is primarily used by diagnostic manufacturers to check potency assignment of their commercial calibrators. The material is also used by some therapeutic manufacturers for measurement of protein C in their products.		
Number of products or methods	This standard will have two analytes – functional activity and antigen assigned to it. A fill of 6000 ampoules will be prepared to last about 20 years. Stability will be continuously monitored, with data from the current standard indicating stability		

	for ambient shipment and no loss of activity to date (over 18 years) when stored at -20°C.
Public health importance	For diagnostic purposes – this standard is the primary material for value assignment in patients of Protein C for thrombophilia determination. Additionally used for Protein C functional measurement in virus inactivated fresh frozen plasma.
Global importance	Accurate determination of patient levels of protein C will be required for therapeutic interventions, and to ensure the activity of therapeutics.
Global need from regulatory & scientific considerations	The International Standard ensured the continuity of the International Unit to ensure the traceability of diagnostic manufacturers own calibrators and for therapeutic preparations.
ECBS outcome	[BLANK]

Proposal (title)	Proposal to develop the 1st WHO IS and reference panel for anti-HEV antibody		
Proposer (name of Institution)	MHRA	**Principal contact**	Catherine Cherry
Rationale	Hepatitis E virus (HEV) is of global heath significance yet clinically underdiagnosed. The WHO estimates that there are 20 million HEV infections worldwide each year. The infection is normally resolved within weeks but an estimated 3 million cases can develop into acute hepatitis. It is transmitted by the fecal oral route, primarily through contaminated water or food products. Hyperendemic regions in developing countries are most at risk of HEV outbreaks, in particular areas prone to flooding and areas with poor sanitation and water access. There are 4 genotypes that infect humans. Genotypes 1 and 2 are associated with large scale outbreaks with transmission primarily through contaminated water. Genotypes 3 and 4 are zoonotic and cases are found worldwide following consumption of raw or undercooked meat products. There is a high rate of infection and morbidity in pregnant women. HEV diagnosis can be confirmed by a variety of methods including the detection of IgM (acute infection) and IgG antibodies. The WHO global hepatitis strategy aims to reduce new hepatitis infections by 90% by 2030. There remains a difficulty in interpreting disease burden due to the lack of consistency of reported data and poor interassay concordance. The 2015 WHO position paper on HEV noted that 'the performance characteristics of various currently available commercial assays…are suboptimal.' Therefore, there is a need to replace the WHO Reference Reagent for anti-HEV (95/584) which was prepared in 1997, as stocks are now depleted. In addition, a reference panel to represent the different genotypes remains of value. In 2015 the WHO endorsed the preparation of an anti-HEV reference panel by PEI (WHO/BS/2015.2275). We aim to include the production of this reference panel alongside the development of the 1st International Standard.		
Anticipated uses and users	Standardisation of diagnostic assays, seroepidemological studies and analysis of the protective level of anti-HEV. Anticipated users include clinical and public health laboratories, assay kit manufacturers, research laboratories and HEV vaccine developers.		
Source/type of materials	Serum has been sourced and characterised by PEI from PCR confirmed HEV infection cases from either clinical cases or blood donors. This will be evaluated to serve as either a reference panel member or candidate IS. Additionally, candidate IS sera will be sourced from individuals vaccinated as part of a clinical trial in Pakistan (anticipated 2025).		

Outline of proposed collaborative study	A collaborative study will evaluate a panel of sera samples covering multiple genotypes and both IgG and IgM reactive sera to select a final panel with good reactivity in a range of assays. From this panel, a candidate IS of good volume, reactivity and demonstrating the ability to reduce inter-laboratory variation will be chosen and an assigned unitage proposed. A pilot study using the material from clinical cases/blood donors may be run in advance of receiving the material from the vaccine trial. The study will involve 10-20 laboratories worldwide, performing a range of HEV serological assays, and include control labs, kit manufacturers and clinical and academic labs. Laboratories will be recruited by contacting the global network of HEV laboratories.		
Issues raised by the proposal	Transfer of material from PEI to MHRA will require new MTAs. There may be difficulty in sourcing material to cover all 4 genotypes for the reference panel.		
Action required	ECBS to endorse proposal		
Proposer's project reference		**Date proposed:**	October 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	There are commercial serological kits available for the detection of anti HEV IgM and IgG. A recombinant vaccine known as Hecolin, was produced by Xiamen Innovax Biotech Co., Ltd. in Xiamen, China. The vaccine is only licensed for use within China and is not available worldwide. However, in 2022 following an HEV outbreak at the Bentiu Camp, South Sudan, MSF and WHO led the first reactive vaccination campaign using Hecolin.		
Number of products or methods	The anti-HEV antibody IS and reference panel will be used in commercial kits and in-house binding ELISA assays. At least 2000 vials will be produced of the IS.		
Public health importance	The standard will enable both the validation of and establishment of consistency across HEV serological assays. This will allow for a deeper global understanding of HEV seroprevalence and disease burden.		
Global importance	As above		

Global need from regulatory & scientific considerations	Standardisation and calibration of serological assays for HEV will support vaccine development, case management, seroepidemological studies and surveillance. The IS and reference panel will support the WHO global hepatitis strategy.
ECBS outcome	[BLANK] Please see above comments.

Proposal (title)	International Standards for potency assays of novel Oral Polio Vaccines (nOPVs)		
Proposer (name of Institution)	MHRA	**Principal contact**	Javier Martin
Rationale	OPV has been the preferred vaccine throughout the WHO Global Poliovirus Eradication Initiative. However, the Sabin poliovirus strains in OPV are genetically unstable and have been shown to lose their attenuating mutations and revert to a neurovirulent phenotype during passage causing vaccine-derived poliovirus (VDPV) outbreaks in areas of low immunity. For this reason, genetically-modified novel oral polio vaccines (nOPVs) with improved genetic stability, less likely to revert to a neurovirulent phenotype, have been developed. The use nOPV against type 2 poliovirus (nOPV2) under WHO Emergency Use Listing authorization for the control of VDPV outbreaks due to type 2 poliovirus (VDPV2) is proving successful at limiting the extent of VDPV2 transmission and will likely be the vaccine of choice to continue global polio eradication efforts. The potency evaluation of nOPV2 during development and in current batch release involves the use of proprietary in-house homologous reference reagents and standards. As more manufacturers begin to develop nOPVs, it will be important for homologous reference standard and reagents to be readily available through the Medicines and Healthcare Products Regulatory Agency (MHRA-formerly NIBSC) portal to support lot release of vaccine materials. Additionally, as we progress towards WHO prequalification of nOPV2, and continue to advance type 1 and 3 nOPV (nOPV1 and nOPV3) development, the availability of appropriate homologous International Standards will be critical for setting up vaccine quality control (QC) potency assays that can be used across manufacturers and National Control Laboratories. MHRA intends to produce WHO International Standards for monovalent nOPV1, nOPV2 and nOPV3. A WHO International Standard for trivalent nOPV will also be produced if suitable materials are available.		
Anticipated uses and users	The study will result in the establishment of WHO International Standards to be used for potency assays of nOPV products, which involves the assessment of virus titres of monovalent and trivalent vaccine preparations for the control of vaccine bulks and finished products. Anticipated users are vaccine manufacturers, National Control Laboratories and research and development consortiums developing new safer vaccine strains.		
Source/type of materials	Candidate vaccine materials will be provided by manufacturers. Filling at the manufacturer might be considered.		

Outline of proposed collaborative study	The collaborative study would assign potency in $Log_{10}/CCID_{50}$ for each of the serotypes based on infectivity assays using Hep2C cell system.		
Issues raised by the proposal	Urgently needed to support the endgame of polio eradication		
Action required	ECBS to endorse proposal		
Proposer's project reference	To be added	**Date proposed:**	October 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	nOPV2 is being used under WHO Emergency Use Listing and it is currently being evaluated for WHO prequalification. nOPV1 and nOPV3 are currently under clinical evaluation.		
Number of products or methods	Monovalent nOPV1, nOPV2 and nOPV3 and trivalent nOPV.		
Public health importance	Provision of these WHO International Standards will support the standardization of potency assays for nOPV globally and help manufacturers and National Control Laboratories in the quality control and batch release process to ensure safe and effective vaccines are available for disease prevention and control.		
Global importance	Prevention of paralytic poliomyelitis disease caused by poliovirus and contribution to global polio eradication.		
Global need from regulatory & scientific considerations	Provision of these WHO International Standards will support the quality control and batch release process of nOPV products globally		
ECBS outcome	TBD		

Proposal (title)	First WHO International Reference Panel for adventitious virus detection in biological products using high-throughput sequencing technologies		
Proposer (name of Institution)	FDA/CBER	**Principal contact**	Arifa S. Khan, Ph.D.
Rationale	HTS has been introduced in revised ICH Q5A(R2) as an alternative assay for replacing or supplementing the currently recommended *in vivo* and *in vitro* assays for adventitious virus detection. With the finalization of the ICH guideline (Nov 2023), it is expected there will be increased interest globally for using HTS by regulators and industry, since the currently used virus detection assays have failed to detect some known and novel viruses, are not standardized, and need $\geq$ 28 days to obtain results. Furthermore, replacement of the *in vivo* assays by HTS could facilitate achieving the global objective of minimizing the use of animals in research. However, implementation of HTS for adventitious virus testing of biologics requires method validation and assay qualification. Therefore, reference reagents are needed to demonstrate the sensitivity and specificity of the key steps in the HTS workflow, namely, sample processing, cDNA synthesis, library preparation, sequencing and bioinformatics analysis. A panel of live viruses, representing virus families with diverse physical, chemical and genomic properties, could allow for assessment of the entire HTS workflow in terms of sensitivity and breadth of adventitious virus detection. The current international reference reagents were established at the 72nd meeting of the WHO ECBS in October 2020. Five viruses were selected based on their diverse physicochemical and genomic properties for testing the broad detection of adventitious viruses by HTS. Around 400–500 vials of each virus were prepared and are currently being distributed internationally to health agencies, manufacturers, CROs and academia. However, requests have been increasing due to growing interest in using HTS for adventitious virus detection in biologics, and the use of HTS to replace or supplement one or more adventitious virus detection assays has increased in regulatory submissions. To ensure the future supply of reference reagents in this field, CBER has produced 1000 vials of each of the original five viruses and expanded the representation of virus families by including a coronavirus (OC43) and a parvovirus (MVM). These seven new virus stocks were characterized similarly to the original five reference reagents. This included the determination of infectious titre, viral copies per mL and total viral particle count. A Certificate of Analysis will be provided for each virus.		
Anticipated uses and users	The availability of the larger number of vials of the seven new viruses constituting the proposed virus panel will help ensure its long-term use for HTS qualification and validation studies. The inclusion of the two new virus families will broaden the scope of the proposed panel in demonstrating the breadth of adventitious virus detection by HTS. At the same time, the current five virus reagents could, following their disestablishment as WHO reference reagents, be made available to new users to help further develop and establish the use of HTS for adventitious virus detection.		

	The use of the virus reference panel for HTS qualification and validation could facilitate implementation of the technology as a rapid method for replacing or supplementing the currently used adventitious virus detection assays. The use of a common reference virus panel could also facilitate the international harmonization of the regulatory review of HTS data submitted by different regional authorities. Live viruses can facilitate evaluation of the various steps in the entire HTS workflow for adventitious virus detection, from sample preparation through to bioinformatics. The reference virus panel would be made available to all appropriate HTS users for method qualification and validation. Anticipated users include regulatory agencies, manufacturers (vaccines, gene therapies and biotherapeutics), contract research organizations (CROs) and academia.
Source/type of materials	The seven viruses in the proposed panel were selected to represent families with distinct physical and chemical properties, and different types and sizes of virus genome for demonstrating HTS capabilities for broad adventitious virus detection. The viruses and cell lines used for their propagation were the same as were used for the original five virus reagents and were obtained from the American Type Culture Collection (ATCC). Large-scale virus stocks were prepared to meet the criteria for infectious titre and genome copy number. Stocks were tested for sterility, mycoplasma, adventitious viruses and host cell DNA. Each virus stock was individually vialed and is distributed with the ATCC Certificate of Analysis. The proposed panel consists of: Epstein-Barr virus (HHV-4), strain B95-8; human respiratory syncytial virus, strain A2; mammalian (human) orthoreovirus type 1, strain Lang; feline leukemia virus, strain Theilen; porcine circovirus type 1; human coronavirus OC43; and minute virus of mice (MVM).
Outline of proposed collaborative study	A collaborative study will be designed to demonstrate HTS virus detection of the seven-virus panel, similar to the previous study for the five reference reagents. The study will include HTS users with demonstrated experience the Illumina HTS platform, and will also try to include users of Oxford nanopore technology. The seven viruses will be spiked at 10^4 viral copies per mL in a high-titre adenovirus background (10^9) using independent protocols, sequencing platforms, and bioinformatics pipelines. The study will include regulatory/public health agencies, industry and CROs.
Issues raised by the proposal	There should be no gap in the availability of an international reference material to ensure the continued application of HTS. The current five reference reagents and the new seven-virus panel should be very clearly demarcated as separate resources with distinct purposes and having different international standing. This will require disestablishment of the original five viruses as WHO reference reagents and removal of all mention of WHO in their names should the WHO international reference panel be established.
Action required	ECBS to endorse proposal

Proposer's project reference		**Date proposed:**	May 13, 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES			
Approval status of medicine or in vitro diagnostic method	Due to the need for urgent and rapid vaccine development during the COVID-19 pandemic there has been significant progress in the use of HTS for adventitious virus detection. The availability of the five WHO reference reagents has proved to be important for demonstrating method qualification and validation. The results obtained have provided confidence in the broader use of HTS as an alternative to conventional assays for adventitious virus detection, particularly animal assays. This could in turn also aid in the global objective of minimizing the use of animals in research. Furthermore, it was widely recognized at the 3rd NGS meeting in Rockville, MD held in Sept 2022 that the use of HTS can help to reduce the testing time of the *in vitro* cell culture assays as well as overcome the challenges of assay interference in cases when the vaccine virus cannot be effectively neutralized. Additionally, HTS can serve as a single broad virus detection assay to replace the multitude of virus-specific PCR assays. There is currently general acceptance by regulators and industry that HTS can be used for broad adventitious virus detection, subject to demonstration of method qualification and validation using suitable reference standards. A common set of virus standards therefore needs to be available for international use to further strengthen confidence in HTS data for adventitious virus detection in biologics and to harmonize the related regulatory review processes.		
Number of products or methods	HTS for adventitious virus detection is encouraged in the EP and WHO documents and is accepted by the EMA and the US FDA; in the case of the latter, HTS applications have been reviewed on a case-by-case basis for vaccines as well as for gene therapy and biotherapeutic products. Use of HTS as an alternative to current virus detection assays has been introduced in the revised ICH Q5QA(R2). There is also a new Ph. Eur. guideline on HTS under development.		
Public health importance	The detection of porcine circovirus (PCV1) in the licensed Rotarix vaccine demonstrated the limitations of the currently recommended adventitious virus detection assays and highlighted the importance of HTS for broad virus detection to industry and regulators. The discovery of a novel rhabdovirus in Sf9 cells further demonstrated the potential of HTS in the detection of unknown viruses for increased assurance of product safety and enhanced public health.		
Global importance	The global availability of an international virus standard would facilitate HTS implementation as an alternative assay for rapid and broad adventitious virus testing. This could result in the accelerated development of vaccines with enhanced safety, including during public health emergencies, as current *in vivo* and *in vitro* adventitious virus detection assays take at least 28 days to perform, and in some cases cannot be performed at all due to interference by the vaccine virus. Furthermore, the use of HTS to replace *in vivo* adventitious virus detection assays will help to minimize the use of		

	animals for testing thus helping to achieve the 3Rs objectives on a global scale.
Global need from regulatory & scientific considerations	The availability of the proposed international reference panel would help to globally harmonize HTS testing for adventitious virus detection in biologics. Such live virus standards would be used to demonstrate the performance of the entire HTS workflow from sample preparation to bioinformatics for virus detection. The use of HTS could then: 1) help assure product safety by demonstrating the absence of known and unknown adventitious viruses; 2) help to minimize animal use on a global scale by replacing *in vivo* adventitious virus detection assays and testing for rodent viruses (MAP, HAP, RAP); 3) provide more consistent and reliable results since the current *in vivo* animal assays and *in vitro* cell culture assays are subject to significant biological variability, and assay performance and interpretation can only be done by trained individuals; 4) allow for the inclusion of HTS data using common standards across industry thus aiding consistent review across regulatory agencies; and 5) accelerate the development of vaccines against emerging and re-emerging diseases. Such gains may in turn facilitate the harmonized development of international guidance, including WHO guidance, on the use of HTS for adventitious virus detection in biologics.
ECBS outcome	[BLANK]

Proposal (title)	WHO 6th International Standard for Thromboplastin, Human, Recombinant, Plain		
Proposer (name of Institution)	MHRA	**Principal contact**	Craig Thelwell
Rationale	Stocks of the current WHO 5th International Standard for Thromboplastin, Human, Recombinant, Plain (RTF/16) are almost depleted and a replacement is needed. Approximately 1100 ampoules of RTF/16 are distributed each year.		
Anticipated uses and users	The IS is used for the International Sensitivity Index (ISI) calibration of secondary standards or commercial thromboplastin preparations, used to monitor patients undergoing treatment with vitamin K antagonists (VKA, e.g. warfarin) with the one-stage prothrombin time (PT) assay. The PT measured in seconds depends on the thromboplastin reagent and the technique in the device; the PT measured in seconds should be transformed to the International Normalised Ratio (INR) by means of a mathematical formula to achieve equivalence between the various reagents and techniques. The mathematical formula is based on the ISI of the thromboplastin reagent and the Mean Normal Prothrombin Time (MNPT).		
Source/type of materials	The source material should be a commercial human recombinant thromboplastin preparation. A potential donor was identified; the material was assessed in a trial fill study and is progressing towards a planned definitive fill of 25,000 ampoules. The material will be filled at 1 ml per ampoule at 2X concentration, and will therefore have a reconstitution volume of 2 ml.		
Outline of proposed collaborative study	A working group of stakeholders was formed under the auspices of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (SSC/ISTH). The members of the working group represent calibration laboratories, SSC/ISTH, MHRA, external quality assessment organizations, and manufacturers of reference materials and measurement procedures. The primary purpose of the working group is to: • develop a harmonized reference measurement procedure based on the WHO manual tilt tube technique (MTT); • establish a complete reference measurement system for global standardization of the PT/INR test • establish a network of at least three calibration labs running the harmonized MTT method for certifying the calibration of commercial thromboplastins from IVD-manufacturers;		

	• evaluate the establishment of a single WHO IS for thromboplastin to replace the current two which co-exist (rFT/16 and RBT/16) Based on the recommendations of the working group, the collaborative study is proposed for the assignment of an ISI value to the 6th IS by the network of four calibration labs established by the working group, using the harmonised MTT method, relative to the current 5th IS for recombinant human thromboplastin (rTF/16) and not the current 5th IS for rabbit brain thromboplastin (RBT/16)		
Issues raised by the proposal	Current WHO guidelines: 'Guidelines for thromboplastins and plasmas used to control oral anticoagulant therapy' (WHO, 2013) states that each collaborative study for the replacement of a thromboplastin IRP should include the testing of all existing IRPs, and that the ISI assigned to the replacement should be the mean of the ISIs of all existing IRPs, which would include the current WHO 5th IS for human recombinant thromboplastin (RTF/16) and the current WHO 5th IS for rabbit brain thromboplastin (RBT/16). The conclusions of the working group are: • the ISI value assignment of a given thromboplastin is more precise if performed against a reference preparation of similar composition and from the same species. • the availability of two reference preparations (i.e. human and rabbit) is not absolutely necessary. • a manufacturer's selected procedure with a rabbit thromboplastin could be calibrated against reference preparation of human thromboplastin, and the number of unlike calibrations (i.e. rabbit against human) would be the same as in an alternative route using a reference preparation of rabbit thromboplastin • the preparation and availability of a reference thromboplastin from a single source (human, recombinant) would be preferable to establish an unequivocal calibration hierarchy according to ISO 17511:2020. In following these recommendations the current WHO Guidelines, in which the ISI to be assigned to the replacement IRP is the mean of the ISIs obtained by calibration with all existing IRPs, would need to be amended. The proposal of the working group also means only four calibration labs would take part in the calibration exercise, using the harmonised WHO MTT method, rather than the >20 laboratories previously employed using MTT methods without harmonisation.		
Action required	ECBS to endorse proposal		
Proposer's project reference		**Date proposed:**	October 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			

Approval status of medicine or in vitro diagnostic method	WHO guidelines define the manual tilt tube PT method (MTT)
Number of products or methods	Large number of commercial thromboplastin products, value assigned for ISI using the WHO MTT method, for use with a range of different PT methods and devices. The IS is also used to calibrate POC devices that use whole blood.
Public health importance	The PT/INR system is essential for monitoring patients on anticoagulant therapy (VKA), for appropriate dosing adjustments and improved patient management.
Global importance	Anticoagulant therapy using VKAs is of significant global importance
Global need from regulatory & scientific considerations	There is a global need for standardisation to establish consistent treatment guidelines, ensure regulatory oversight, facilitate research and clinical trials, enhance quality assurance and contribute to global health surveillance efforts.
ECBS outcome	[BLANK] Please address my comments