Medicines & Healthcare products Regulatory Agency

NIBSC
Confidence in Biological Medicines

Best Practices for the Use of International Standards in Vaccine Testing

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DCVMN Regional Training Workshop Hyderabad, 07 -10 May, 2018

Biologicals

"substances which cannot be fully characterized by physicochemical means alone, and which therefore require the use of some form of bioassay"

- The includes, but is not restricted to proteins, antigens, vaccines, antisera, blood products and nucleic acids.

Biological

- Complex composition
- Requires biological or immunological assay for characterization.
- The assays are usually comparative rather than absolute
- A reference standard is critical in defining the **qualitative nature** or **relative magnitude** of the biological or immunological response

WHO Biological Reference Standards

- classed as “biological”
- Developed to enable the results of biological assay or immunological assays to be expressed in the same way throughout the world
- i.e. Global Assay harmonisation

WHO Nomenclature for Biological References

- International Standard (IS)
- International Reference Reagent (IRR)
- Secondary Standard or other Reference Material

WHO International Standard (IS)

- Highest order of reference for biological materials and medicines
- Project initiation is endorsed by WHO ECBS
- Quantifies “relative potency” in a specific but arbitrarily defined International Unit (IU)
- Allows direct comparison between different assays and methodologies
- Controls all steps of the assay, ideally
- Behaves in similar way to the clinical or biological material under test

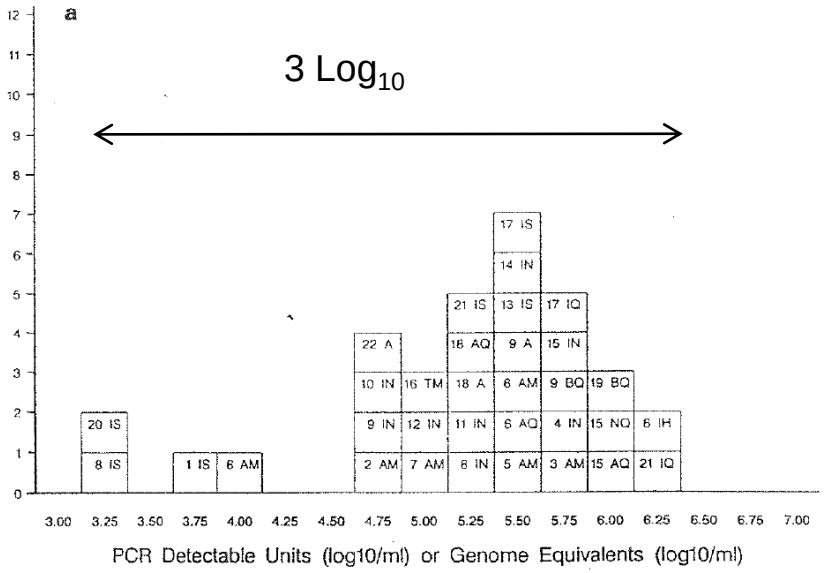
WHO International Standard (IS)

- Freeze-dried for long-term stability and shipment at ambient temperature

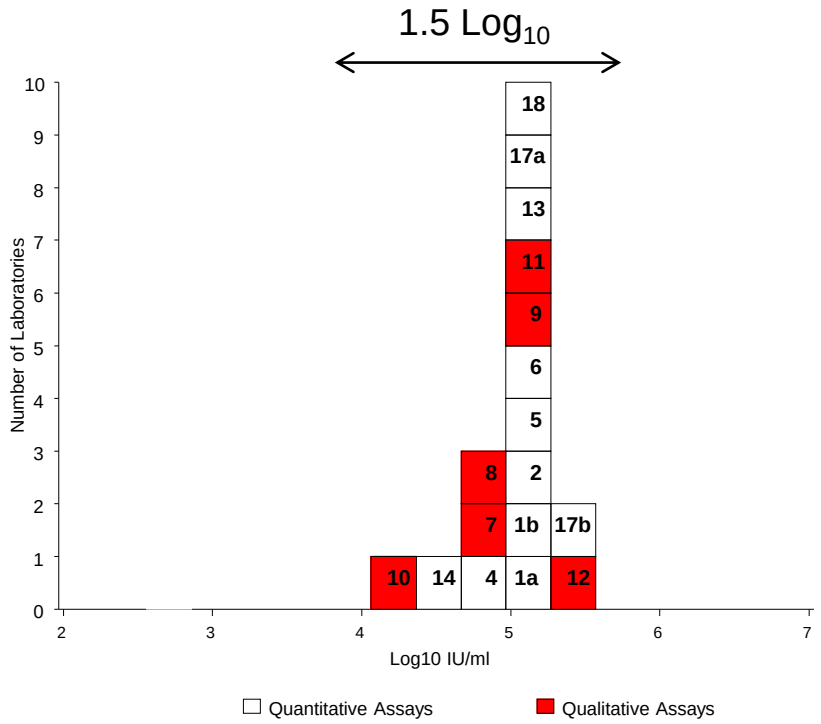
WHO International Standards

Example of assay harmonisation over time

1st HCV RNA NAT study (copies/ml) 1996



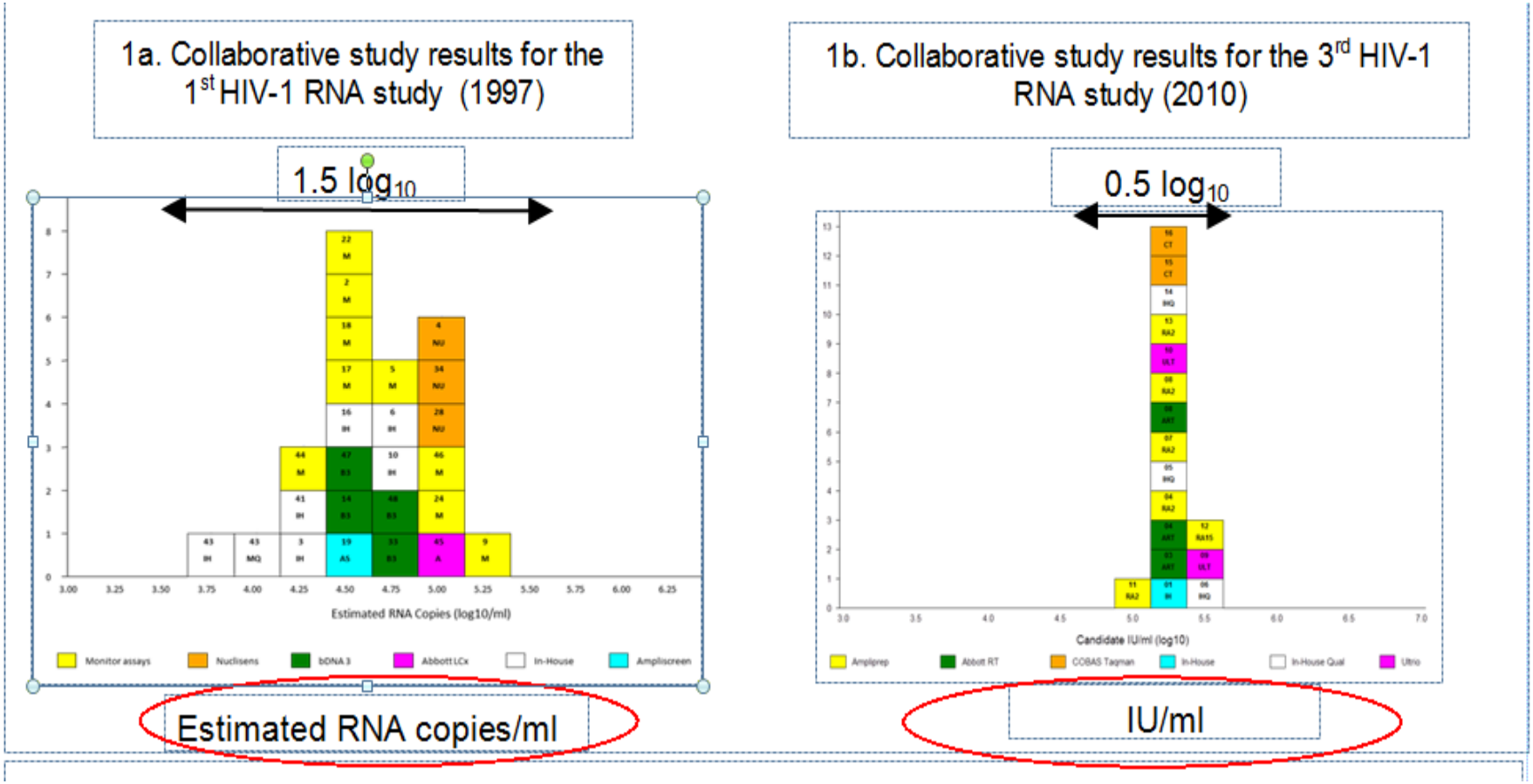
5th HCV RNA NAT study (IU/ml) 2011



Example courtesy of Clare Morris, Virology NIBSC

WHO International Standards & Assays Can Evolve

Example



Based on subtype B virus

Example courtesy of Clare Morris, Virology NIBSC

WHO International Reference Reagent (IRR)

- WHO biological reference standard, the activity of which is defined by WHO in terms of a “unit”
- This category of reference standard is intended to be interim and replacement of the reference reagents may not envisaged.
- May not fully meet the requirements to become an IS, but serves to be useful in assay standardisation and control

WHO International Reference Reagent (IRR)

IRR Examples

- Anti-HPV 16 was initially established by ECBS as an IRR (10 “units” per ampoule) until additional stability studies could be performed.
  It was subsequently established as an IS with 10 IU per ampoule
- JE antibody preparation- No unitage assigned
- EBOV RNA IRRs - freeze-dried synthetic preparations assigned “units” but development needed for long-term stability
- anti-EBOV antibody IRR – limited numbers of frozen aliquots. This served as the interim standard while we continued to establish the anti-EBOV antibody IS (Now in the catalogue).

Secondary Standard or other Reference Material

- A reference material that has been directly calibrated against the WHO International Standard and is itself assigned a unitage in IU
- A secondary standard may be used in the calibration of tertiary reference materials (calibration and validation of assay systems)
- e.g. assay calibrators, in-run controls, proficiency study samples
- The IU is traceable back to the International Standard and carries with it an uncertainty of measurement.

WHO Publications on the development and establishment of biological reference preparations…..

WHO Biological Reference Standards

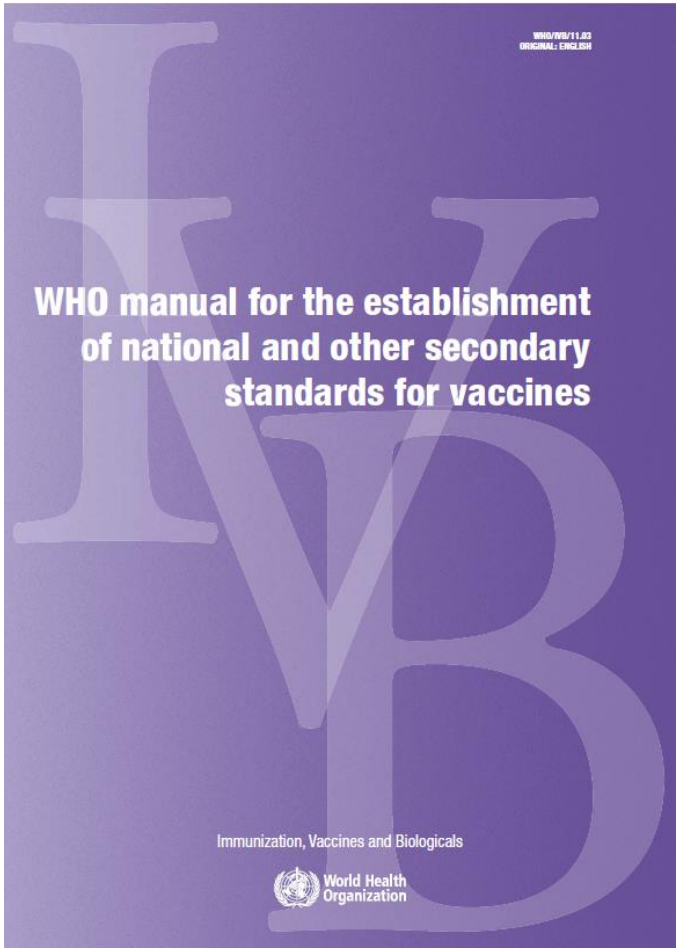
http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf?ua=1

WHO Technical Report Series, No. 932, 2006

Annex 2

Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004)

WHO Manual for the establishment of national and other secondary standards for vaccines WHO/IVB/11.03



Planning

Material selection and processing

Quality aspects

Calibration against current IS

Statistical analysis Stability

Collaborative study report and other documents

Storage and stability monitoring

Custodian laboratory responsibilities

Labelling

Instructions for use Dispatch of standards

Planning for batch replacement

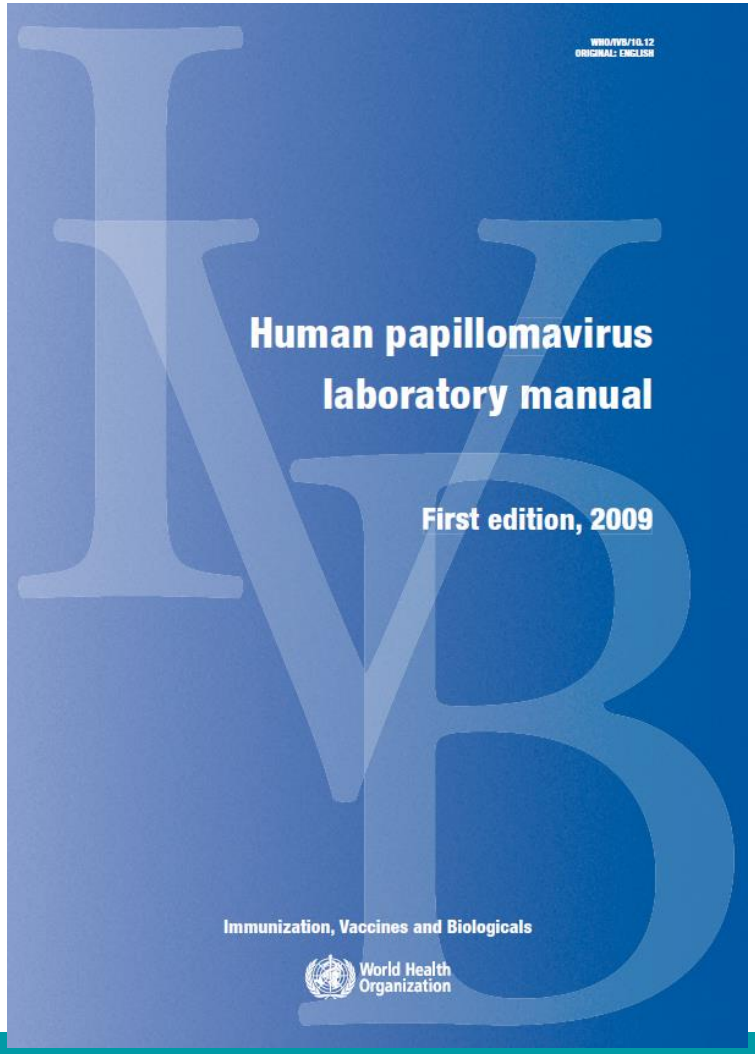
http://apps.who.int/iris/bitstream/handle/10665/70669/WHO_IVB_11.03_eng.pdf;jsessionid=1605A0D549785A3CC95B8650B7ED58DD?sequence=1

WHO manual for the preparation of secondary reference materials for in vitro diagnostic assays designed for infectious disease nucleic acid or antigen detection: calibration to WHO International Standards

WHO Expert Committee on Biological Standardization Sixty-seventh report

http://www.who.int/bloodproducts/norms/SecStandManWHO_TRS_1004_web_Annex_6.pdf?ua=1

Calibration of Secondary Standards for HPV assays



Chapter 9

9. International standards and secondary standards

9.5 Preparation of secondary standards for HPV antibodies and their calibration in IU

http://www.who.int/immunization/hpv/learn/hpv_laboratory_manual__who_ivb_2009_2010.pdf

9.4 Preparation of secondary standards for HPV DNA and their calibration in IU

Calibration of Secondary Standards for Specific Vaccines

World Health Organization

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Biologicals

- Biologicals
- Vaccines
- Biotherapeutics
- Reference preparations
- Publications
- About
- Blood products and related biologicals

Vaccine Standardization

The World Health Organization brings together international experts in specific fields through its biological standardization programme to develop and revise specific recommendations for the production and quality control of vaccines of major international public health importance. Authoritative, harmonized guidelines and recommendations, for use by manufacturers and regulatory authorities, are published in the reports of ECBS meetings in the WHO Technical Report Series.

http://www.who.int/biologicals/vaccines/en/

ECBS Reports for Collaborative Studies

WHO International Biological Reference Preparations

Held and Distributed by the WHO International Laboratories for Biological Standards

PREPARATION	STANDARD	MATERIAL	HELD AT	CODE	WHO/BS DOCUMENT
A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs 13 (ADAMTS13), plasma, Lyophilized., Function: 0.91 IU/ampoule Antigen: 0.92 IU/ampoule	1st International Standard, 2014	Blood products and related substances	NIBSC	12/252	2014.2246
Acellular pertussis vaccine for potency assay by modified mouse challenge test, Lyophilized, 34 IU / ampoule	1st International Standard, 2008	Vaccine	NIBSC	JNIH-3	08.2086
Activated coagulation factor XI, Lyophilized., 9.8 IU/ampoule	1st International Standard, 2014	Blood products and related substances	NIBSC	13/100	2014.2245
Activin A, human, recombinant, Lyophilized, 5 units / ampoule.	1st Reference Reagent, 1998	Recombinant hormone	NIBSC	91/626	98.1882
Alpha-1-antitrypsin, (2008), plasma derived, Lyophilized, 243	1st International Standard, 2008	Purified plasma protein	NIBSC	05/162	08.2092

http://www.who.int/bloodproducts/catalogue/Alph2017.pdf?ua=1

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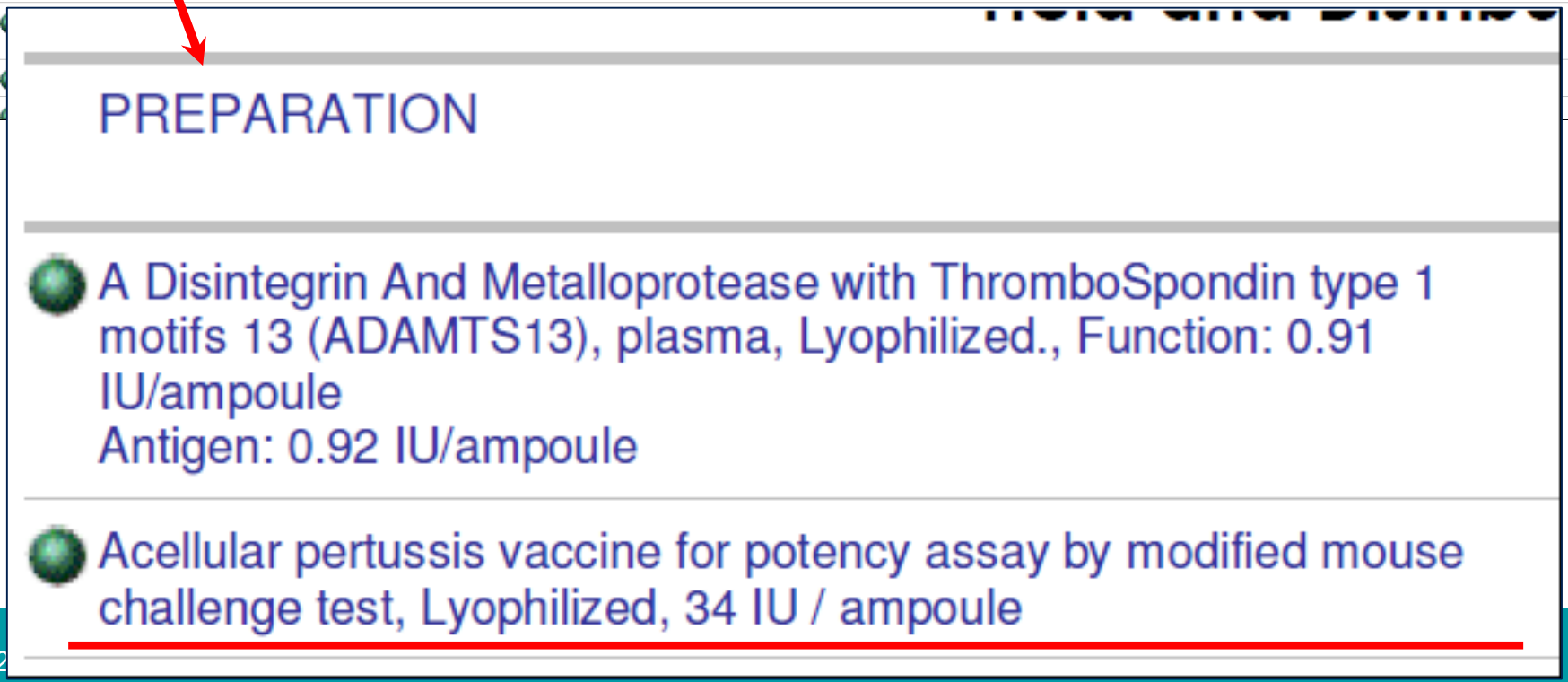

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STANDARD	MATERIAL
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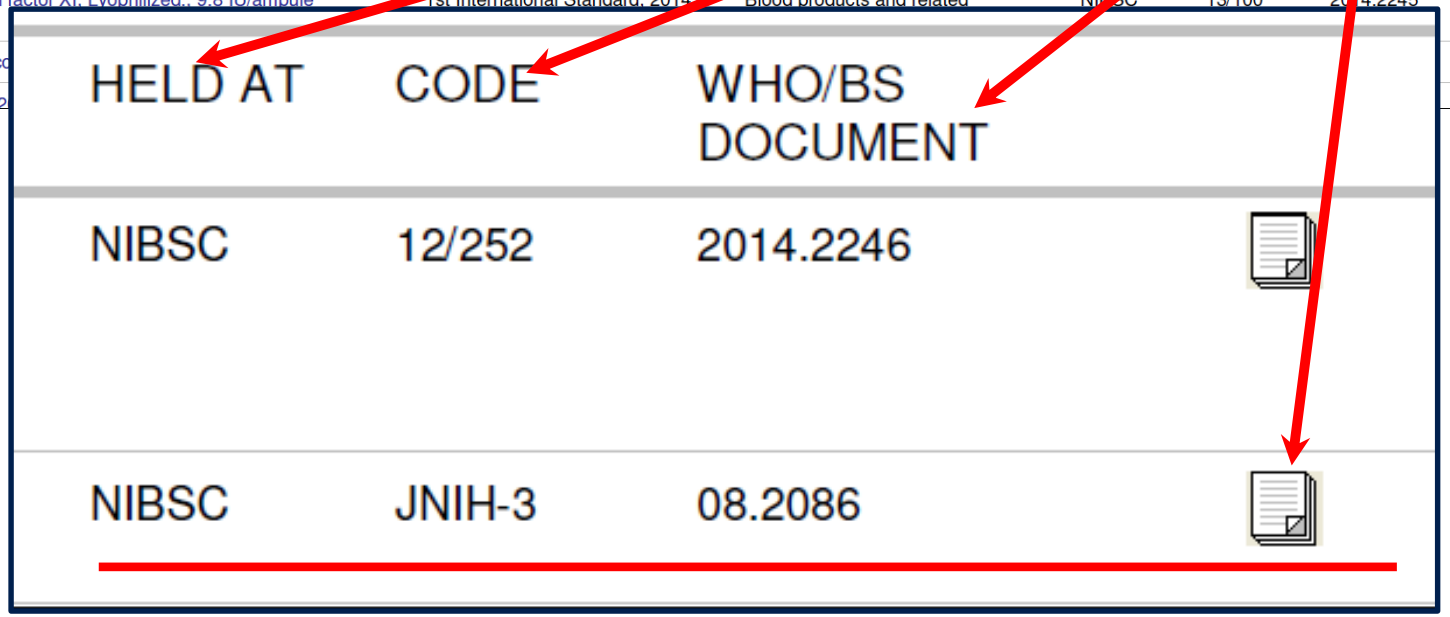
Activated coagulation factor XI, Lyophilized., 9.8 IU/ampoule	1st International Standard, 2014	Blood products and related	NIBSC	13/100	2014.2245
Activin A, human, recc					
Alpha-1-antitrypsin, (2					



WHO International Standard (IS)

- Are quantified in International Units (IU's) assigned following a **multicentre collaborative study** using **multiple assays**
- Study Report presented to WHO ECBS
- ECBS endorses the establishment of the IS
- Supply should last 10-20 years
- Intended for calibration of secondary references

Calibration of Secondary Standards

- Secondary Standards may be prepared and calibrated at the
  Regional level
  National level
  Multi-centre level
  Within a single lab
- Batch may be large or small, but should be large enough to avoid frequent replacement
- Freeze-dried, frozen liquid, liquid but should be suitably stable
- Should follow the principles (If not the stringent requirements) of establishing WHO reference preparations
- Commutable

Calibration of Secondary Standards

A protocol should be prepared and agreed

The design of the study should consider

- representation of all assays that will be using the 2^{ndary} standard
- repeat testing using fresh samples
- simultaneous testing of all materials within each assay
- replicates of at least one sample should be included to enable estimation of the within-assay variability
- Choice of diluent(s) (matrix effects) and dilution series
- Calibration software- e.g. Parallel line analysis. Requires statistical support

Calibration of Secondary Standards

Flow chart of process

(annex A WHO/IVB/11.03)

Planning – e.g. calculation of amount of material required based on anticipated usage; deciding on assay methods; gathering details of prospective participants

⬇

Selection of source vaccine(s)

⬇

(pre-fill) **Characterization** of source bulk vaccine(s). Performed by one or a few labs (e.g. the donor of the material)
If there are multiple candidate vaccines, then each candidate should be characterised. One or more candidate vaccines may subsequently be selected for the collaborative study

⬇

Processing of final container (e.g. filling vials or ampoules with known precision, freeze-drying if applicable). Multiple candidates may need to be prepared.

⬇

Continued

Calibration of Secondary Standards

Flow chart of process

(annex A WHO/IVB/11.03)
Continued

(Pre-study) Characterization of candidate secondary standard(s) e.g. appearance and potency – to ensure that the candidate(s) is/are suitable for evaluation

⬇

Calibration against IS
(i.e. collaborative study or validation if on a smaller scale)

⬇

Statistical analysis

⬇

Evaluation of estimated **stability** (if undertaken)

⬇

Preparation of collaborative **study report**
(or **validation report** if smaller scale study)

⬇

Establishment as reference standard

⬇

Preparation of **Instructions for use** and **Distribution** e.g. cold chain

Calibration of Secondary Standards

Flow chart of process

(annex A WHO/IVB/11.03)

Continued

↓

Consideration of how the Batch will be replaced

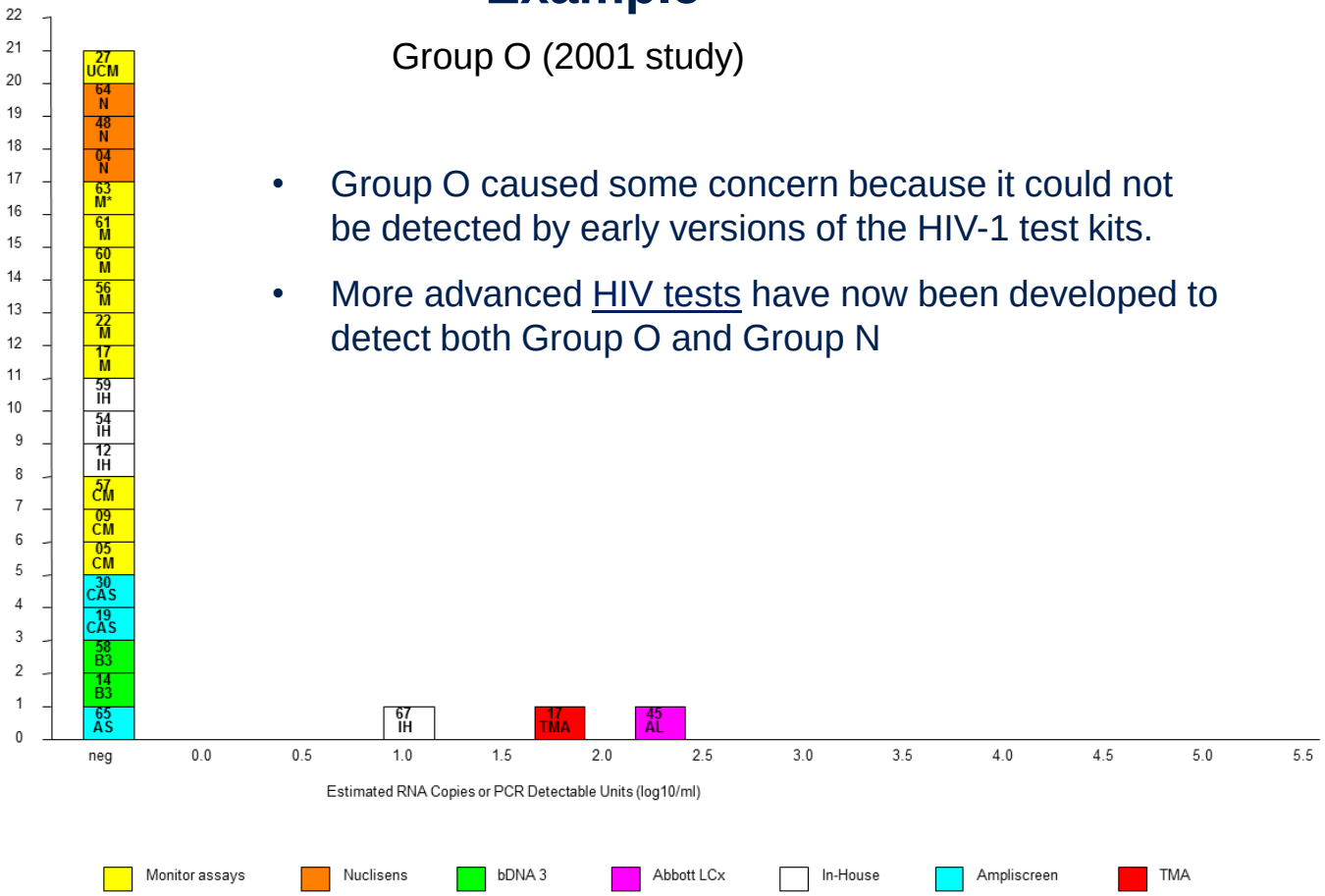
Some Issues and Difficulties in Biological Standardisation

Issues and Difficulties

Beware of the variants

Example

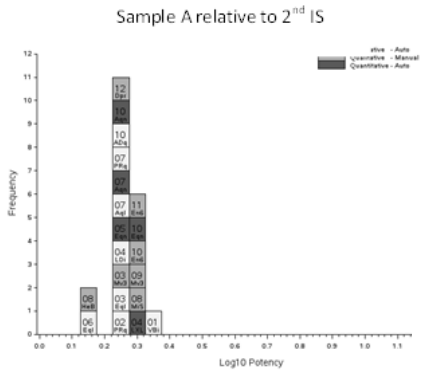
Group O (2001 study)

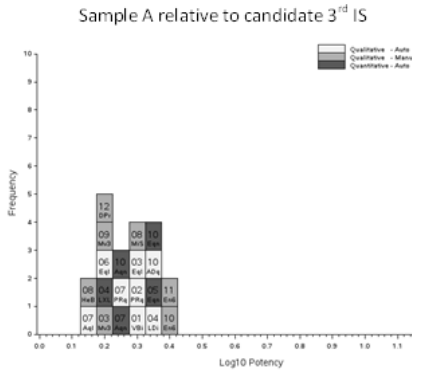


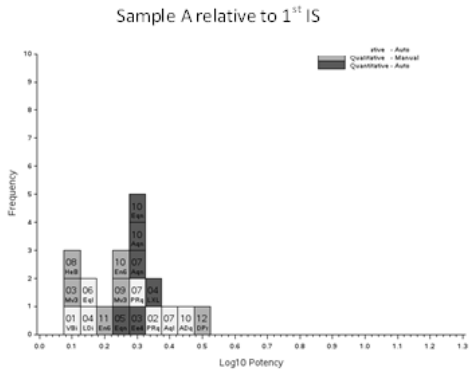
- Group O caused some concern because it could not be detected by early versions of the HIV-1 test kits.
- More advanced HIV tests have now been developed to detect both Group O and Group N

Example courtesy of Clare Morris, Virology NIBSC

Issues and Difficulties







Beware of the variants

Example HBsAg

Table 10. Overall mean estimates (IU/mL) for potency and inter-laboratory variation (%GCV) for samples A-D relative to the 2nd IS, the candidate 3rd IS or the 1st IS.

Reference		2nd IS (33 IU/mL)		Candidate 3rd IS (50 IU/mL)		1st IS (100 IU/mL)	
Sample	No. of assays	Overall GM	Overall % GCV	Overall GM	Overall % GCV	Overall GM	Overall % GCV
A	56	1.8	11	1.9	18	1.9	29
C	63	46.2	15	47.2	10	46.8	37
D	60	80.9	24	83.1	23	80.2	44
E	63	105.2	29	107.9	34	106.2	39

Abbreviations: GM = Geometric mean; %GCV = Geometric coefficient of variation.

- Some genotypic differences observed
- Importance of multiple assay representation
- WHO HBsAg genotype panel is available (PEI)

Issues and Difficulties

Reference vaccines may be product specific e.g. HepB and HPV vaccines

i.e. It may not be suitable or possible to establish an International Standard for all vaccines for a given pathogen

Issues and Difficulties

Cases where there is no International Standard for a given vaccine

It may be necessary to assign an "other" unit to the secondary standard until an IS is established.

Issues and Difficulties

Commutability

Issues and Difficulties

Lack of source material

E.g. Rabies IgG replacement material

WHO Collaborative Study to establish the 7th IS for Rabies Vaccine

UPDATE

WHO Collaborative Study to establish the 7th IS for Rabies Vaccine

- **15 participants from 13 countries**

  Argentina Belgium Canada France (2)
  Germany India (2) Mexico Russia
  Serbia S Africa Thailand UK USA

- **25 data sets returned on NIH (10), ELISA (8), SRD (6) and serology (1) assays**
- **Study samples included**

  6th IS as calibrant
  Coded duplicates of the candidate 7th IS
  Coded liquid bulk (for in *in vitro* assays only)
  5th IS (limited numbers were available for NIH)

WHO Collaborative Study to establish the 7th IS for Rabies Vaccine

- **Data analysis is underway**
- **Draft report to be sent to participants for comments**
- **Stability study is underway**
- **Study report to be submitted to WHO ECBS in October 2018**
- **~10,000 ampoules have been produced**

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THANK YOU

Dianna Wilkinson

DCVMN Regional Training Workshop Hyderabad,

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