World Health Organization

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# EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
**Geneva, 24 to 28 August 2020**

## 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 **10 August 2020** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

## Proposed new projects

1. WHO International Standard for anti-SARS-CoV-2 antibodies
2. WHO 1st International Standard for SARS-CoV02 RNA
3. WHO 1st International viral reference panel A for adventitious virus detection by next-generation sequencing (NGS) technologies, (NIBSC)
4. WHO 1st International virus reference standard for adventitious virus detection in biological products by next-generation sequencing (NGS) technologies, (CBER)

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| | 1st WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibodies | | |
| **Proposer (name of Institution)** | NIBSC | **Principal contact** | Giada Mattiuzzo |
| **Rationale** | Severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2) previously known as novel coronavirus 2019 (nCOV-2019) is the aetiological agent of the Coronavirus Disease 2019 (COVID-19). It causes mild symptoms in the majority of cases, however ~10% cases are requiring medical intervention, with a small percentage progressing to severe pneumonia and death. The World Health Organization declared COVID-19 a Public Health Emergency of International Concern on 30th January 2020, and a Pandemic on 11th March 2020. As of 25th June 2020, there are over 9 million confirmed cases and 485,000 deaths. Serological assays are needed to understand the real impact of COVID-19, as most of the cases with mild symptoms are undetected. Urgent and rapid vaccine development is underway; currently there are 128 vaccine candidates in pre-clinical phase and 13 vaccines have entered clinical trials (see: https://www.who.int/teams/blueprint/covid-19). The scientific and clinical community requires a COVID-19 antibody standard urgently for serological assay development, evaluation of vaccine efficacy, and for epidemiological studies. Plasma or serum from convalescent patients is the preferred candidate standard as these are commutable, due to most closely representing clinical samples that are analysed in the assay. These samples have consistently been able to reduce inter-assay variability when used as a calibrant for a range of tests, as shown for many other viruses, including MERS-CoV. | | |
| **Anticipated uses and users** | Standardisation of serological assay (e.g. ELISA, neutralisation assays) for identification and/or potency test of anti-SARS-CoV-2 antibodies in:<br>Clinical and public health laboratories<br>Vaccine manufacturers - Vaccine studies<br>Therapeutic Ab producers<br>Assay Kit manufacturers<br>Research laboratories | | |
| **Source/type of materials** | Donated human serum or plasma from convalescent individuals will be preferred. The project is supported by the Coalition for Epidemic Preparedness Innovations. Donors have been identified from affected countries (UK, Norway,USA). | | |

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| | Materials issued by NIBSC will have undergone treatment steps for virus inactivation, to be shipped as non-infectious. All clinical and other samples will have undergone screening for blood-borne viruses. | | |
| **Outline of proposed collaborative study** | Collaborative study will involve 50 laboratories worldwide, performing a range of serological assays for SARS-CoV-2, and representing control laboratories, vaccines and kit manufacturers, clinical and academic laboratories.<br>The aims will be to assess the suitability of different antibody preparations to serve as the International Standard with an assigned unitage per ampoule for use in the harmonisation of SARS-CoV-2 serology assays by:<br>• characterisation of the antibody preparations in terms of reactivity/specificity in different assay systems.<br>• assessing each preparation's potency i.e. readout in a range of typical assays performed in different laboratories<br>• assessing commutability i.e. to establish the extent to which each preparation is suitable to serve as an interim standard for the variety of different samples and assay types. | | |
| **Issues raised by the proposal** | The study is taking place during COVID-19 pandemic, and logistics could be difficult. Personnel may also be an issue due to mobility restrictions and self-isolation. | | |
| **Action required** | ECBS to endorse proposal | | |
| **Proposer's project reference** | | **Date proposed:** | 25 June 2020 |
| **CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)** | | | |
| **Approval status of medicine or in vitro diagnostic method** | Several licensed drugs are under investigation to be re-purposed for COVID-19 treatment. Clinical trials are taking place.<br>On the 25th June 2020, 128 vaccine candidates have been developed and in pre-clinical phase and 13 of them are in clinical trial (see: https://www.who.int/teams/blueprint/covid-19) | | |
| **Number of products or methods** | Commercial kits and in-house assays are available; some CE-marked, EQA scheme has been organised.<br>Majority of in-house assays are neutralisation assay using either the virus in CL3 or pseudotyped systems. ELISA format is the most prevalent commercial assay format. Rapid point of care testing is also being developed. | | |

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| **Public health importance** | The World Health Organization declared COVID-19 a Public Health Emergency of International Concern on 30th January 2020, and a Pandemic on 11th March 2020. |
| **Global importance** | The World Health Organization declared COVID-19 a Public Health Emergency of International Concern on 30th January 2020, and a Pandemic on 11th March 2020. Proposed standard is essential for evaluation of vaccines and other biologicals developed for prevention and control of COVID-19 disease. |
| **Global need from regulatory & scientific considerations** | Standardised and calibrated assays are vital for accurate evaluation of treatments, including antibody therapies and vaccine, and for case management and surveillance. |
| **ECBS outcome** | [BLANK] |

Running Title: **SARS-CoV-2 antibody standard**

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| **Proposal (title)** | 1st WHO International Standard for SARS-CoV-2 RNA | | |
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| **1st WHO International Standard for SARS-CoV-2 RNA** | | | |
| **Proposer (name of Institution)** | NIBSC | **Principal contact** | Giada Mattiuzzo |
| **Rationale** | Severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2) previously known as novel coronavirus 2019 (nCOV-2019) is the aetiological agent of the Coronavirus Disease 2019 (COVID-19). It causes mild symptoms in most cases, however ~10% cases are requiring medical intervention, with a small percentage progressing to severe pneumonia and death. The World Health Organization declared COVID-19 a Public Health Emergency of International Concern on 30th January 2020, and a Pandemic on 11th March 2020. As of 25th June 2020, there are over 9 million confirmed cases and 485,000 deaths. Accurate diagnosis of the infection is essential not only for patient treatment, but also to contain the outbreak and to inform governmental quarantine and isolation procedures. The WHO has published in their technical guidance for novel coronavirus 2019 a list of in-house developed molecular assays. A common reference reagent 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. | | |
| **Anticipated uses and users** | Standardisation of diagnostic assays based on nucleic acid amplification techniques (NAT) (e.g. PCR, quantitative PCR, digital PCR, etc) for identification of SARS-CoV-2 in:<br>Clinical and public health laboratories<br>Vaccine manufacturers - Vaccine studies<br>Assay Kit manufacturers<br>Research laboratories | | |
| **Source/type of materials** | High titer of SARS-CoV-2 England/2/20 from Public Health England will be grown at NIBSC within CL3 lab and the virus in the supernatant will be inactivated by a validated treatment, with minimal disruption of the RNA.<br><br>In the study, an alternative preparation will be also included. SARS-CoV-2 RNA packaged inside lentiviral particles. These chimeric constructs will be designed to be safe, non-infectious, non-replicative. Single nucleotide mutations will be introduced in the SARS-CoV-2 sequences to prevent the production of any viral protein. This | | |

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| | approach has already been applied for the International Reference Reagent for Ebola virus RNA established by ECBS in 2015. A research reagent using the same composition was made available end of March (NIBC cat no. 19/304) and feedback has been received. | | |
| **Outline of proposed collaborative study** | Collaborative study will involve 25 laboratories worldwide, performing nucleic acid amplification technology (NAT) based assays for SARS-CoV-2, and representing control laboratories, manufacturers, clinical and academic laboratories. The aim will be to assess the suitability of different preparations to serve as the International Standard for use in the harmonisation of SARS-CoV-2 diagnostics assays by:<br>• characterisation of the candidate preparations in terms of reactivity/specificity in different assay systems.<br>• assessing each preparation's potency i.e. readout in a range of typical assays performed in different laboratories | | |
| **Issues raised by the proposal** | Validated inactivation protocol is not available and will need optimisation- may take time.<br>Commutability- the lentivirus vector (LVV) system has not been compared to the real virus. A collaborative study to include both preparations will be very useful to provide more insight into this approach. | | |
| **Action required** | ECBS to endorse proposal | | |
| **Proposer's project reference** | | **Date proposed:** | 25 June 2020 |
| **CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)** | | | |
| **Approval status of medicine or in vitro diagnostic method** | Several licensed drugs are under investigation to be re-purposed for COVID-19 treatment. Clinical trials are taking place<br>On the 25th June 2020 there are 128 vaccine candidates developed and13 of them are in clinical trials (see: https://www.who.int/teams/blueprint/covid-19).<br>12 diagnostics kits are listed in the WHO EUL; 9 are under review and 12 manufacturers have expressed interest | | |
| **Number of products or methods** | CE-marked commercial kits and in-house assays are available and have been published. Mainly these assays are based on reverse transcriptase polymerase chain reaction (RT-PCR) assay. | | |
| **Public health** | The World Health Organization declared COVID-19 a Public Health | | |

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| **importance** | Emergency of International Concern on 30th January 2020, and a Pandemic on 11th March 2020 |
| **Global importance** | The World Health Organization declared COVID-19 a Public Health Emergency of International Concern on 30th January 2020, and a Pandemic on 11th March 2020. Proposed standard is essential for evaluation of vaccines and other biologicals developed for prevention and control of COVID-19 disease. |
| **Global need from regulatory & scientific considerations** | Clinical symptoms of COVID-19 are not specific, an accurate diagnosis is essential for early identification of the disease, to respond and control outbreak. Furthermore, a reference material will assure harmonisation in the evaluation of vaccine/treatments in clinical studies. |
| **ECBS outcome** | [BLANK] |

| Virus reference panel for adventitious virus detection | | | |
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| **Proposal (title)** | *WHO international viral reference panel A for adventitious virus detection by next-generation sequencing (NGS) technologies* | | |
| **Proposer (name of Institution)** | NIBSC | **Principal contact** | Edward Mee |
| **Rationale** | Development of deep sequencing (or NGS)-based metagenomics methods offers great potential for improvements in adventitious agent detection in biological products. To realise this potential, appropriate reference materials are required to allow for optimisation and meaningful comparison of different methods. A multiplex reference material (NIBSC reference 11/242-001) has been available since 2016. Feedback from the collaborative study performed for 11/242-001 and discussions with potential end users have highlighted a number of desirable improvements. A new reference panel is proposed, with reduced complexity, improved purity and characterisation relative to 11/242-001, to facilitate optimisation and comparison of AV detection methods. | | |
| **Anticipated uses and users** | Used as a parallel or spiked-in run control, in viral metagenomic (NGS) studies aiming to detect viruses in vaccines, cell lines and other biological products.<br>End users will likely be vaccine/biological manufacturers, contract research/testing laboratories, regulatory agencies and academic laboratories. | | |
| **Source/type of materials** | Seven individual virus stocks representing a wide range of virus genome and particle properties have been sourced from donors or in-house stocks. Material are being expanded, purified and characterised in-house. The panel will comprise:<br><br>Human herpesvirus 1<br>Porcine circovirus 1<br>SV40 polyomavirus<br>Canine parvovirus 2b<br>Feline leukaemia virus<br>Simian rotavirus SA11<br>Influenza A H1N1 | | |
| **Outline of proposed collaborative study** | The material will be distributed to 6-20 participating labs who will be asked to process it using their adventitious agent detection methods. Results will be collated by NIBSC. A report summarising the viruses detected will be distributed to participants and published as a scientific article. | | |
| **Issues raised by the proposal** | | | |

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| **Action required** | ECBS to endorse proposal | | |
| **Proposer's project reference** | NIBSC VIR00105 | **Date proposed:** | 24.6.20 |
| **CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)** | | | |
| **Approval status of medicine or in vitro diagnostic method** | The proposal does not relate to a specific medicine. AV detection by deep sequencing is most obviously relevant to live viral vaccines, recombinant products produced in mammalian cell culture and cell therapies. | | |
| **Number of products or methods** | Multiple products within the categories described above. The material is designed to support NGS-based AV detection but will be suitable for use in infectivity and PCR-based assays as well as comparison of different types of assays. | | |
| **Public health importance** | Medicines produced on mammalian cell substrates or derived from human blood carry an increased risk of contamination with adventitious viruses. Traditional tests have served well but several cases (e.g. PCV contamination of Rotarix) highlight certain limitations as well as demonstrating the potential of NGS to serve as a more comprehensive test. An essential component of widespread and accurate use of NGS is the assurance of satisfactory assay performance through common reference materials. | | |
| **Global importance** | Novel vaccines, antibodies and recombinant therapeutics are in development worldwide. Improved viral safety testing will support the biologicals industry in multiple countries/regions as well as enhancing product safety for patients worldwide. | | |
| **Global need from regulatory & scientific considerations** | NGS-based detection methods are complex and involve multiple steps, each of which can significantly impact the accuracy and sensitivity. While some individual steps (e.g. nucleic acid extraction, sequencing) are partly standardised through the use of kits, overall sample-to-result methods are largely unique to an individual laboratory. The availability of common reference materials will enable meaningful comparison of results obtained using different methods and assurance to a minimum standard. The proposed material will also support wider efforts to implement NGS-based testing as an alternative to animal testing for adventitious virus detection (the requirement for suitable reference materials having been highlighted in Ph. Eur. general texts 5.2.14).<br><br>The proposed panel, or a traceable derivative material, may also serve as a proficiency testing panel once the technology becomes established in testing laboratories. | | |

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| **ECBS outcome** | [BLANK] |

| Virus reference standards for adventitious virus detection by NGS | | | |
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| **Proposal (title)** | WHO 1st international virus reference standards for adventitious virus detection in biological products by next-generation sequencing (NGS) technologies | | |
| **Proposer (name of Institution)** | CBER | **Principal contact** | Arifa S. Khan |
| **Rationale** | NGS is recognized by regulators and industry as a potential rapid, alternative assay for replacing or supplementing the currently recommended adventitious virus detection assays, which are not standardized and need ≥ 30-days to obtain results. Additionally, replacement of the *in vivo* assays can facilitate achieving the global objective of reducing animal use. However, to implementation of NGS for adventitious virus testing of biologics requires standardization and validation of NGS. Therefore, reference reagents are needed to demonstrate the sensitivity, specificity and reproducibility of the key steps in NGS: sample processing, cDNA synthesis, library preparation, sequencing, and bioinformatics analysis. Live viruses, representing diverse virus families relevant to potential virus contamination in biologics, could aid in evaluating the entire NGS workflow. | | |
| **Anticipated uses and users** | Anticipated users include regulatory scientists, manufacturers (vaccines, gene therapies, and biotherapeutics), contract research organizations (CROs), and academia. Use of virus reference reagents can expedite NGS standardization and validation to facilitate its implementation as a rapid alternative method to replace or supplement currently used adventitious virus detection assays. Use of shared virus standards can internationally harmonize regulatory review of NGS data submitted by different sponsors. Live, virus reagents can evaluate the entire NGS workflow for adventitious virus detection, from sample preparation through bioinformatics. The reference virus reagents are being made available to all NGS users for workflow and platform evaluation and for method standardization and validation. | | |
| **Source/type of materials** | Five viruses were selected to represent families with distinct physical and chemical properties and different types of genome structures for demonstrating NGS capabilities for broad adventitious virus detection. The viruses and cell lines for propagation were available at the American Type Culture Collection (ATCC), where large-scale virus stocks were prepared to meet the criteria for infectious titer and genome copy number. Stocks were tested for sterility, mycoplasma, and were characterized by ATCC, by outside commercial labs, and by CBER. Each virus stock was individually vialed and is distributed with an ATCC Certificate of Analysis. The 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 Thielen, and porcine circovirus type 1. | | |
| **Outline of proposed collaborative study** | A collaborative study is near-completion, which involves 8 international laboratories, including vaccine manufacturers, CROs, NIBSC, and CBER/FDA performing spiking studies to evaluate detection of the 5 reference virus stocks using independent protocols, sequencing platforms, | | |

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| | and bioinformatics pipelines. All of the study participants used the 5 virus stocks to spike at different concentrations into a background of a high-titer virus to mimick testing of a viral vaccine seed for detection of adventitious virus contamination. Two spiked concentrations were used by all of the labs and additional ones were included by some. The data is currently being discussed and will be prepared for publication (expected in late 2020). | | |
| **Issues raised by the proposal** | N/A | | |
| **Action required** | ECBS to endorse proposal | | |
| **Proposer's project reference** | | **Date proposed:** | May 12, 2020 |
| **CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES** | | | |
| **Approval status of medicine or in vitro diagnostic method** | NGS emerged as a new technology in 2007 and is still evolving; however, much progress has been made for demonstrating its capabilities for broad virus detection in biologics, including novel viruses. NGS has been extensively been used as a research tool and also as an investigational tool for applications in biologics. Currently, there is much interest by industry and regulatory authorities for use of NGS as an alternative method for the conventional assays for adventitious virus detection, particularly the in vivo animal assays which will reduce use of animals in testing. Furthermore, NGS can aid 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, NGS can serve as a single broad virus detection assay to replace the multitude of virus-specific PCR assays. There have been individual efforts by industry for developing reference materials (viruses and databases) for in-house standardization and even validation. However, common virus standards need to be developed and used internationally to generate confident NGS data for adventitious virus detection in biologics and to harmonize regulatory review. | | |
| **Number of products or methods** | NGS for adventitious virus detection is encouraged in the EP and WHO documents and is currently being accepted on a case-by-case basis in the US FDA. | | |
| **Public health importance** | The detection of PCV1 in the licensed Rotarix vaccine demonstrated the limitations of the currently used virus detection assays and highlighted the importance of NGS for broad virus detection to industry and regulators. The discovery of a novel rhabdovirus in Sf9 cells further demonstrated the potential of NGS for detection of unknown viruses for assuring product safety and enhancing public health. Availability of international reference virus standards for implementation of NGS as a rapid adventitious virus detection assay can aid in accelerating new vaccines against the current COVID-19 pandemic. | | |
| **Global importance** | Global availability of international virus standards to facilitate NGS implementation as an alternative assay for rapid and broad adventitious virus testing can help address the current COVID-19 pandemic by | | |

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| | accelerating the development and enhancing the safety of SARS-Cov-2 vaccines, since the current in vivo and in vitro adventitious virus detection assays can take at least 28-days and in some cases cannot be performed due to interference by the vaccine virus. Furthermore, use of NGS to replace the in vivo adventitious virus detection assays will reduce use of animals and help reach the 3Rs objectives globally. |
| **Global need from regulatory & scientific considerations** | The availability of international reference virus standards can help establish a universal set of reagents to globally harmonize NGS testing for adventitious virus detection in biologics. Live, virus standards can be used to demonstrate performance of the entire NGS workflow from sample preparation to bioinformatics for virus detection. The use of NGS virus standards can: 1) assure product safety for known and unknown adventitious viruses; 2) reduce 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 confident results since the current in vivo animal assays and in vitro cell culture assays are subject to biological variability and assay performance and interpretation can only be done by trained individuals; 4) including NGS data using common standards across industry can aid in consistent review across regulatory agencies, which may harmonize development of guidelines internationally for using NGS for adventitious virus detection in biologics; 5) availability of international NGS virus standards can facilitate NGS implementation as an alternative rapid assay for adventitious virus detection, thereby accelerating development of vaccines against emerging and re-emerging diseases |
| **ECBS outcome** | [BLANK] |