EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 24 to 28 October 2022

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 23 September 2022 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.
This draft does not necessarily represent the decisions or the stated policy of the World Health Organization.

**Proposed new projects**

1. WHO 3rd International Standard for Protein S Plasma
2. WHO 2nd International Standard for HIV-1 p24 antigen
3. WHO 2nd International Standard for SARS-CoV-2 RNA
4. WHO 2nd International Standard for Serum Amyloid A
5. WHO 2nd International Standard for Yellow Fever vaccine for potency assays
6. WHO Reference Reagent for the characterization of Fc binding domain containing biotherapeutic proteins
7. WHO Reference Reagent for flow cytometry analysis of pluripotent stem cells (revised proposal)
8. WHO Reference Reagent for flow cytometry analysis of mesenchymal stromal cells (revised proposal)
## Running Title: 3\textsuperscript{rd} IS Protein S Plasma

| Proposal (title) | The 3\textsuperscript{rd} IS Protein S, plasma |
|------------------|---------------------------------------------|
| Proposer (name of Institution) | NIBSC/MHRA | **Principal contact** | John Hogwood |
| **Rationale** | The Protein S standard is required for the laboratory diagnostics of protein S in patients as part of a screen should they present with thrombophilia, and for the measurement of protein S in the therapeutic products, virus inactivated fresh frozen plasma and prothrombin complex concentrates. There are sufficient stocks to last two years based on the current unrestricted usage. |
| **Anticipated uses and users** | Expect use is 400 ampoules per year, with diagnostic and therapeutic manufacturers the main users. |
| **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 30 laboratories, will be carried out to assign value to the three analytes associated with the material – functional, free antigen and total antigen. |
| **Issues raised by the proposal** | None – replacement of existing reference material. |
| **Action required** | ECBS to endorse proposal |
| **Proposer's project reference** | BIO000149 | **Date proposed:** | 05-Jul-2022 |

### 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 S in their products. |
|----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| **Number of products or methods** | This standard will have three analytes – functional activity, free antigen and total antigen assigned to it. A fill of 8000 ampoules will be prepared to last 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\textdegree C. |
| **Public health importance** | For diagnostic purposes – this standard is the primary material for value assignment in patients of Protein S for thrombophilia determination. Additionally used for Protein S functional measurement in prothrombin complex concentrates and virus inactivated fresh frozen plasma. |
|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Global importance**        | Accurate determination of patient levels of protein S 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) | Proposed 2\textsuperscript{nd} International Standard for HIV-1 p24 Antigen |
|-----------------|---------------------------------------------------------------------|
| Proposer (name of Institution) | NIBSC | Principal contact | Graham Prescott |
| Rationale | The WHO International Standard for HIV-1 p24 Antigen is used by In vitro diagnostic manufacturers to access sensitivity of test devices where the common technical specification (CTS) published in the European union states that IVD medical device must detect at least 2 International units for p24 Antigen. As of June 2022, the existing stock of the current 1st IS for HIV-1 p24 Antigen (90/636) is ~300 vials. At current rates of issue stocks are likely to be exhausted in 1 year; stock have therefore been restricted to 1 vial per customer/year. The replacement will require up to 2 years for completion. |
| Anticipated uses and users | Used to calibrate secondary reference materials and in the validation of 4\textsuperscript{th} generation combination assays, antigen only p24 assays and some rapid detection assays. Anticipated users are IVD manufacturers, reference laboratories, diagnostic labs, EQA providers and OMCL’s |
| Source/type of materials | The 1\textsuperscript{st} IS for HIV-1 p24 Antigen (90/636) is derived from peptide isolated from detergent-treated HIV-1 virus (Subtype B) and diluted in human serum albumin and glass distilled water. Candidate source material will need to be sourced and characterized. |
| Outline of proposed collaborative study | Study (comprising 10-20 participants) to calibrate the new candidate against 90/636. |
| Issues raised by the proposal | None |
| Action required | ECBS to endorse proposal |
| Proposer's project reference Date proposed: | | June 2022 |

**CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)**

| Approval status of medicine or in vitro diagnostic method | Commercially available methods to detect HIV-1 p24 Antigen in human serum and plasma. |
| Number of products or methods | A range of commercial Immunoassays p24 antigen only and combination assays for the detection and quantification of HIV-1 p24 Antigen are available. |
| Public health importance | Early detection of HIV is paramount in the effectiveness of administering antiretroviral therapy. Although molecular methods are known to reduce the... |
window period further still, the cost is often significantly higher which can be a concern for Low Middle Income Countries (LMIC). Therefore, assays that can detect viral antigen is both time and cost effective, with antigen being detected 5-7 days before the presence of antibodies.

It is essential that the International standard is replaced to provide a reference material that can ensure high analytical sensitivity of the p24 antigen component of 4th generation immunoassays/p24 specific assay systems.

| Global importance          | HIV infection continues to be a major public health concern around the world with more than 35 million people infected worldwide. Although treatment management of HIV has significantly improved over the last couple of decades, accurate qualification is highly important to ensure drug therapies remain effective. |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Global need from regulatory & scientific considerations | CTS guidelines outline the need for assays to have a minimum limit of detection of 2IU/mL |
| ECBS outcome               | [BLANK]                                                                                                 |


| Proposal (title) | Second WHO International Standard for SARS-CoV-2 RNA |
|-----------------|------------------------------------------------------|
| Proposer (name of Institution) | NIBSC | Principal contact | Emma Bentley |
| Rationale | In December 2019 the WHO was notified of an outbreak of pneumonia which went on to be identified as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and by March 2020 a global pandemic was declared. To support the development of diagnostic molecular assays, a WHO collaborative study was rapidly undertaken to establish the First WHO International Standard (IS) for SARS-CoV-2 RNA (NIBSC code 20/146) in December 2020. A common standard will facilitate development, assessment of assays and allow for comparability of the assays, including determining the limit of detection, and ultimately the most reliable result. As of May 2022 over 1,485 vials of 20/146 have been distributed, with a stock of 580 vials remaining. Whilst sales have declined, we have continued to distribute approximately 50 vials per month in Q1 2022. With more than 518 million COVID-19 cases recorded globally, and the near worldwide endemcity of SARS-CoV-2, the demand for molecular diagnostics will continue for the foreseeable future. The timely replacement of the WHO IS for SARS-CoV-2 RNA will ensure continuity in the International Unit (IU). |
| Anticipated uses and users | Standardisation of diagnostic assays based on nucleic acid amplification techniques (NAT) (e.g. quantitative RT-PCR, digital RT-PCR etc.) for identification of SARS-CoV-2 infection by: Clinical and public health laboratories Vaccine manufacturers - Vaccine studies Assay kit manufacturers Research laboratories |
| Source/type of materials | The replacement IS will be based on inactivated virus, following the procedure of acid-heat treatment used to prepare 20/146. The strain used will be representative of the most widely circulating SARS-CoV-2 variant at the time (https://www.who.int/activities/tracking-SARS-CoV-2-variants), likely Omicron. Stocks of this virus are already held at NIBSC, provided under Material Transfer Agreement from UK Health Security Agency which includes the preparation of WHO reference material. |
| Outline of proposed collaborative study | The collaborative study will involve 20-30 laboratories worldwide, to both recapitulate assays used in the previous evaluation of the First WHO IS for SARS-CoV-2 RNA and to capture the variety of assays currently in use |
globally. The study panel will include inactivated virus of different SARS-CoV-2 strains/formulation to provide an assessment of commutability, following the same approach as the previous collaborative study. The possibility to include clinical samples or live SARS-CoV-2 virus will also be considered.

The aim will be to assess the suitability of the candidate replacement to serve as the Second WHO IS for SARS-CoV-2 RNA by:

- Characterising the candidate preparation’s reactivity/specificity in different assay systems
- Evaluating the potency/readout in a range of typical assays performed in different laboratories
- Assessing commutability i.e. to establish the extent to which the candidate is suitable to serve as a standard for a variety of different samples/strains
- Proposing a unitage for the Second WHO IS to ensure continuity with the First WHO IS

| Issues raised by the proposal | None |
|-----------------------------|------|
| Action required              | ECBS to endorse proposal |
| Proposer's project reference | Date proposed: | June 2022 |

| CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932) |
|-----------------------------------------------|
| **Approval status of medicine or in vitro diagnostic method** | Over 190 countries have approved vaccines, with 38 platforms having been approved and including 11 with WHO EUL. More than 160 are undergoing clinical trials. 26 in vitro diagnostic tests have WHO EUL status, with a further 14 undergoing assessment. Within the UK the MHRA has approved 11 tests. |
| **Number of products or methods** | There are 361 CE-IVDs on the market, this includes Real-Time RT-PCR, digital, Transcription Mediated Amplification (TMA) and Loop-mediated Isothermal Amplification (LAMP) technology. |
| **Public health importance** | The COVID-19 pandemic has so far caused over 518 million cases and 6 million deaths. Countries continue to report a high level of cases, with 15th June 2022 epidemiological situation report showing cases had increased between 13-58% across three WHO regions. The global vaccination rate (at least one dose) stands at 65.9% and weekly fatalities remain in the thousands. Further, there is a global effort to monitor for new viral variants and evaluate the risk they may pose to public health, either through vaccine/therapeutic... |
escape or altered transmission or disease characteristics. As such, the public health importance remains high.

| Global importance | As above |
|-------------------|----------|
| Global need from regulatory & scientific considerations | Standardised and calibrated assays are vital for the comparability and control of platforms being used to detect SARS-CoV-2 RNA, assisting the definition of parameters such as analytical sensitivity/limits of detection. Continuity in the unitage and availability of the IS remains important, for continued assessment and approval of new/updated platforms by regulatory bodies. |
| ECBS outcome | [BLANK] |

Running Title: **Second WHO International Standard for SARS-CoV-2 RNA**
| Proposal (title)                      | The 2\textsuperscript{nd} WHO International Standard for serum amyloid A |
|--------------------------------------|--------------------------------------------------------------------------|
| Proposer (name of Institution)       |MHRA/NIBSC | Principal contact | Ben Cowper |
| Rationale                            | Serum amyloid A (SAA) is an important marker of acute phase inflammation due to infection, trauma, or chronic inflammatory diseases such as rheumatoid arthritis. SAA levels rise rapidly (up to 1000-fold) following an inflammatory stimulus and quickly return to “normal” levels following resolution of the inflammatory event. SAA, along with C-reactive protein (CRP), is therefore a useful tool for monitoring inflammation.  

The 1\textsuperscript{st} WHO IS for SAA, coded 92/680, has been available for almost 30 years, and is used to calibrate immunoassays measuring SAA in patient serum/plasma. Immunoassays typically report SAA levels in units of mg/L. Although 92/680 is assigned in IU, this value is an arbitrary conversion derived from measurements in SI units (1 µg = 1 mIU), and the material is generally used to calibrate assays in SI units.  

A low seller for many years, sales of 92/680 began to increase approximately four years ago, and have reached ~250 ampoules per year. This has led to rapid depletion of stocks, and despite restriction of sales (currently 2 ampoules per lab per year) stocks are expected to be exhausted in 1-1.5 years. As a result, it is proposed to prepare a replacement IS to ensure continued availability to support SAA immunoassay development, calibration and harmonisation. |
| Anticipated uses and users            | The standard will be used by manufacturers of immunoassays for serum amyloid A measurements, clinical and quality control laboratories. |
| Source/type of materials              | As for 92/680, Professor Sir Mark Pepys (UCL) has agreed to donate anonymised clinical remnants of high SAA (>400 mg/L) serum. These samples will be viral marker tested, and negative samples pooled and lyophilised to prepare the candidate standard. |
| Outline of proposed collaborative study| The candidate IS will be shipped alongside the 1\textsuperscript{st} IS to multiple (>10) expert laboratories for immunoassay analysis, to evaluate the suitability of the material to serve as an IS, and assign a value in terms of the 1\textsuperscript{st} IS. Serum/plasma samples covering a range of SAA concentrations will be included in the study, to enable assessment of the commutability of the candidate IS. An accelerated thermal degradation study will also be carried out through a smaller number of laboratories. |
**Issues raised by the proposal**  
Despite the attempt to introduce an IU through assignment to the 1st IS in the early 1990s, SAA immunoassays continue to be calibrated in SI units. In evaluating and value assigning a candidate replacement IS, it must be ensured that a transition to a new IS causes no/minimal disruption to users of the material.

**Action required**  
ECBS to endorse proposal

**Proposer's project reference**  
TBC  
**Date proposed:**  
April 2022

| CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932) |
|-----------------------------------------------------|
| Approval status of medicine or in vitro diagnostic method | Multiple immunoassay kits marketed globally. |
| Number of products or methods | >10 |
| Public health importance | Serum SAA measurements are used to monitor inflammation due to infection, trauma, or chronic inflammatory diseases such as rheumatoid arthritis. |
| Global importance | Particularly strong sales activity in China, but numerous customers based in Europe/North America in addition. |
| Global need from regulatory & scientific considerations | Continued availability of an IS required to support SAA immunoassay development, calibration and harmonisation by manufacturers across continents. |

**ECBS outcome**  
[BLANK]
| Proposal (title) | 2nd International Standard for Yellow Fever Vaccine for Potency assays. |
|-----------------|---------------------------------------------------------------------|
| Proposer (name of Institution) | e.g. NIBSC | Principal contact | Gillian Cooper / Laura Stephens |
| Rationale | Yellow Fever vaccine Live attenuated vaccine, grown in eggs and the final presentation is freeze dried. The vaccine is crucial in the prevention of Yellow Fever which is endemic in parts of Africa and Central and South America. It is a WHO Prequalified vaccine and central to the WHO surveillance program to control the disease. Yellow Fever vaccines requirements for potency testing is that they should be assayed in cell cultures or mice or deemed sensitive and that the titer of the vaccine should not be <1000 mouse LD50 or its equivalent in PFU. The collaborative study to establish the 1st IS 99/616 assessed the relationship between LD50 – PFU with a view to validating the standard to assign IU to vaccines to improve reproducibility between vaccines. The 1st IS 99/616 was established in 2003 and has successfully been used by producers and testing NCL’s. Stocks of this IS are critically low and needs replacing. A replacement strategy has now been initiated. |
| Anticipated uses and users | Manufacturers and National Control Laboratories for the calibration / validation of their in-house references used in the QC testing of production lots. |
| Source/type of materials | NIBSC would need to secure donations of final YFV bulk from global manufacturers of the vaccine. |
| Outline of proposed collaborative study | The collaborative study would assign IU to the YFV standard by in vitro testing (cell based PFU). |
| Issues raised by the proposal | Sourcing the material as there is a global storage of vaccine and currently there are studies to evaluate using fractional doses. |
| Action required | ECBS to endorse proposal The standardization of YFV discussed at the recent WHO TRS meeting and endorsed by group to replace. |
| Proposer’s project reference | Date proposed: July 2021 |

CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)
| Approval status of medicine or in vitro diagnostic method | Licensed Yellow Fever Vaccine. |
|----------------------------------------------------------|--------------------------------|
| **Number of products or methods**                        | WHO PQ vaccine. Globally produced by 5 manufacturers. 1 and multi dose presentations. |
| **Public health importance**                             | Provision of the IS will support the standardization of the potency of YF vaccines globally and aid NCL’s in the control of YF vaccines. This will ensure the safety of the vaccine for use in disease control and prevention. |
| **Global importance**                                    | Prevention of Yellow Fever disease globally. |
| **Global need from regulatory & scientific considerations** | Provision of the IS will support the standardization of the potency of YF vaccines globally and aid NCL’s in the control of YF vaccines. |
| **ECBS outcome**                                         | |

**Running Title:** 2nd *International Standard for Yellow Fever Vaccine for Potency assays. Replacement for 99/616*
The number of approved biotherapeutic monoclonal antibodies (mAb) and associated modalities, re-purposed and under development is increasing, and has most extraordinarily in the light of COVID-19 pandemic. A continued interest in these products is driven by improved understanding of disease targets and their clinical success, given their specificity, stability, long half-life, and relatively good safety profile.

Human immunoglobulins have 2 functional domains: the antibody binding region (Fab), responsible for the antigen specificity, and the crystallizable fragment (Fc), that drives the antibody function. The Fc fragment mediates interactions with soluble and cellular components of the immune system and also impacts on their clearance from the blood stream. Fc-domain effector functions such as complement mediated cytotoxicity (CDC), antibody dependent cell-mediated cytotoxicity (ADCC) and antibody dependent phagocytic killing (ADCP), contribute to the mechanism of action (MoA) and clinical therapeutic activity of many biotherapeutic mAbs. Conversely, significant efforts in the design of these medicines can be also directed towards silencing the Fc interactions with the immune system that may compromise their safety or efficacy in certain indications.

Structural differences in the Fc domain define the immunoglobulin isotype and subclasses and have a great impact on the mAb flexibility, stability, half-life and functional affinity. In addition, glycosylation and other post-translational modifications also affect the interactions of the Fc with the complement system and the various Fc receptors (FcR). Although the links between Fc structure and function are widely recognised, they are not yet completely understood and often the relative contribution of various Fc-interactions and associated effector functions on the clinical effects of mAb products are unclear. For many, N-glycosylation of the Fc and Fc-effector functions need careful control as critical quality attributes.

Orthogonal methods are used to characterise the Fc domain, its interaction with the immune system and associated functional consequences. A plethora of physicochemical, biochemical and cell-based assays are available and the number of novel tools and assay technologies continues to increase. Surrogate cell-based assays and/or biochemical assays are increasingly pursued to facilitate validation, quality control and high throughput in lieu of otherwise complex, technically demanding and often variable bioassays. Amongst these, platforms to assess binding to FcRs (FcγRI, FcγRII, FcγRIII, FcRn) and complement (C1q) such as ELISA, flow cytometry, surface plasmon resonance (SPR), biolayer interferometry (BLI), Kinetic exclusion binding assays (KinExA), and proximity–based assays (i.e. Alpha technology, FRET and HTRF) receive much interest. These assays are becoming broadly used in non-clinical in vitro programs and are critical in their biological characterization to understand and control mAb products through their life-cycle. Some of these methods are also used to interrogate Fc-interactions of the immune-complexes, where the Fc-bearing therapeutic is bound to the target.

In view of the broad use and growing interest in assays to characterize Fc domain interactions for mAbs and other Fc-bearing modalities, irrespective of their primary molecular target, we propose to develop WHO reference reagents to support development, performance, qualification and validation of Fc-binding assays and platforms. This proposal is in alignment with WHO recognized need for standardization of biotechnology products contributing to appropriate control measures to ensure safety, quality and efficacy (WHO TRS, 56th Report, 941: 12-13, 2007).

| Proposal (title) | WHO Reference reagents for the characterisation of Fc-domain interactions in biotherapeutics |
|------------------|--------------------------------------------------------------------------------------------------|
| Proposer (name of Institution) | NIBSC | Principal contact | Sandra Prior |

| Rationale | The number of approved biotherapeutic monoclonal antibodies (mAb) and associated modalities, re-purposed and under development is increasing, and has most extraordinarily in the light of COVID-19 pandemic. A continued interest in these products is driven by improved understanding of disease targets and their clinical success, given their specificity, stability, long half-life, and relatively good safety profile. Human immunoglobulins have 2 functional domains: the antibody binding region (Fab), responsible for the antigen specificity, and the crystallizable fragment (Fc), that drives the antibody function. The Fc fragment mediates interactions with soluble and cellular components of the immune system and also impacts on their clearance from the blood stream. Fc-domain effector functions such as complement mediated cytotoxicity (CDC), antibody dependent cell-mediated cytotoxicity (ADCC) and antibody dependent phagocytic killing (ADCP), contribute to the mechanism of action (MoA) and clinical therapeutic activity of many biotherapeutic mAbs. Conversely, significant efforts in the design of these medicines can be also directed towards silencing the Fc interactions with the immune system that may compromise their safety or efficacy in certain indications. Structural differences in the Fc domain define the immunoglobulin isotype and subclasses and have a great impact on the mAb flexibility, stability, half-life and functional affinity. In addition, glycosylation and other post-translational modifications also affect the interactions of the Fc with the complement system and the various Fc receptors (FcR). Although the links between Fc structure and function are widely recognised, they are not yet completely understood and often the relative contribution of various Fc-interactions and associated effector functions on the clinical effects of mAb products are unclear. For many, N-glycosylation of the Fc and Fc-effector functions need careful control as critical quality attributes. Orthogonal methods are used to characterise the Fc domain, its interaction with the immune system and associated functional consequences. A plethora of physicochemical, biochemical and cell-based assays are available and the number of novel tools and assay technologies continues to increase. Surrogate cell-based assays and/or biochemical assays are increasingly pursued to facilitate validation, quality control and high throughput in lieu of otherwise complex, technically demanding and often variable bioassays. Amongst these, platforms to assess binding to FcRs (FcγRI, FcγRII, FcγRIII, FcRn) and complement (C1q) such as ELISA, flow cytometry, surface plasmon resonance (SPR), biolayer interferometry (BLI), Kinetic exclusion binding assays (KinExA), and proximity–based assays (i.e. Alpha technology, FRET and HTRF) receive much interest. These assays are becoming broadly used in non-clinical in vitro programs and are critical in their biological characterization to understand and control mAb products through their life-cycle. Some of these methods are also used to interrogate Fc-interactions of the immune-complexes, where the Fc-bearing therapeutic is bound to the target. In view of the broad use and growing interest in assays to characterize Fc domain interactions for mAbs and other Fc-bearing modalities, irrespective of their primary molecular target, we propose to develop WHO reference reagents to support development, performance, qualification and validation of Fc-binding assays and platforms. This proposal is in alignment with WHO recognized need for standardization of biotechnology products contributing to appropriate control measures to ensure safety, quality and efficacy (WHO TRS, 56th Report, 941: 12-13, 2007). |
| Anticipated uses and users | These reference reagents will be used as a benchmark of activity and to assist the development, optimisation, performance and qualification of assays used in the characterisation of Fc-domain interactions for biotherapeutic products. |
| **Source/type of materials** | We will be seeking donations from manufacturers or through collaboration with other institutions. In-house production and/or purchasing of material to re-formulate and/or modify as may be required will also be explored. |
|----------------------------|---------------------------------------------------------------------------------------------------------------|
| **Outline of proposed collaborative study** | It is anticipated that the study will incorporate a number of independent and/or overlapping stages depending on the successful sourcing and/or preparation of candidate material, availability of assays, interest and recruitment of laboratories. In first instance, the preliminary screening and characterisation of a number of potential candidates will be performed in-house and/or with the involvement of a small number of collaborators as needed, in order to select the most appropriate lead candidate preparation panel representing a broad range of functionalities and characteristics. Subsequently, an International multicentre collaborative study will be designed to evaluate the suitability of the candidate panel of reference standard preparations where we will aim at comparing their relative bioactivity and performance in as broad a number of assays and assay platforms as possible, interrogating a variety of FcR and immune effectors. |
| **Issues raised by the proposal** | None to date |
| **Action required** | ECBS to endorse proposal |
| **Proposer's project reference** | TBD | **Date proposed:** 5th June 2022 |

**CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)**

| **Approval status of medicine or in vitro diagnostic method** | There are more than 100 biotherapeutic monoclonal antibodies and fusion proteins approved with indications in the field of cancer, immune disease and infection, in excess of 30 biosimilar mAbs, and hundreds of others are under regulatory review and development. Of significance is the extraordinary speed in the development of mAbs therapies and associated modalities for COVID-19 where there is an urgent need and interest to understand the contributions of Fc-interaction and associated functions. Nevertheless, the interest is not only limited to SARS-CoV2 treatment, mAbs-based biotherapeutics constitute one of the most promising therapeutic interventions of today. A number of cell-based and non cell-based biochemical assays and analytical platforms are available and under development to characterise Fc-interactions, in particular Fc binding assays. To name a few, ELISA, electrochemiluminescence (ECL), mesoscale discovery (MSD), Alpha technology-based, TR-FRET technology-based, flow cytometry, surface plasmon resonance are platforms currently used, however this is a field of continual development underpinned by the need of sensitive, fast and high-throughput analytical tools. |
| **Number of products or methods** | The many manufacturers of mAbs and associated modalities invest significant efforts to develop reagents and platforms within their non clinical characterisation programs at various stages of development and marketing of their products. An increasing number of service providers and developers of reagents for biotherapeutics also perform and develop assays to assess Fc-domain interactions. The field is promoted by advances in technology and availability of sensitive read-outs, and the development of reagents and new chemicals to evaluate ligand binding interactions. |
| **Public health importance** | High |

Support the availability of well characterised products (with enhanced or reduced Fc effector functions and defined pharmacokinetics), increase the confidence in the available platforms to assess Fc-binding, and facilitate harmonisation and integrated understanding of Fc-interaction characterisation data. In turn this will assist manufacturers and regulators at improving biotherapeutics.
### Global importance

|                     | High |
|---------------------|------|
| Ensuring the quality safety and efficacy of biotherapeutic products and the availability of suitable methods to develop and control these are paramount. These reference reagents may also support the work of other public bodies that work towards medicine harmonisation and development of global standards. |

### Global need from regulatory & scientific considerations

|                     | The WHO has raised concerns over the quality and safety of biotechnology products. The proposed panel of reference preparations will further contribute to efforts of WHO in the field of biotherapeutic monoclonal antibody and associated modalities and their biosimilars. |

### ECBS outcome

| [BLANK] |
| Proposal (title) | The first WHO Reference Reagent for Pluripotent Stem Cell Identity for Flow Cytometry |
|-----------------|-------------------------------------------------------------------------------------|
| Proposer (name of Institution) | MHRA | Principal contact | Katherine Warre Cornish |
| Rationale | Pluripotent Stem Cells (PSCs) offer enormous potential as starting materials for cell therapies due to their ability to generate all the cell types of the adult body and expand indefinitely. There are currently 54 clinical studies employing PSC derivatives, with this number forecast to increase (Kobold et al., Stem Cell Reports, 2020). Currently no physical standards or reference reagents specific for PSCs exist. Ongoing global investment has resulted in numerous PSC lines classified as clinical grade. However, consensus is lacking around the critical attributes defining this label. Considerable efforts are underway to harmonize and achieve transparency around manufacturing and quality parameters required for clinical grade PSCs (e.g. Anthony et al., Cell Stem Cell, 2021; Stacey and Healy, Stem Cell Research, 2021; Lovell-Badge, Nature, 2021). Flow cytometry is among the most used techniques in establishing PSC identity. A PSC reference reagent for flow cytometry would streamline these efforts, allow comparison between PSC lines and promote consensus in quality assurance. Furthermore, the capacity of PSCs to multiply and differentiate raises concerns of tumorigenicity after transplantation. For any PSC-derived therapy, it will be essential to demonstrate that PSCs are absent from the final product. A reference reagent for pluripotent stem cells would increase the accuracy of this assay and therefore safety of the medicine. We propose to develop a reference reagent for PSCs for flow cytometry. The reference reagent will consist of fixed, freeze-dried PSCs, displaying consistently high expression of pluripotency markers, such as Oct4, Sox2, Nanog and Tra-1-60. Cells will be obtained from the UK Stem Cell Bank, with permission from the UK Cell Bank Steering Committee and from depositors. Materials will be distributed among collaborative study participants with instructions to perform in-house flow cytometry assays for PSC markers. We propose to limit batch size of PSC reference reagents to 300-500 vials for several reasons. These include unknown demand for the product and unknown long-term stability of PSC marker epitopes in freeze-dried cells, as well as limits to internal production capacity. We propose to produce a |
master bank of PSCs, from which successive distribution banks can be generated. We will produce at least three such distribution banks and conduct flow cytometry to assess batch-to-batch variation in expression of pluripotency markers. When the collaborative study takes place, participants will receive material from at least three distribution banks, to capture and evaluate batch-to-batch variability across participating groups.

| Anticipated uses and users | The PSC Reference Reagent we propose to develop will consist of fixed, freeze-dried PSCs with consistent, high expression of pluripotency markers. The reference reagent should be used in flow cytometry assays for pluripotency marker expression in clinical grade PSCs. Use of a reference reagent would enable comparison between batches of clinical grade PSCs over time and promote consistency between different groups, despite differing equipment, reagents, instrument settings and data analysis strategies. This will facilitate quality assurance of clinical grade PSCs and promote harmonization between different centres. Furthermore, due to the ability of PSCs to divide indefinitely, it is essential to ensure they are absent from final products. A PSC reference reagent would improve the consistency and accuracy of this assay and therefore the safety of the medicine. Anticipated users are developers and manufacturers of clinical grade PSCs and PSC-derived cell therapy products. |
| Source/type of materials | Fixed and freeze-dried PSCs. |
| Outline of proposed collaborative study | Material from at least three distribution banks will be shipped to 15-20 laboratories where the material will be reconstituted following instructions provided. Collaborators will immuno-stain the material for pluripotency markers and run flow cytometry using in house reagents, equipment and protocols. |
| Issues raised by the proposal | We will select a PSC line with consistent high expression of pluripotency markers and conduct quality control experiments on biological replicates to ensure consistency between batches. However inter-batch variation remains a possibility. We will use our preliminary data to calculate appropriate numbers of replicates to be distributed in our collaborative study, so that batch-to-batch variation can be captured and evaluated. |
| Action required | ECBS to endorse proposal |
| Proposer's project reference | PSC Reference Reagent | Date proposed: | 01/07/2022 |
| CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932) |
|------------------------------------------------------|
| **Approval status of medicine or in vitro diagnostic method** | At least 54 PSC-derived products are in clinical trials. |
| **Number of products or methods** | No licensed products. |
| **Public health importance** | Cell therapies use living human cells to replace or repair damaged tissue. This novel treatment paradigm has the potential to effectively treat a wide variety of conditions. For cell therapies to have a meaningful impact on public health, they need to be scalable. PSCs’ ability to expand indefinitely removes issues around sources of primary cells such as donor availability and senescence of cultured cells. For PSCs to be impactful in addressing these challenges, they must be quality assured, readily available and transferrable across manufacturing centres. A PSC reference reagent for flow cytometry could be used to: 1. Assure the safety and quality of clinical grade PSCs as starting materials for cell therapies; 2. Allow comparison between different PSC lines or batches, increasing availability and broadening access to quality assured PSCs; 3. Assure absence of PSCs from final products, increasing product safety. |
| **Global importance** | Cell and gene therapies are now the fastest growing therapeutic area, with nine cell-based advanced therapies approved by the European Medicines Agency and thousands of active clinical trials globally. Among these are at least 54 trials of PSC-derived products. This number is set to increase with many more PSC-derived cell therapy products in pre-clinical development. High profile examples include treatments for Parkinson’s disease, affecting up to 10 million people globally (Ou et al., Frontiers in Public Health, 2021), and age-related macular degeneration, estimated to affect 170 million people (Wong et al., Lancet Global Health, 2014). |
| **Global need from regulatory & scientific considerations** | While PSC-derived cell therapies are expanding, the field is still in its infancy and has yet to result in significant public health impact. A PSC reference reagent would facilitate comparison and assure quality of clinical grade PSCs across different batches, different cell lines and different laboratories. This would enable harmonisation across different manufacturing centres worldwide, opening the door to wider global access. Availability of a reference reagent would support the regulatory advancement of PSC-derived cell therapies in the following ways: |
| ECBS outcome | [BLANK] |
|--------------|---------|

1. Through assurance of the quality of the starting material PSCs  
2. Through assurance of the absence of PSCs from the final product.

Running Title: Please add short/abbreviated name of the reference preparation along with the status, e.g. Anti-HPV-18 (1st IS) and delete this line.
| Proposal (title) | The first WHO Reference Reagent for Mesenchymal Stromal Cell Identity for Flow Cytometry |
|-----------------|--------------------------------------------------------------------------------------|
| Proposer (name of Institution) | MHRA | Principal contact | Katherine Warre Cornish |
| Rationale | There are currently over 1,000 Mesenchymal Stromal Cell (MSC) clinical trials registered for a large variety of diseases (https://clinicaltrials.gov/). However, there are no standards or reference reagents designed for MSC-based therapies. In 2006 the International Society for Cellular Therapy (ISCT) issued recommendations defining minimal criteria for MSC identity (Dominici et al., Cytotherapy 2006). These criteria include ≥95% of the cell population expressing MSC positive markers, CD73, CD90 and CD105 and ≤2% of the cell population expressing MSC negative markers, CD45, CD34, CD14, CD11b, CD19 and HLA-DR, as assessed by flow cytometry. However, a survey of manufacturers of MSCs for clinical applications (Trento et al., Blood, 2016) highlighted considerable variation in testing parameters and release criteria for MSC marker detection by flow cytometry. Such inconsistencies are likely to affect quality of the product and clinical outcomes. The need for standards and reference reagents is therefore high. The UK Stem Cell Bank conducted a successful first WHO collaborative study for a flow cytometry reference reagent for MSC identity in 2018-2019. Fixed, freeze-dried MSCs derived from human pluripotent stem cells (hPSCs) were distributed to collaborators and tested for MSC markers using in-house flow cytometry protocols. Results were highly consistent between participants and indicated expected expression of positive and negative markers, complying with ISCT identity requirements. However, with currently available facilities at the MHRA, the maximum number of vials of the hPSC-derived MSC reference reagent that can be produced is 70-100. These are largely expended in a single collaborative study. Adequate facilities to scale up production are not currently available. To address these limitations, we have carried out a study comparing hPSC-derived MSCs with MRC-5 cells – a readily expandable human fibroblast cell line, which has previously been shown to express the required profiles of MSC identity markers (Zhang et al., Vaccine, 2014). Our study indicated similar expression of MSC identity markers between MRC-5 cells and hPSC-derived MSCs, consistent across three biological replicates. These results support use of MRC-5 cells to develop an MSC reference reagent. Using MRC-5 cells increases our production capacity to batches of 500 vials. Nonetheless, a single batch of cells is unlikely to be sufficient to produce a long-lasting reference reagent, due to unknown demand and shelf life. We therefore propose to produce a master bank of MRC-5 cells, from which successive distribution banks can be generated. We propose to produce at least three such distribution banks of fixed, freeze-dried MRC-5 |
Collaborators will receive vials from each distribution bank to assess batch-to-batch variation in MSC marker expression.

**Anticipated uses and users**

We propose to generate an MRC-5 derived MSC reference reagent for flow cytometry with consistent high expression of ISCT-defined MSC identity markers.

The reagent should be used for identity assessment of MSC products for clinical applications. Use of a reference reagent would enable comparison between batches of MSCs and promote consistency in flow cytometry assessment between different laboratories, despite differing equipment, reagents, instrument settings and data analysis strategies, ultimately aiding harmonization of MSC products.

Anticipated users are developers and manufacturers of MSC clinical products.

**Source/type of materials**

Human fibroblast cell line, MRC-5, fixed and freeze-dried.

**Outline of proposed collaborative study**

Material from at least three distribution banks will be shipped to 15-20 laboratories where the material will be reconstituted following instructions provided. Collaborators will immuno-stain the material for positive and negative MSC markers and run flow cytometry using in house reagents, equipment and protocols.

**Issues raised by the proposal**

MRC-5 is a stable and well-established cell line and is likely to show similar strong expression of MSC markers between batches. Our preliminary study supported this, showing consistent expression in three biological replicates. However inter-batch variation remains a possibility. We will use our preliminary data to calculate appropriate numbers of replicates to be distributed our second collaborative study, so that batch-to-batch variation can be captured and evaluated.

**Action required**

ECBS to endorse proposal

| Proposer's project reference | Date proposed: |
|-----------------------------|----------------|
| MSC Reference Reagent       | 01/07/2022     |

**CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)**

**Approval status of medicine or in vitro diagnostic method**

Several allogenic MSC products have been licensed for use as medicines. Alofisel was approved by the European Medicines Agency in 2018 as a treatment for rectal fistulas in adults with Crohn's disease.
TEMCELL® HS Inj. was approved by the Pharmaceuticals and Medical Devices Agency in Japan in 2015 for acute graft-versus-host disease. Stempeucell® was licensed by the Drugs Controller General of India in 2017 for critical limb ischemia due to Buerger’s disease. Remestemcel-L was approved in 2019 in Canada by the Health Products and Foods Branch and in New Zealand by Medsafe for acute graft-versus-host disease in pediatric patients.

| Number of products or methods | Several allogenic MSC products have been licensed (see above). Over 1,000 clinical trials of MSC products have been registered. |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Public health importance      | The public health importance of MSCs is high. MSCs have shown promise in the treatment of a wide range of disorders for which effective therapies are currently limited. These include auto-immune diseases such as Crohn’s disease and systemic lupus erythematosus, conditions involving tissue damage such Chronic Obstructive Pulmonary Disease and diseases of uncontrolled inflammation such as graft versus host disease. |
| Global importance             | Over 1,000 clinical trials of MSC products have been registered globally (www.clinicaltrials.gov). Currently no MSC standards are available. |
| Global need from regulatory & scientific considerations | Identity assessment of cell therapies is a core requirement for products entering clinical trials. As such, developers and manufacturers of clinical MSC products are expected to demonstrate ISCT-defined MSC flow cytometry marker expression within their product. As a technique, flow cytometry is inherently difficult to standardize due to user assignment of instrument settings for data acquisition and thresholds for data analysis. Standards and reference reagents are therefore of high importance. A reference reagent for MSC identity would allow users to validate their equipment and results, providing assurance of MSC identity despite variations in user settings, equipment, reagents, manufacturing process and sources of MSCs. The provision of an MSC reference reagent for flow cytometry would thus aid in harmonizing the quality of MSC therapies. |

Running Title: 1st WHO Reference Reagent for MSCs