NIBSC

Assuring the quality of biological medicines

Biological Reference Preparations

What are biological reference preparations?

- Biological medicines are complex and usually require biological assays.
- Biological assays employ either living animals, tissues, cells or other materials of biological origin. These are naturally subject to individual variation. As a consequence, assays dependent on them are inherently variable.
- To minimize the effects of biological variation, assays usually compare response of similar groups to test preparation and a reference preparation of the same or similar material.
- Reference preparations may be used as positive or negative controls and/or for calibration of biological assays.

Types of reference preparations

- International Standards and Reference Preparations approved by recognized international organization eg WHO, FAO etc. ISs for biological assays are usually calibrated in IU.
- Regional Standards
- National Standards
- In house reference preparations

The latter categories are secondary to the primary International Standards and are used as working references. These should be calibrated against the IS

Sources of biological reference materials

- **IS and IRPs from WHO approved laboratories**.
  (Currently >90% supplied by NIBSC, UK)

- **Subsidiary standards**

  – Pharmacopoeial Standards: - PhEur, USP &c

  – Regional WHO Standards-appointed regional laboratories

  – National Standards-OMCLs, Reference labs.

  – In house standards- testing laboratories, manufacturers QC labs. &c

WHO Collaborating Centres

- Currently 2 International Laboratories and 3 CCs for biological standardization
  - NIBSC, UK; *(Sanquin, NL)*
  - CBER/FDA, USA; NIID, Japan; PEI, Germany
- WHO long-term aim to foster broader geographic representation
  - Encouragement of developing regional working references
  - To develop a more networking style of working

Can WHO International Standards be used routinely?

- WHO International Standards are distributed free of charge to national control laboratories
- But supplies are limited and not sufficient for use in routine assays
- The IS should be used for calibration of a secondary reference material - costly and complex task, thus WHO advise to prepare regional working reference materials

Regional working standards/references

- Each working standard/reference would be prepared as a large batch, characterised and calibrated in term of IS in international collaborative study

- The regional standard/reference would be stored and distributed by a regional reference laboratory

Types of products covered

- Vaccines
- Antisera, antitoxins, antivenoms
- Blood products
- Hormones
- (Antibiotics)
- Cytokines and growth factors
- Monoclonal antibodies
- Genetic reference materials
- Diagnostic materials

Preparation of reference materials 1.

- Identify public health need
- Identify material required and find suitable source
- Obtain material in suitable form-usually liquid or frozen bulk preparation
- Characterize material to confirm identity, composition and suitability for purpose
- Perform trial lyophilization study to optimize conditions
- Confirm stability of material
- Fill bulk material into ampoules for maximum stability, observing required precision and reproducibility of fill
- Lyophilize and seal under optimum conditions
- Confirm activity and set up accelerated degradation and real time stability studies
- Set up international collaborative studies to assess value of standard and determine unitage

Centre for Biological Reference Materials

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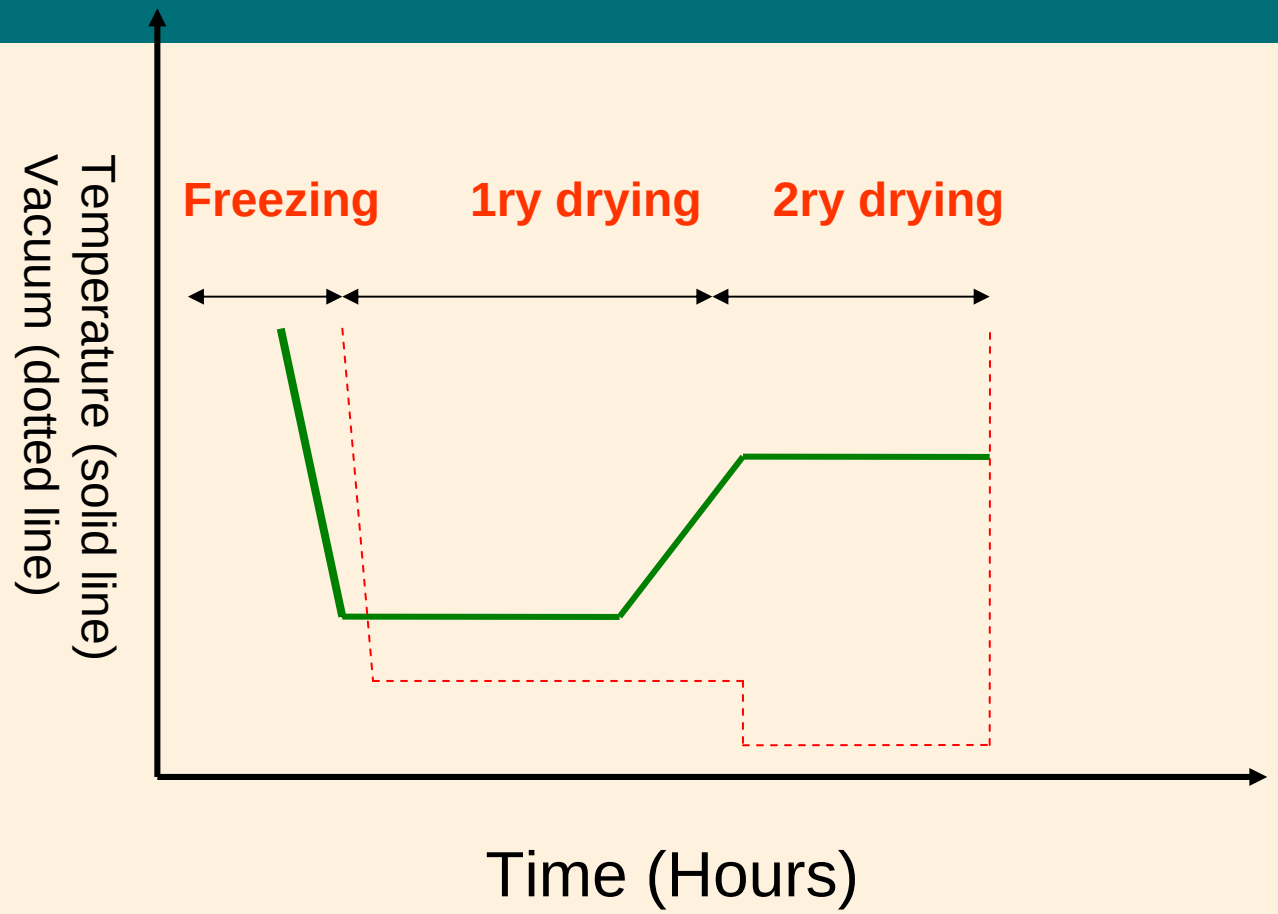
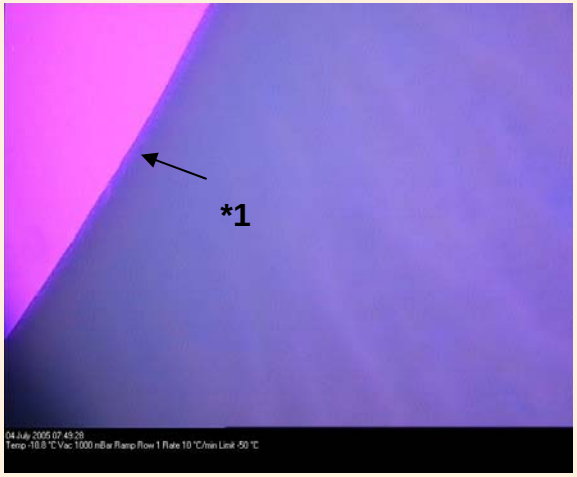
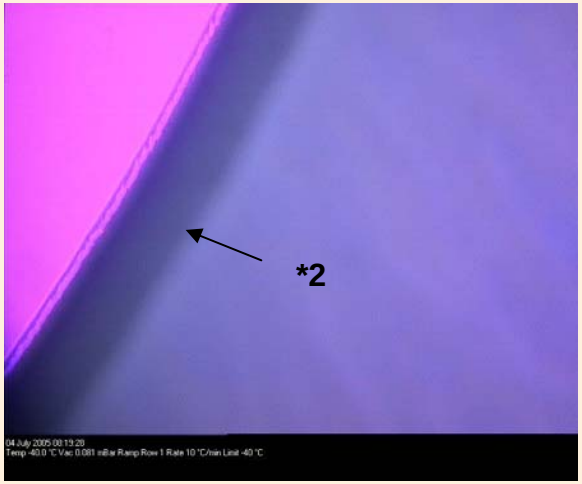
How do we freeze dry?

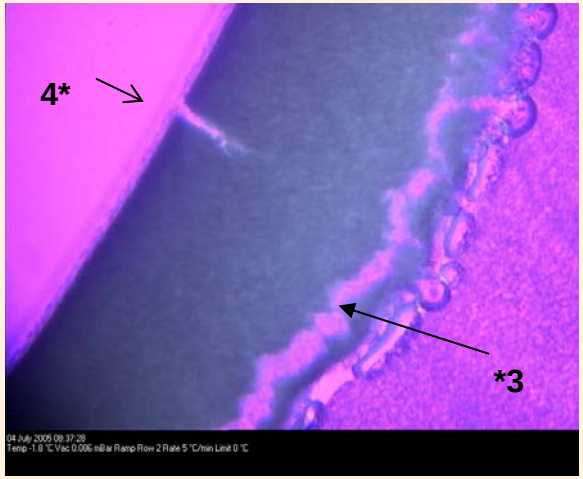


- Process Variables: Temperature, Time, Vacuum
- Product Variables: Formulation, container, volume of fill

Freeze drying microscopy







Freezing

Primary drying freeze
Drying front progressing

Collapse – temperature too high
Also burst at interface
Due to skin formation

Pilot scale freeze dryer

Typical trial lyophilization run

Thermogravimetric analyser with mass spectrometer interface

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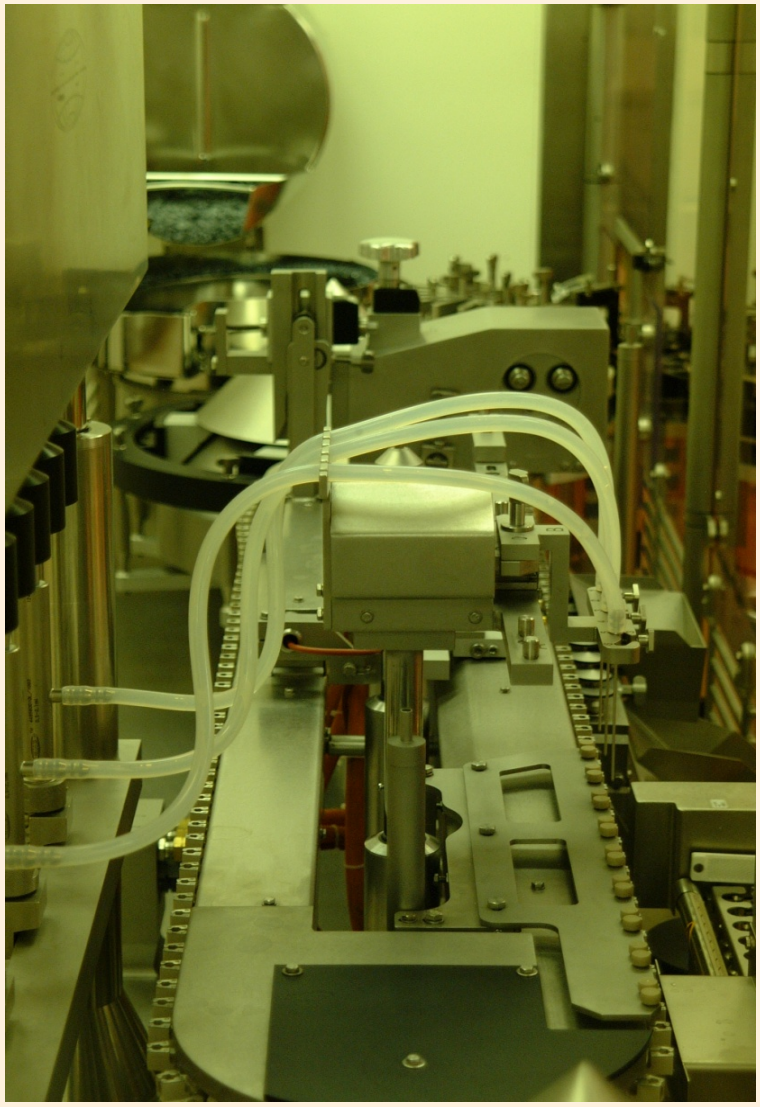
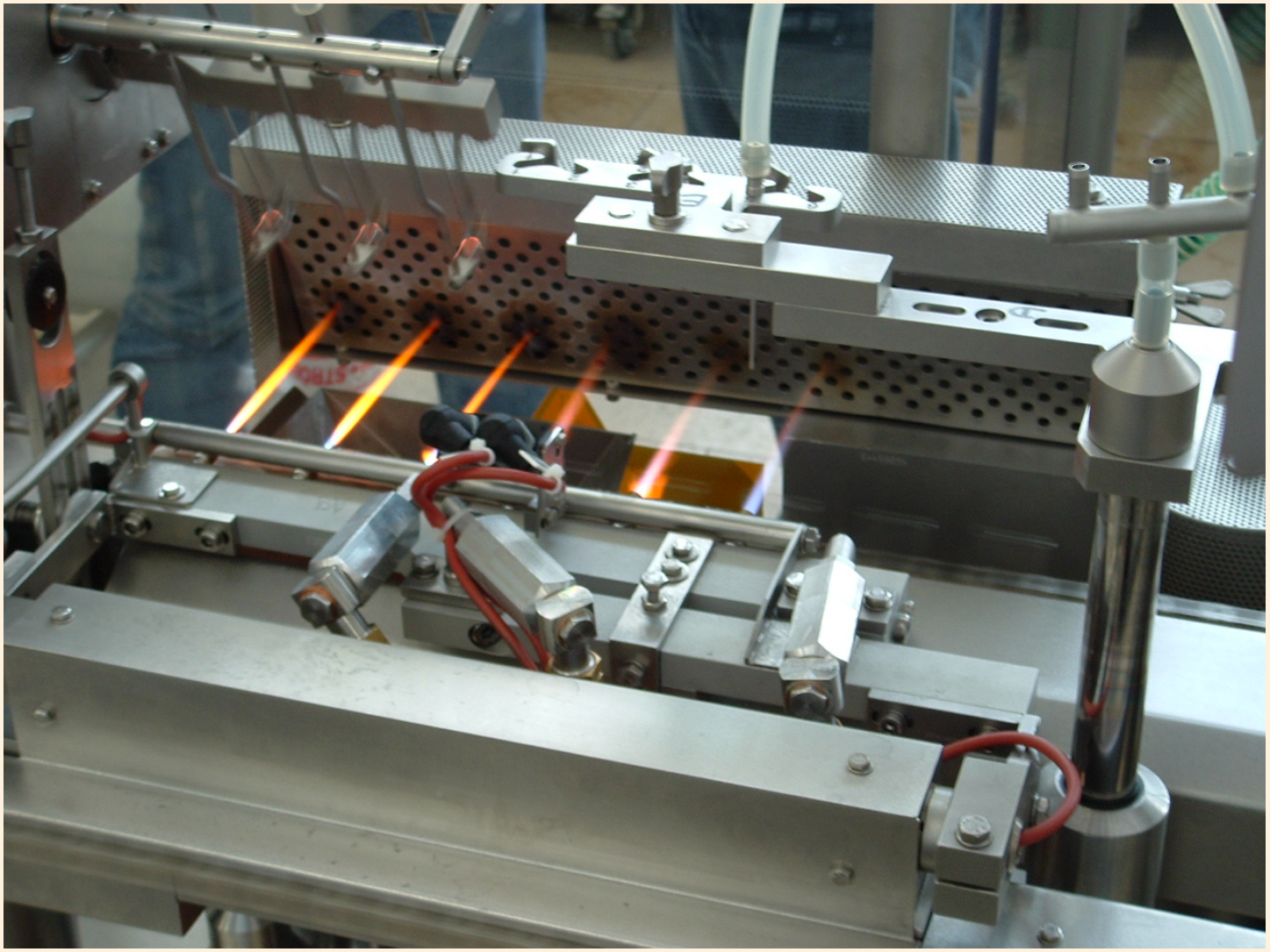
CBRM

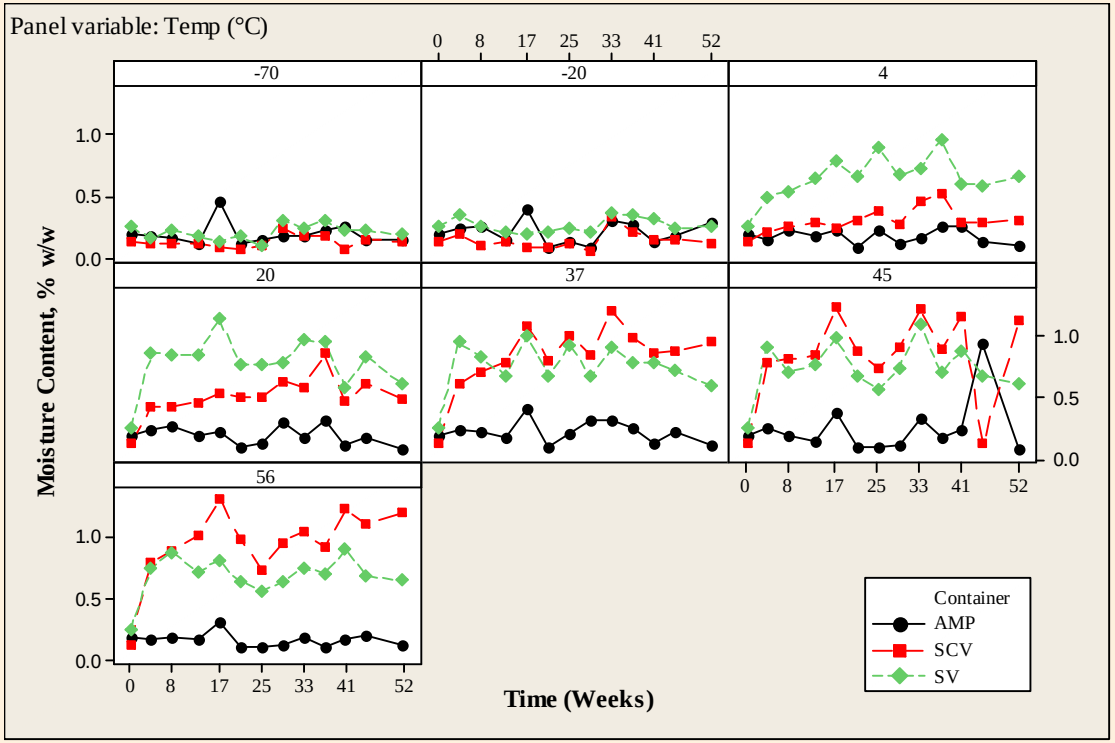
CBRM



CBRM



Comparison of Ampoules with vials for long term storage stability of a model biological material



From Matejtschuk et al (2005) *Biologicals* 33;63-70

Preparation of standards 2.

- International collaborative studies
  - Design study in consultation with statistician
  - Select and invite participants from regulatory laboratories, manufacturers, academia with experience of tests. They should give wide geographical representation.
  - Send blinded samples including negative and positive control and existing reference and instructions to participants
  - Participants perform tests according to instructions-may follow standard protocol, use in house methods or both-and return results to statistician
  - Analysis of results, assignment of unitage and cv (if relevant)
  - Referral of report to WHO ECBS for approval
  - If approved, reference is established as WHO IS or RP

Benefits of international collaboration

- Performance of laboratories is compared-helps to achieve common standard
- Promotes harmonization of procedures
- Labs with limited experience/facilities gain access to technical advice/training
- Helps to eliminate local problems/variations in performance
- Improves confidence in regulatory process
- Facilitates regulatory process for manufacturer
- Helps development of products in developing countries

Future challenges for standards

- New vaccines
- Growth factors and cytokines
- Plasma derived products
- Diagnostic kits eg for blood factor analysis
- rDNA standards eg for rDNA blood products (complex proteins difficult to characterize and standardize)
- Genetic reference materials incl. DNA references, synthetic gene sequences, SNPs, multiple tandem repeat sequences, for detecting genetic disorders; vectors for gene therapy with or without replacement genes

Past trends and future issues: WHO Standard-setting process and future challenges

- Participation of developing countries in the **standard-setting** process
- Promote developing country **compliance** with international standards
- Assist developing countries in the implementation of international standards

Future approaches to reference materials

- Preparation of stable freeze-dried standards is expensive, slow and requires dedicated facilities
- Simpler procedures for stabilizing reference materials should be developed
- New technology developed in the food and pharmaceutical industries needs to be assessed eg water-soluble glasses, liquid stabilizers, non-aqueous systems.

Summary 1.

- Reference materials are developed to ensure the safety and efficacy of biological medicines
- Biological materials are inherently variable. Medicinal products formulated from these may vary widely in potency and toxicity between batches
- Biological assays are needed to monitor activity
- Standardized reference materials are essential to ensure reproducibility of assays and allow inter-laboratory comparison
- Where feasible International Standards or Reference Preparations should be developed as primary standards for calibration of Working Standards or in house references
- In some situations eg high throughput, Regional Standards may established to supplement International Standards. These must be calibrated accurately against the relevant primary standard (IS or RP)

Summary 2.

- Calibration of International or Regional Standards must be achieved through a properly designed international collaborative study
- Data must be analyzed by an independent statistician
- Any unitage assigned must be consistent with units approved by WHO
- The collaborative study process is beneficial in establishing inter laboratory cooperation and harmonization of procedures
- The range and demand for reference materials is expanding
- Simpler methods for preparing stable reference materials should be developed

Acknowledgements

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