WHO perspectives on the use of HTS for detection of adventitious agents in biologicals

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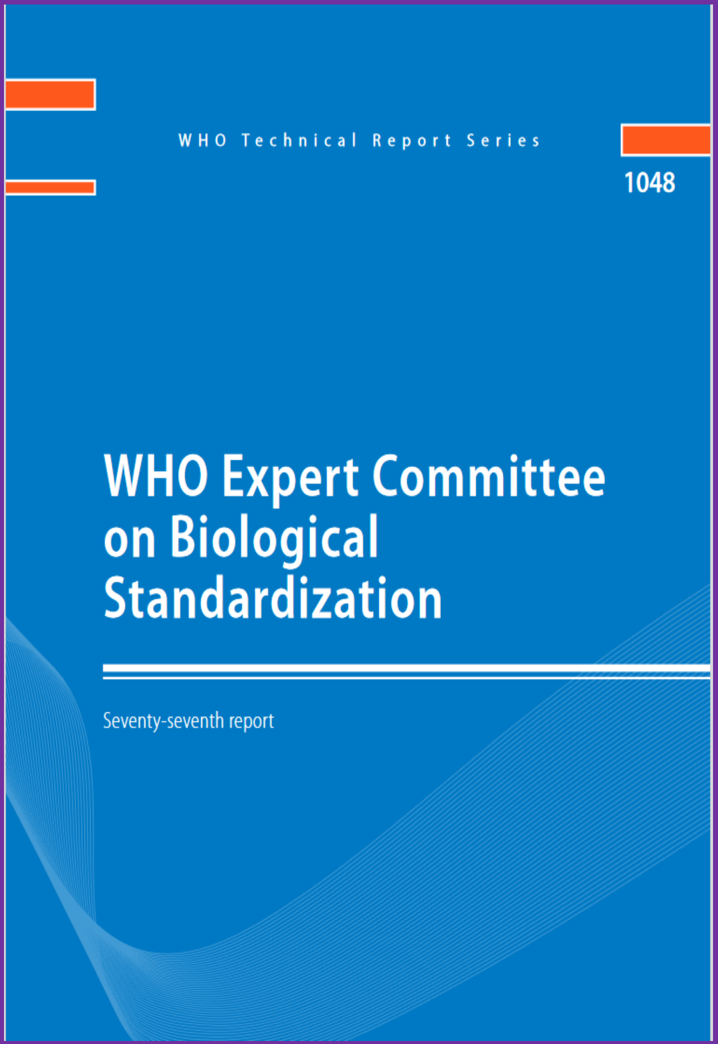
Outline of the presentation

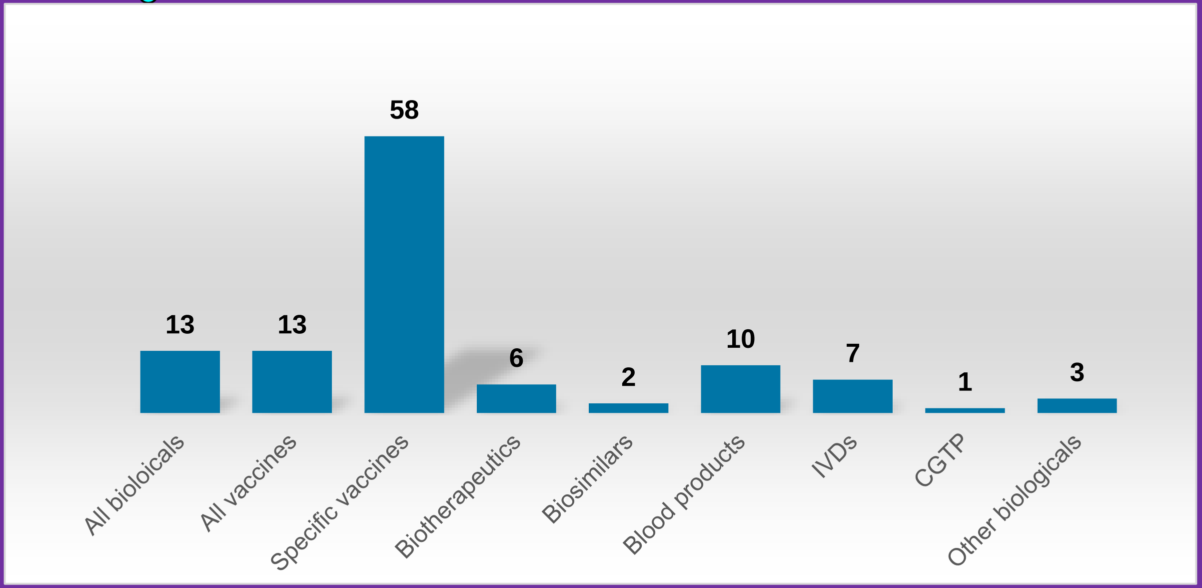
- WHO standards for biological products
  - written standards
  - measurement standards
- Expert Committee on Biological Standardization
- Standards for high throughput sequencing
- Feedback from regulators on HTS for detection of adventitious agents
- Forthcoming events

WHO norms and standards for biologicals

Global written standards (113)

https://www.who.int/groups/expert-committee-on-biological-standardization





Global measurement standards (more than 500)

Measurement standards: essential elements for development, licensing and lot release

Scientific evidence

1) Standardization of assays
2) Further development and refinement of QC tests
3) Scientific basis for setting specifications

Concept of WHO Guidelines

1) Provide key principles for evaluation of biologicals as a basis for setting national requirements;

2) Leave space to NRAs to formulate additional/ more specific requirements;

3) Living document that will be developed further in line with the progress in scientific knowledge and experience

4) Assist with the implementation of the guidelines into regulatory and manufacturers practice through:

- Global, regional and national workshops involving regulators, manufacturers and other relevant experts
- Trainings, advisory groups

5) Consider guidance issued by other bodies – intention to complement them, not to create a conflict.

Science based regulation

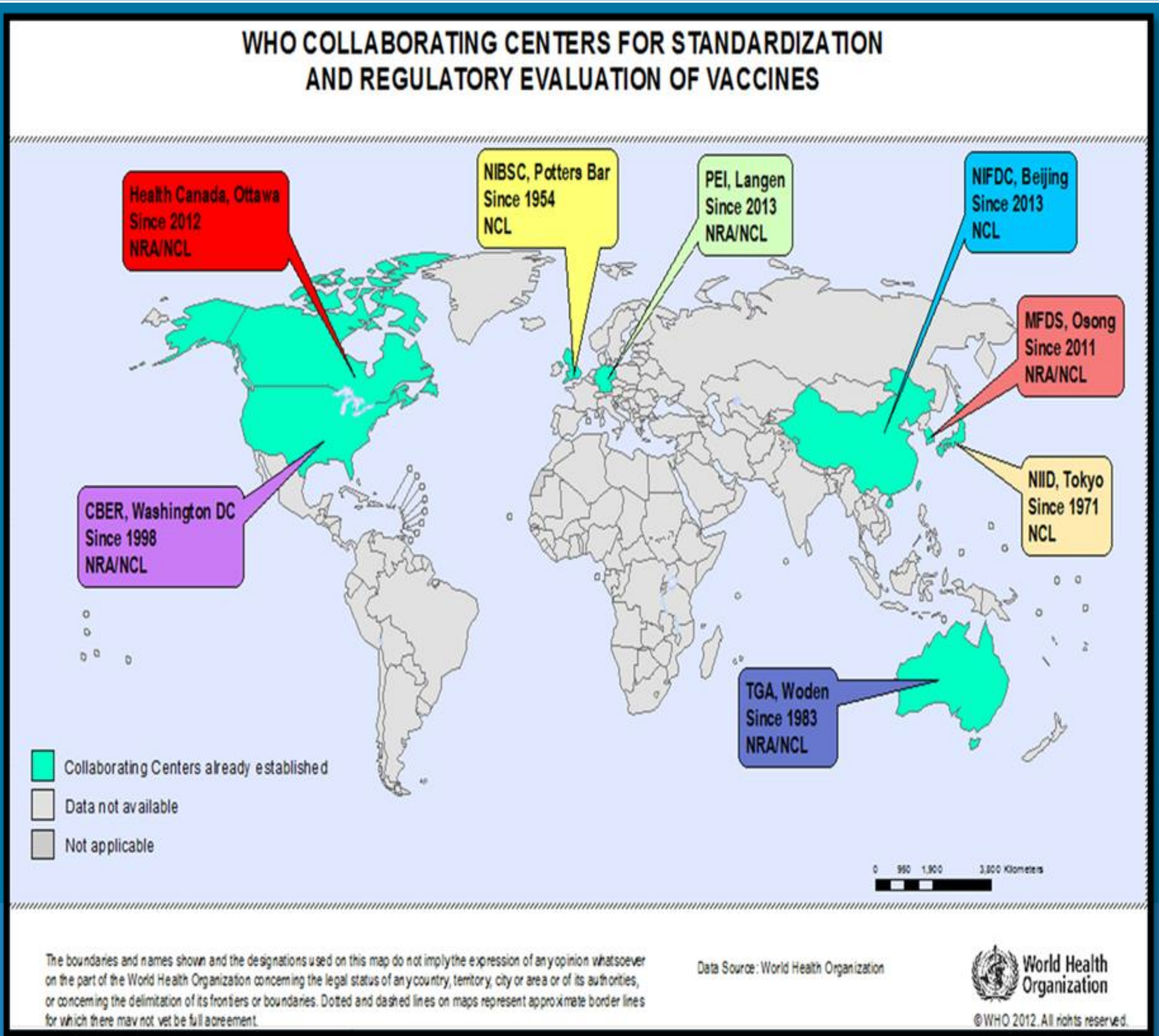
WHO CCs and Custodian Laboratories for Biological Standardization

- **Input provided to the following:**
  - Measurement standards: MHRA (NIBSC) with the input from CCs and other laboratories
    - Vaccines and related substances
    - Biotherapeutics
    - Blood products and related substances
    - In vitro diagnostics
    - Cell and gene therapies
    - PHE (eg, SARS-CoV-2)
    - high-throughput sequencing standards
  - Written standards
  - Implementation workshops

- ***CBER re-designation completed in February 2024***
- ***Health Canada re-designation completed in Oct 2024***

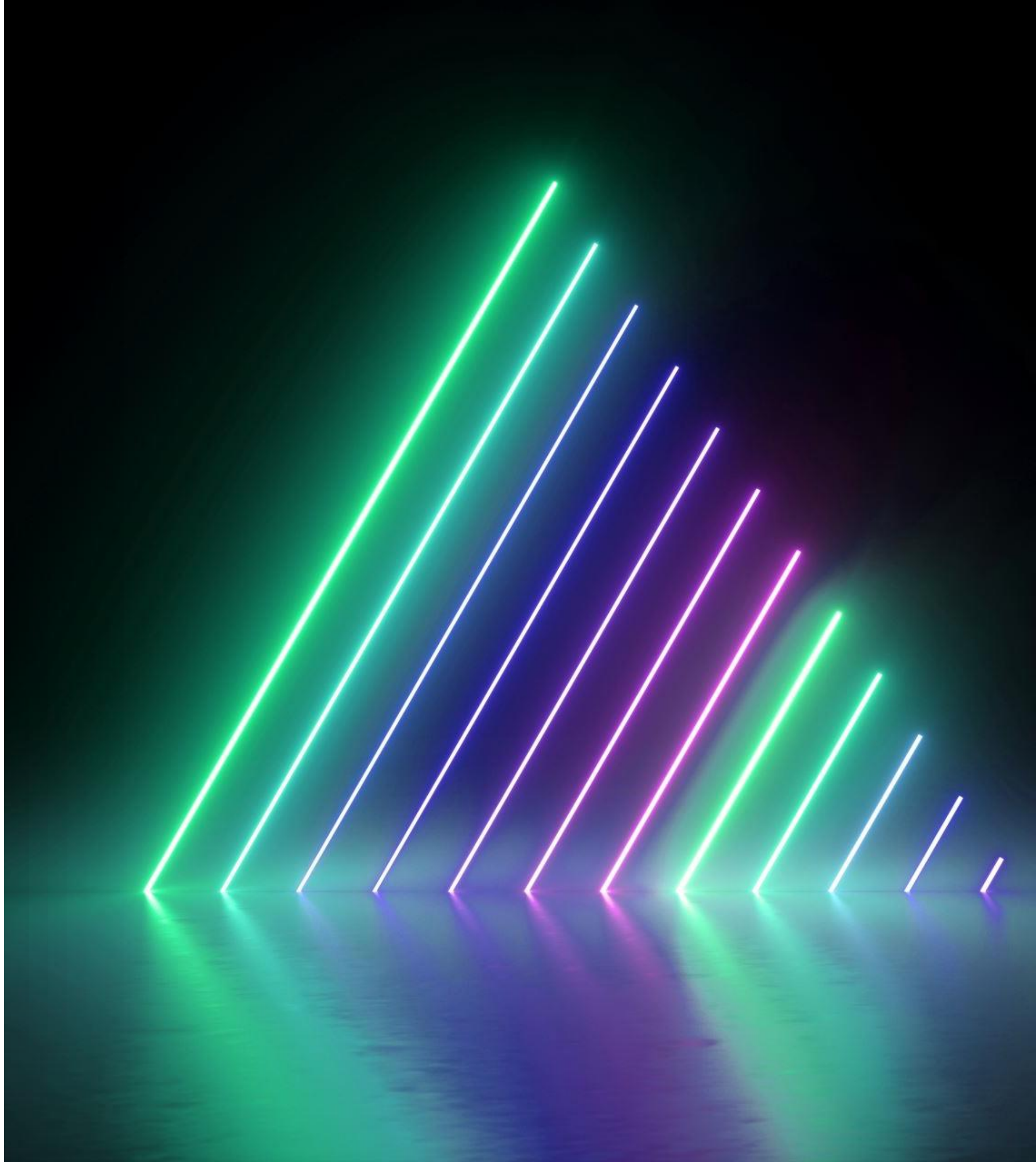
- **WHO custodian laboratories for measurement standards:**

1) MHRA (NIBSC), 2) PEI, 3) CBER/US FDA and
4) EDQM (antibiotics)



New methods in WHO Recommendations for Cell Substrates WHO TRS 978, 2010 (1)

"New, sensitive, molecular methods, with broad detection capabilities are being developed. These are not yet in routine use, but as they become widely available and validated, they will play an increasing role in the evaluation of cell substrates. The sensitivity of these methods as well as their breadth of detection should be considered when evaluating their applicability. One of the advantages of some of these new methods is that they have the potential to discover new viruses. These new approaches involve either degenerate PCR for whole virus families or random-priming methods, which do not depend on a known sequence. Analysis of the resulting amplicons has employed sequencing, hybridization to oligonucleotide arrays, and mass spectrometry [102, 103, 104]. The new generation of massively parallel (deep) sequencing (MPS) methods may have particular utility. They can be applied to detect virions after nuclease treatment to remove cellular DNA and unencapsidated genomes. Used in this mode, MPS has been used to discover new viruses in serum and other tissues and has revealed the contamination of human vaccines by porcine circovirus [102, 105, 106, 107, 108, 109]."



New methods in WHO Recommendations for Cell Substrates WHO TRS 978, 2010 (2)

"MPS can also be employed to screen cell substrates for both latent and lytic viruses by sequencing the transcriptome. In this mode, enormous quantities of data are generated, and robust bioinformatic methods are required to detect viral sequences by either positive selection against viral databases or negative selection to remove cellular sequences [102, 109, 110].

Care is required to exclude false “hits” to viruses due to recognition of transduced cellular sequences present in some viral genomes or due to viral genes like virokines that have a close homology to cellular genes.[102, 104, 110] It is probable that application of methods of this type will be expected or required by regulatory agencies in future. At present they have not been evaluated for sensitivity and specificity and should be thought of as powerful investigational tools that can reveal issues to be explored by more established methods."

Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine (WHO TRS 993, Annex 2)

FINDING OF AN AA HAS BEEN OF CONCERN TO REGULATORY AGENCIES, MANUFACTURERS AND PUBLIC HEALTH OFFICIALS SINCE THE EARLY 1900S

SIGNAL DETECTED FOR A POTENTIAL AA AS A CONTAMINANT OF A MARKETED PRODUCT; THE MOST RECENT IN 2010

REQUESTS TO WHO TO PROVIDE GUIDANCE TO COUNTRIES ON THE DEVELOPMENT AND IMPLEMENTATION OF REGULATORY RISK-EVALUATION STRATEGIES DURING THE POST-MARKETING PERIOD

IN 2013, DOCUMENT WAS ADOPTED BY THE ECBS AND PUBLISHED IN TRS 993, ANNEX 2

IN 2014, REVIEW PAPER WITH 4 CASE STUDIES PUBLISHED IN BIOLOGICALS

79th ECBS meeting held on 11-14 March 2024

1. Executive Summary published on WHO web site on 20 March 2024:

Page link:

https://www.who.int/publications/m/item/79th-ecbs-meeting-march-2024

Document link:

https://www.who.int/publications/m/item/79th-ecbs-meeting-march-2024

2. ECBS report (TRS 1059) published on 5 August 2024 on WHO web site

Main outcomes of 79th ECBS meeting (11-14 March 2024)

World Health Organization

Table 1
WHO international reference materials established by the ECBS in March 2024

Material	Unitage	Status
Biotherapeutics other than blood products		
Golimumab	500 IU/ampoule TNF neutralizing activity 500 IU/ampoule of TNF binding activity 500 IU/ampoule of FcγRIII binding activity 500 IU/ampoule of ADCC activity 50 µg/ampoule for therapeutic drug monitoring	First WHO International Standard
In vitro diagnostics		
HIV-1 p24 antigen	44 IU/ampoule	First WHO International Standard
Lassa virus RNA for NAT-based assays Lineages II, III, V and VII	No unitage assigned	First WHO International Reference Panel
Standards for use in high-throughput sequencing technologies		
Adventitious virus detection in biological products using HTS technologies	CBER-FSCUST-90 (hCoV) 2.6 x 10^{10} genome copies/mL CBER-FSCUST-91 (PCV1) 8.1 x 10^{9} genome copies/mL CBER-FSCUST-92 (REO) 1.5 x 10^{10} genome copies/mL CBER-FSCUST-93 (FeLV) 4.0 x 10^{10} genome copies/mL CBER-FSCUST-94 (EBV) 2.8 x 10^{7} genome copies/mL CBER-FSCUST-95 (RSV) 5.5 x 10^{10} genome copies/mL CBER-FSCUST-96 (MVM) 1.2 x 10^{10} genome copies/mL	First WHO International Reference Panel
Vaccines and related substances		
Diphtheria antitoxin for use in flocculation test (equine)	No unitage assigned	WHO International Reference Reagent

Table 2
WHO international reference materials proposed for discontinuation in March 2024

Material	Rationale	Proposed discontinuation pathway
First WHO International Standard for calcitonin, ASU 1–7 eel calcitonin analogue (elcatonin) **NIBSC code 84/614**	Low, and highly geographically restricted, demand	Decision by the ECBS in October 2024 based on the outcomes of stakeholder consultation and feedback
First WHO International Standard for human C-reactive protein **NIBSC code 85/506**	Low demand as a result of limited adoption of the assigned IU	Decision by the ECBS in October 2024 based on the outcomes of stakeholder consultation and feedback
WHO international reference reagents for adventitious virus detection by high-throughput sequencing Porcine circovirus type 1 **CBER code SC-VR-6000P** Mammalian orthoreovirus type 1 **CBER code SC-VR-6001P** Feline leukaemia virus **CBER code: SC-VR-6002P** Human respiratory syncytial virus **CBER code: SC-VR-6003P** Epstein-Barr virus **CBER code: SC-VR-6004P**	Superseded by the establishment of the First WHO International Reference Panel (see Table 1 above)	Recommendation by the ECBS to establish the reference panel at the current meeting resulted in immediate discontinuation of all five WHO international reference reagents and their reassignment as CBER reagents for research purposes only

First WHO International reference panel – WHO TRS 1059

World Health Organization

- **At 79th ECBS meeting in March 2024, first WHO international reference panel for adventitious virus detection in biological products using HTS was established. This reference panel including 7 viruses superseded a set of 5 individual virus preparations established in 2020 as WHO international reference reagents for adventitious virus detection by HTS, which will be discontinued in line with the proposed succession plan.**

7. International reference materials – standards for use in high-throughput sequencing technologies

7.1 WHO international reference standards for use in high-throughput sequencing technologies

7.1.1 First WHO International Reference Panel for adventitious virus detection in biological products by high-throughput sequencing

HTS technologies have the ability to detect both known and novel adventitious viruses in biological products. As an alternative to conventional adventitious virus detection, which relies on in vivo animal testing and in vitro cell culture assays or on polymerase chain reaction (PCR) assays, HTS has the potential to expand the breadth of virus detection while also significantly shortening the time required for the quality control testing of biological products. Importantly, the increasing implementation of HTS for this purpose aligns with, and provides support for, the shift towards reducing the use of animals in such testing. Encouraged by regulatory guidance worldwide, the need for international viral standards for HTS process qualification and validation to support the application of HTS to biological product virus safety testing has long been recognized.

An international collaborative study involving nine laboratories in six countries had therefore been conducted to evaluate the suitability of seven viruses representing diverse virus families for use as a potential WHO international reference panel for adventitious virus detection in biological products by HTS. The following viruses had been selected based on their distinct physicochemical and genome properties:

- human betacoronavirus OC43 (hCoV)
- porcine circovirus type 1 (PCV1)
- mammalian orthoreovirus type 1 (REO)
- feline leukemia virus (FeLV)
- Epstein-Barr virus (EBV)
- human respiratory syncytial virus (RSV)
- minute virus of mice (MVM).

Feedback from regulators on HTS status

- Questions sent to regulators in August 2024, aiming to:
  - Understand the current situation regarding the use of High-Throughput-Sequencing (HTS) technologies in regulatory evaluation of biological products
  - Identify the area where technical support is required for use of HTS
  - Expectations regarding the amendment to WHO recommendations on cell substrates: TRS 978 Annex 3 and TRS 993 Annex 2

- 16 NRAs responded:

AFR	AMR	EMR	EUR	SEAR	WPR
Ghana South Africa	Brazil Canada* USA*	Egypt Tunisia	Germany* UK*	Bangladesh India Indonesia	Australia* China* Japan* Korea*

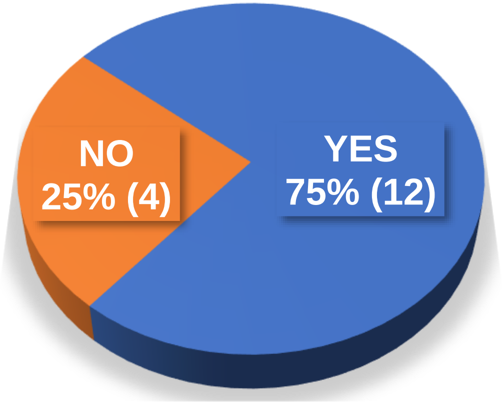
* WHO Collaborating Centres for Biological Standardization (8)

- The results shown in this presentation are based on the answers.

Alternative use of HTS

Q1. Does your country accept HTS as an alternative to existing in vitro and in vivo adventitious virus detection methods for viral safety test of biological products in marketing authorization and/or clinical trials?

- India
- Japan
- Korea
- South Africa



- Australia
- Bangladesh
- Brazil
- Canada
- China
- Egypt
- Ghana
- Germany
- Indonesia
- Tunisia
- UK
- USA

- Countries have **already adopted HTS as an alternative** or **in the process of adopting/discussing** its adoption.
- When accepted HTS as an alternative,
  - **Most countries**: alternative for both in vitro and in vivo assays
  - **China**: alternative for in vivo assay only
  - **Ghana**: alternative for in vitro assay only

  (Tunisia: details not provided)

Challenges for not accepting HTS

Q2. If you answered ‘No’ to question 1, what is the most challenging reason for not accepting HTS for viral safety test?

India	• Lack of WHO documents with request for mandatory HTS testing for AAs detection • No validated methods for HTS
Japan	• Lack of experience and/or performance in country
Korea	• Lack of regulations and/or guidelines • Lack of experience and/or performance in country • NGS technology and criteria for assessing the validity of HTS-based methods (DNA/library quality, data quality etc.) have not yet been fully standardized. For cell and gene therapy products, validating HTS methods is challenging due to limitations such as interference from genomic nucleic acids (DNA and RNA) present in samples. • HTS data is considered supplementary
South Africa	• Not been adopted as policy – remains under discussion

HTS in Biological products

Q3. If you answered ‘Yes’ to question 1, which products has your country authorized the use of HTS for viral safety test?

Vaccines only	Vaccines, Biotherapeutics	Vaccines, CGTPs	Vaccines, Biotherapeutics, CGTPs
• China (for other biologicals, no approval so far) • USA • Indonesia (Poliovirus type2/nOPV2)	• Tunisia	• Germany (for biotherapeutics, no application so far)	• Australia, • Bangladesh • Brazil (no approval so far) • Canada • Egypt • UK

*Ghana: no application so far

HTS in Manufacturing step(s)

Q4. In which step(s) of product manufacturing does your country accept the use of HTS for viral safety testing?

a) Cell lines and other raw materials	b) Appropriate step(s) in the process of the production	c) Final products
Bangladesh, India	Japan, Indonesia (nOPV)	
Australia, Brazil, China, Ghana, Germany	Australia, Brazil, China, Ghana, Germany	
Canada, Egypt, Tunisia, UK, USA	Canada, Egypt, Tunisia, UK, USA	Canada, Egypt, Tunisia, UK, USA

*No response: Korea, South Africa

Rationale for use of HTS

Q5. By which rationale does your country accept HTS?

Q6. If you selected a) or b), please specify the relevant regulations or guidelines

a) International guidelines (e.g. WHO, ICH, etc)	b) National/regional regulations or guidelines (e.g. Pharmacopoeia, etc.)	c) Submitted method validation report of HTS by manufacturer
Egypt (ICH)		Indonesia, Tunisia, USA
Brazil (ICH, Ph Eur), Japan (ICH, National regulation[1)]), Korea (ICH, National guideline[2)])	Brazil (ICH, Ph Eur), Japan (ICH, National regulation[1)]), Korea (ICH, National guideline[2)])	
Australia (ICH), Bangladesh, Canada (ICH, Ph Eur, WHO), China[3)] (ICH, Ph Eur), Ghana (ICH, WHO), Germany, India (ICH, WHO), South Africa (under discussion), UK (ICH, Ph Eur)	Australia (ICH), Bangladesh, Canada (ICH, Ph Eur, WHO), China[3)] (ICH, Ph Eur), Ghana (ICH, WHO), Germany, India (ICH, WHO), South Africa (under discussion), UK (ICH, Ph Eur)	Australia (ICH), Bangladesh, Canada (ICH, Ph Eur, WHO), China[3)] (ICH, Ph Eur), Ghana (ICH, WHO), Germany, India (ICH, WHO), South Africa (under discussion), UK (ICH, Ph Eur)

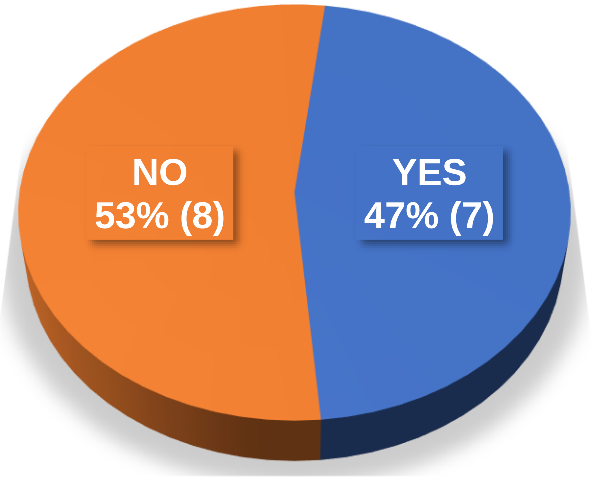
1) Japan: Minimum requirements for biological products
2) Korea: National guideline will be updated by December 2024
3) China: HTS is recommended in Chinses Pharmacopoeia 2025 version - Requirements for Animals Used for the Production of Biologics

Consideration of WHO recommendations for HTS

Q7. Regarding the acceptance or suitability of HTS for adventitious virus detection in the evaluation of biological products, did you consider the WHO recommendations provided in the following documents?

- 'Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks' (TRS 978 Annex 3)
- 'Scientific principles for regulatory risk evaluation on finding an adventitious agent in a marketed vaccine' (TRS 993 Annex 2)

- Australia
- Bangladesh
- Brazil
- Germany
- Korea (biotherapeutics, gene therapy products)
- Tunisia
- UK
- USA



- Canada
- China
- Egypt
- India
- Indonesia
- Japan
- South Africa

***Ghana:** Not applicable as HTS for viral safety evaluation has not been submitted. Upon submission, Ghana FDA will reference WHO recommendations along with other relevant guidelines.

Need for updates to WHO recommendations on adventitious virus detection

Q8. If you answered 'Yes' to question 7, please provide your feedback regarding the need for updating or elaborating more on adventitious virus detection.

- Five countries responded that the WHO documents **need to be updated or elaborated:**

China	• Include a detailed description of the use of HTS method and a new method appendix with a high-quality reference viral genome database	*Answered 'Yes' to Q7
Egypt	• Add a comparative table describing the pros and cons of each method, indicating relevant and feasible methods for each manufacturing step and for different types of raw material. • Describe the state of art methods used.	
India	• Revise to align with ICH Q5A(R2) and provide information about the reference panel. • Develop sequence database and bioinformatics tool.	
Indonesia	• Updated guidelines on viral safety test using HTS	
USA	• Update the WHO document to align using HTS for adventitious virus testing with other current ones (e.g. ICH Q5A(R2), new HTS chapter from EDQM 2.6.41.)	Answered 'No' to Q7

* Japan - immediate update is not necessary; South Africa - under discussion; Canada - No response

World Health Organization

WHO international reference panel

Q9. Are you aware that the first WHO international reference panel for adventitious virus detection by HTS is available ?

- First WHO International Reference Panel (WHO_TRS_No 1059, 2024 March)

Material	Unitage	Virus
CBER-FSCUST-90 (hCoV)	2.6 x 10^{10} genome copies/mL	human betacoronavirus OC43
CBER-FSCUST-91 (PCV1)	8.1 x 10^{9} genome copies/mL	porcine circovirus type 1
CBER-FSCUST-92 (REO)	1.5 x 10^{10} genome copies/mL	mammalian orthoreovirus type 1
CBER-FSCUST-93 (FeLV)	4.0 x 10^{10} genome copies/mL	feline leukemia virus
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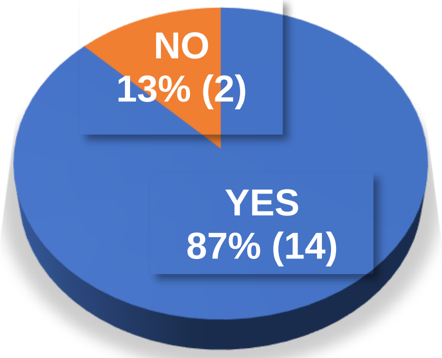
CBER-FSCUST-95 (RSV)	5.5 x 10^{10} genome copies/mL	human respiratory syncytial virus
CBER-FSCUST-96 (MVM)	1.2 x 10^{10} genome copies/mL	minute virus of mice

- Australia
- Tunisia



- Most countries (87%) are aware of the first WHO Internation Reference Panel

- Available at BEI repository: Catalogue number NR-59630 (https://www.niaid.nih.gov/research/bei-resources-repository)

WHO events in 2024 and 2025

- 80th meeting of the Expert Committee on Biological Standardization (ECBS): 7-11 October 2024, Geneva
- 19th International Conference of Drug Regulatory Authorities: 14 - 18 October 2024, New Delhi, India
- WHO Drafting Group meeting to discuss revision of Recommendations for the preparation, characterization, and establishment of international and other biological reference standards, 24-26 February 2025, WHO, Geneva, Switzerland
- Informal consultation on WHO Guideline on the phasing out animal tests for the quality control of biological products, 27-28 February 2025, WHO, Geneva, Switzerland – Document is subject of public consultation from 2 December 2024 to 10 January 2025 **New**
- Implementation workshop on cell and gene therapy products – April 2025
- Implementation workshop on the evaluation of biosimilars – July 2025
- 81st meeting of the Expert Committee on Biological Standardization (ECBS): 13-17 October 2025, Geneva

Special thanks to colleagues who provided feedback to the questionnaire on HTS

- Ramkishan Ajmeer (CDSCO, India)
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- Johannes Bluemel (PEI, Germany)
- Juliati Dahlan (Indonesian FDA, Indonesia)
- Delese Mimi Darko, Rita Osekre (Ghana FDA, Ghana)
- Dennis Dowhan (TGA, Australia)
- Asmaa Fouad, Hadeer Mohamed El-Sayed Abosalem (EDA, Egypt)
- Janet Glassford, Chris Burns (MHRA, UK)
- Koji Ishii (NIID, Japan)
- Arifa Khan (US FDA, USA)
- Quinton Meyer, James Southern (SAHPRA, South Africa)
- Seung-Rel Ryu (MFDS, Korea)
- Chittra Sarkar (NDCL, Bangladesh)
- Silmara Silveira (ANVISA, Brazil)
- Michael Wall (Health Canada, Canada)
- Miao Xu (NIFDC, China)

Many thanks to…:

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…members of WHO drafting and Working Groups

…colleagues from Collaborating Centers and Custodian Laboratories

…many individual experts

…stakeholders

Further information and contact

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Biological standardization website: Expert Committee on Biological Standardization (who.int)

Dr Eun Kyung Kim (eunkim@who.int) – for further information on HTS