Selection, preparation and calibration of secondary reference materials

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Secondary reference materials

- Development and establishment of a working guide for the preparation and calibration of secondary reference materials against the WHO international standards (IVD area).

- Proposal endorsed by ECBS in Oct 2012
WHO International Standard (IS) materials are highest order standards for the calibration of in vitro measurement procedures used for diagnosis, detection and management of infectious diseases.

These preparations play a critical role in the standardization, harmonization and quality control of diagnostic procedures and patient management.

WHO ISs are fundamental for the regulation of in vitro diagnostic (IVD) tests in the blood safety area and have enabled standardized treatment approaches.

Limited availability of WHO IS preparations.

These materials are intended to be used for calibration of secondary standards.
Secondary standards defined as
- Regional or national reference preparations or
- Working reference materials used routinely by laboratories

Used
- For validation of blood screening and diagnostic assays
- As run controls
- For batch verification of IVD products
Typical analytes for IVD assays

- Antigens, or nucleic acids of infectious agents
- Antibodies directed against infectious agents

- In contrast to purely chemical substances, there is no reference method for the measurement of these analytes of biological origin.

- To ensure the comparison of test systems, the biological standards have been assigned with arbitrary units, i.e. International Units (IU)

- Correspondingly the values assigned to secondary reference materials have also to be defined in units traceable to the higher order IS, i.e. in IU.
**IS vs secondary standards**

- Detailed procedures for the production of ISs have been defined:
  - These high-level requirements for an international biological reference material may apply to secondary biological standards, too
  - There are important differences between ISs and secondary standards which may require modifications to the approach taken for ISs

Commutability

- Important point
- A prerequisite for the calibration of a potential candidate material is that this material will be recognized by a wide range of tests and test platforms with nearly the same efficiency.
- If the material shows a significant inter-method variability in the concentration, it is not suitable as a reference material.
- Potential reference materials, including secondary standards, should be representative of the performance with samples validated for the assays.
General aims of the project

- To create a technical document which describes the principles to be considered in the preparation of secondary standards
- Due to the complexity of the development of secondary standard preparations, practical guidance will facilitate their appropriate design, manufacture and use
- Contribute to the global harmonization and quality assurance of IVDs
IVD tests

- Complex biological assays
- Principles for the preparation and calibration of secondary standards are specific and based on the assay or type of biological standard
The following biological assay types will be considered:

- **NAT-based tests** for the detection of DNA or RNA of infectious agents
- **Immunoassays** for the detection of antigens or antibodies of infectious agents

And …

- **Wide range of test systems** are available
- **Tests represent different technologies and test platforms** and may be either commercial or, e.g. for some NAT tests, in-house assays
Selection, preparation and calibration of secondary standards

- Design of secondary standards
- Specific points to be considered for analyte types (e.g. antibody, antigen, nucleic acid) or pathogens (e.g. virus, bacteria)
- Selection and initial characterization of suitable source material for secondary standards
  - Properties, feasibility and traceability
  - Commutability of biological standard materials
- Preparation / processing of secondary standards
- Assay calibration
  - Testing of the candidate secondary material in parallel with the higher order standard
  - Statistical considerations and data analysis
- Storage and stability testing
- Traceability records
Secondary standards – issues to be considered

- Candidate materials for secondary standards should be tested in at least two additional tests, preferentially assays with different test platforms.

- If candidate material will be lyophilized it must be ensured that the freeze-drying procedure have no influence on the integrity of the target region.

- If candidate material will be inactivated it must be ensured that the inactivation procedure have no influence on the integrity of the target region.
Preparation of secondary standards

- The candidate material can be diluted if necessary
  - Dilution matrix must be characterized

- Should be aliquoted into volumes appropriate for single use

- Screw-capped vials should be used

- Labeling
  - name of the material
  - assigned code number
  - storage temperature
  - material is “not for use in humans”
  - expiry date
Secondary standard calibration - NAT

- Qual assay
  - End-point dilution alongside with the WHO IS
  - Calculation of the concentration by statistical analysis (e.g. probit analysis)
  - Other option could be digital PCR

- Quant assay
  - Parallel line assay alongside with the WHO IS
  - 3 dilution points / replicates
Secondary standard calibration – antigen and antibody assays (1)

Test for antigen detection

- One target protein/peptide

Tests for the detection of antibodies against

- One target protein
- Multiple target proteins
  - Each serum/plasma contains antibodies against a range of proteins and epitopes
  - Every serum/plasma contains these antibodies in different proportions
  - Every form of assay will detect antibodies in the same sera in different proportions
  - The results of all immunoassays are highly dependent on the quality and type of antigens used
Secondary standard calibration – antigen and antibody assays (2)

- **Qual**
  - Dilution alongside with the WHO IS around the assay cut-off (estimating end-point)
  - Statistical analysis

- **Quant**
  - IS is assigned in IU
  - Parallel line assay alongside with the WHO IS
  - 3 dilution points / replicates
Specific aims of the guideline project

- Cooperation between the WHO collaborating centres (NIBSC, CBER and PEI), experts in the field and IVD manufacturers

- WHO collaborating centres’ meeting (April 2013, Geneva)
  - Presentation of the project
  - Evaluation of current procedures for the preparation and calibration of secondary standards
  - Evaluation of the commutability issue and approaches to ensure the suitability of secondary standard materials

- Consultations (to be defined)
- To draft the working guide (PEI; Q2/2014)
- To discuss the working guide (circulation between WHO collaborating centres, experts and IVD manufacturers, Q3/2014)
- To finalize the working guide and submission to ECBS (Jul 2014)
Guideline project

- Any suggestions, contributions, comments and remarks are welcome
- Please contact Michael.Chudy.@pei.de