WHO Biological Standards
And
Genetic Reference Materials

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Genetic Reference Materials
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WHO-
Biological Standardisation Programme

- International Biological Standardization initiated under League of Nations in 1920s - Commission on Biological Standardization

- Part of the constitution of WHO (1946)
WHO-Biological Standardisation Programme

- Develops recommendations (requirements) and guidelines on the production and control of specific biologicals; latest revision - Nov 2004 (Written standards)

- Develops and establishes International Biological Standards and Reference Reagents (Ampouled materials)
Establishment of WHO Standards

• WHO International Laboratories for Biological Standards/ Collaborating Centres

• Biologicals Unit (Quality & Safety of Biologicals/Quality & Safety of Plasma Derivatives) (Secretariat)

• Expert Committee on Biological Standardization, ECBS (1st meeting 1947)
WHO Laboratories and Collaborating Centres

- *National Institute for Biological Standards and Control, Potters Bar, UK (NIBSC)
- *Sanquin-Central Laboratory Netherlands Red Cross Blood Transfusion Service (CLB)
- Center for Biologics Evaluation and Research FDA, Bethesda, USA (CBER)
- Paul Ehrlich Institute (PEI)

* Hold and distribute international standards
Stakeholders/Collaborators

- Other standard setting bodies e.g. Council of Europe/European Department for the Quality of Medicines: USP/International Standards Organization (ISO)
- National Regulatory Authorities/National Control Laboratories
- Scientific Societies/Associations
- Manufacturers Associations (International Federation of Pharmaceutical Manufacturers Associations)
WHO Guidelines

- Quality assurance
  - Quality management system: GLP, GMP, ISO
  - Archive of Documents related to
    - Procedures and tests performed before, during and after filling into containers
    - Stability studies
    - Collaborative studies
    - Storage, inventory and dispatch of the reference material

- Instruction for use and safety data sheets
  - Mean fill volume + CV
  - Residual moisture and oxygen
  - Recommended storage temperature and time
Quality of WHO IS

- With a few exceptions, international standards usually in heat-sealed glass ampoules
  - No exchange of gases and moisture
  - Greater stability over time
- Precision of fill - < 0.25%; 0.05 -0.07% achieved
- Majority are freeze-dried, filled with inert gas before sealing
- Secondary desiccation (for some materials)
- Residual moisture: <1%; <0.05% with secondary desiccation
- Residual oxygen content: <45 µmol/L
Quality of WHO IS

- No declared shelf life
- Stability monitoring:
  - accelerated degradation study at elevated temperatures
    - ampoules stored at +4, +20, +37, +45 and +56°C
    - At various time-points, activities compared with -150°C ampoules
    - Fit in Arrhenius equation for prediction of loss of activity
  - real time degradation monitoring
    - Ampoules at storage temperature (-20°C) against ampoules kept at ultra-low temperatures (-150°C)
Route for Establishment of WHO Biological International Standards and Reference Preparations

- Collaborative study: multi-centre; multi-methods
- Value assignment base on consensus results
- Obtain participants comments and approval on the analysis of data and recommendations
- For some materials, they are reviewed and approved by appropriate expert/professional organisations eg International Society for Thrombosis and Haemostasis (ISTH)
- Final review and establishment by the Expert Committee on Biological Standardisation (ECBS)
WHO Reference Materials

- **International Standards**
  - enable the activity of biological preparations to be expressed in the same way globally
  - mostly in International Units (IUs)

- **Reference Reagents**
  - differ from International Standards in the extent of characterisation and intended use
  - not assigned IU’s
  - Interim

- **International Reference Panels**
  - Group of reference materials established to collectively aid evaluation of assays or diagnostic tests.
  - Comply with requirements for WHO reference standards/reagents
International Standards and Reference Panels

• Intended Use
  - Primary standards and references
  - Promote global harmonisation and consistency of quality and safety of biological medicines and related in vitro biological diagnostic tests

• EU Directive on In Vitro Diagnostics
  - Conventional reference materials of higher order eg International Standard for Hepatitis B (calibrated in IU) as the standard required to fulfil EC Common Technical Document

NIBSC
Ensuring the quality of biological medicines
WHO Laboratories and Collaborating Centres

• *National Institute for Biological Standards and Control, Potters Bar, UK (NIBSC)
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* Hold and distribute international standards
NIBSC - Centre for Biological Reference Materials (CBRM)

- >95% of WHO International Standards/Reference Reagents
- >550 Catalogue items
- 60,000 ampoules/vials distributed p.a. to >60 countries
- New “state of the art” facility
  - Category 3 containment
  - Freeze drying development capability

- AIDS Reagent Repository: 1500 stock items
- CJD Resource Centre
- UK Stem Cell Bank
• Technical Information on WHO International Standards and other NIBSC Biological Reference Materials can be obtained from:

www.nibsc.ac.uk
First international standard for common genetic test approved by WHO

17 NOVEMBER 2004 | GENEVA -- The first international standard for a human genetic test was approved by the World Health Organization (WHO) today. Use of the standard will help to improve the accuracy and quality of laboratory results worldwide from a frequently used genetic test. This test identifies a genetic predisposition to thrombosis -- a potentially life-threatening blood condition -- and could therefore enable people to take preventive measures.

"Establishment of the first international standard for a genetic test is an important milestone. Genetic testing procedures are playing a vital and growing part in clinical medicine. This new standard will help to ensure that the tests are giving accurate results worldwide," said Dr David Wood, Coordinator of Quality Assurance and Safety of Biologicals at WHO.

The newly established standard, formally called an International Reference Panel, relates to the testing of patients for a particular genetic mutation known as Factor V Leiden. Discovered in 1994, this mutation is one of the most common genetic risk factors for venous thrombosis (blood clot), and is involved in 20-40% of all cases. Factor V Leiden induces a defect in the natural anti-coagulation system.
NIBSC approach to making genetic reference materials

- Obtain blood sample from well-characterised, consented patient.
- Make EBV-transformed cell line to ensure continuing supply of DNA
- Large-scale DNA extraction
- Freeze-dried in ampoules
- International collaborative study
- Make material available to all as WHO reference material
Which materials to make?

- Consider impact on public health;
  - Frequency of testing
  - Error rate

- Take advice from:
  - National Genetic Reference Laboratories
  - Professional organisations e.g. ISTH, ISBT
  - NEQAS
  - Clinical laboratories
Where to start?

• Reference Materials already made;
  - FVL
  - Prothrombin mutation 20210

• In the pipeline;
  - Fragile X
  - Haemophilia A,
  - Hereditary Hemochromatosis
  - HLA, HPA and red cell blood groups

• Possible in future;
  - BCR/ABL
  - Fetal DNA in maternal circulation
**Factor V Leiden Polymorphism on Chromosome 1**

- **Position of primers used by participants:**
  - 3
  - 4
  - 6
  - 2
  - 7
  - 4

- **Portion of gene sequenced**

- **Homozygote FVL**

- **Heterozygote FVL**

- **Wild Type**
Are the materials stable?

<table>
<thead>
<tr>
<th>Preparation</th>
<th>04/174</th>
<th>04/194</th>
<th>04/196</th>
<th>MW</th>
</tr>
</thead>
</table>

| Storage temp. | -150 | +45 | +56°C | -150 | +45 | +56°C | -150 | +45 | +56°C |

No degradation detectable after storage at elevated temperatures for 6 months
Collaborative studies

- FVL
  - 3 materials (hetero, homo, WT)
  - 18 blinded samples
  - 41 labs from 16 countries
  - 14 different methods
  - 859 tests
  - 0.7% error rate

- Prothrombin
  - 3 materials (hetero, homo, WT)
  - 18 blinded samples
  - 45 labs from 23 countries
  - 11 different methods
  - 984 tests
  - 0.7% error rate

All participants agreed that the materials are fit for use.
FVL and Prothrombin panels are available from NIBSC

Established by WHO
• FVL 2004
• PT 2005

Others will follow shortly
ACKNOWLEDGEMENTS

- Eurogentest
- CRMGEN
- NGRLs
  - Manchester
  - Wessex
- UK NEQAS for Blood Coagulation
- Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital
- Department of Haematology, University of Cardiff
Fragile X study

• A panel of five gDNA preparations is now on its way, we will run a collaborative study in 2005.
• Anyone interested in participating, please contact Paul Metcalfe or Elaine Gray:

egray@nibsc.ac.uk
pmetcalfe@nibsc.ac.uk
## Steps required to make Genetic Reference Materials

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<tr>
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<tbody>
<tr>
<td>Identify need</td>
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<tr>
<td>Obtain ethics approval</td>
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<td>✓</td>
<td>Not required</td>
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<td>Obtain patient samples</td>
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<td>Make EBV-transformed cell lines</td>
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<td>Characterise DNA</td>
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<tr>
<td>Scale-up cell culture</td>
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<td>✓ (1/5)</td>
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<td>Bulk gDNA extraction</td>
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<tr>
<td>Fill into ampoules &amp; freeze-dry</td>
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<tr>
<td>Carry out collaborative study</td>
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<tr>
<td>Send report to participants and to relevant societies</td>
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<td>Obtain ECBS approval</td>
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<td>Make material available</td>
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