Development and Use of USP Reference Standards for Biologics

Tina S. Morris, Ph. D., Vice President, Biologics & Biotechnology

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The reference materials relate directly to procedures in the USP compendia:
Uses of USP Reference Standards

There are two main types of USP Reference Standards:

**Standards with Quantitative Applications**
- Assays (for drug substances and for formulations)
- Limit tests (e.g., Impurity Reference Standards)

**Standards with only Qualitative Applications**
- Identification tests
- Elution markers
- System Suitability tests
Standard Development

**Bulk Candidate**

**Conventional**

- Collaborative study

**Non-routine**
- Quantity limited
- Proposed RS presentation different from sponsor (liquid vs. solid)

**Formulation/Lyophilisation**

- Pilot Fill

- Content of fill
- Homogeneity
- Stability studies

**Definitive Fill**

- Collaborative study
Collaborative Study Design

Types of Reference Standard

Qualitative application
- Identification, peak identification, system suitability
  - Establish identity of candidate material
  - Evaluate chemical identity by compendial and non-compendial procedures
  - No value assigned to the RS candidate

Quantitative application
- Potency/Assay/Limit
  - Establish identity of candidate material
  - Evaluate chemical identity by compendial and non-compendial procedures
  - Value assignment (mass-balance, bioassay, etc) of the RS candidate
  - Potency RS calibrated relative to the current International Standard
Number of Biological RS in Active Portfolio: > 123

Number of RS in Official Portfolio

<table>
<thead>
<tr>
<th>Class</th>
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<td>Enzyme</td>
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<td>Glycosaminoglycan</td>
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<tr>
<td>Carbohydrate</td>
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<td>Tissue (photomicrograph)</td>
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<td>Cell Line</td>
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</tr>
<tr>
<td>Other</td>
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</table>
Suitability for Compendial Use – What is That?

From the *USP Filgrastim RS* Reference Standard Candidate Evaluation Package (RSCEP)

**OFFICIAL MONOGRAPH USES**

The reference standard is specified in the *PF 36(5)* [Sept. – Oct. 2010], pages 1180-1184, monograph listed below and is suitable for its intended use.

<table>
<thead>
<tr>
<th>Monograph</th>
<th>ID</th>
<th>Assay</th>
<th>Organic Impurities ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filgrastim</td>
<td>Cell-based Bioassay, HPLC, Peptide Mapping</td>
<td>Cell-based Bioassay</td>
<td>RP-HPLC, Isoelectric Focusing, SDS-PAGE, SE-HPLC</td>
</tr>
</tbody>
</table>

¹ Used as a peak identifier, gel band identifier, and in preparation of a resolution solution
Two Components

- Physicochemical tests
  - Peptide Mapping
  - Chromatographic Purity (RP-HPLC)
  - SE-HPLC
  - SDS-PAGE
  - IEF
  - Protein Determination
- Bioassay

Collaborators (International Study)

- 16 collaborators total (some collaborators did both bioassay and bioanalytical tests)
  - Physicochemical tests
    - 8 collaborators total
    - 6 returned results
  - Bioassay
    - 13 collaborator total
    - 11 returned results
FILGRASTIM 0.98 mg
(recombinant methionyl human granulocyte colony-stimulating factor
(r-metHuG-CSF))

DANGER! May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause an allergic skin reaction.


USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666
CAT. NO. 1270435
LOT: F0L526
Each ampoule contains $8.5 \times 10^7$ IU when assayed against the WHO 2nd International Standard for Granulocyte Colony-Stimulating Factor.
Add the following:

**Filgrastim**

MTPLGPA5SL PQ5FLLKCLE QVRKIQGDDGA ALQEKLC4ATY KLCHPEELVL
LHISLGIQPA PLSSCPSGAIE QLAGCILSQGLH SQLFILQGGLL QALEGISPEL
GPTLDTLQOLD VADFATTINQ OMEE LFQ MAPA LOFTQGAMPA FASAFQRRAG
GVLVASHIQS FLEVSYRVL RMLAQPS

C$_{845}$H$_{1339}$N$_{223}$O$_{243}$S$_{9}$ 18,799 daltons
[121171-53-1].

**DEFINITION**

Filgrastim is a recombinant form of human granulocyte colony-stimulating factor (r-methHuG-CSF). It is a single chain, 175 amino acid nonglycosylated polypeptide produced by *Escherichia coli* bacteria transfected with a gene encoding a methionyl human granulocyte colony-stimulating factor. When prepared as a drug substance, it contains NLT 0.9 mg/mL of Filgrastim. Formulation contains one or more suitable buffering and/or stabilizing agents. The presence of host cell DNA and protein in Filgrastim is process-specific. The capability of the process to clear host-derived DNA and protein requires validation and is determined by validated methods. It has a biological potency of NLT 80% and NMT 125% relative to standard on a mass-to-mass basis.
ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers. Store between 2° and 8°. Protect from light during long-term storage.

- **Labeling:** Label to indicate the content of the drug substance in g per container. The labeling states that the material is of recombinant DNA origin.

- **USP Reference Standards (11)**
  USP Endotoxin RS
  USP Filgrastim RS
  USP Filgrastim RS $^{2S}$ (USP36)
From General to Specific – Biological Potency

Overview of Bioassay

- Development of Biological Assays
- Analysis of Biological Assays
- Validation of Biological Assays

Design and Analysis of Biological Assays

Insulin Assays

Insulin Monograph

Guidance & Information

General Requirement

Product-Specific Requirement

Product Quality Attributes
Where possible, a Standard should be prepared using the same manufacturing process as the drug substance.

Storage conditions may vary from drug substance or product:
- Temperature (e.g., −70 °C or −20 °C instead of 2–8 °C)
- Container (e.g., plastic vials instead of syringes)
- Formulation (e.g., lyophilized formulation or addition of carrier proteins)

Test Standard for stability at appropriate intervals.

An initial Standard = Primary Standard
Subsequent Standards = Working Standards
- Separate SOPs usually required
- Trend charts may be useful in identifying the cause of assay drift
A Word on Units

USP General Notices:

5.50.10. Units of Potency (Biological)

For substances that cannot be completely characterized by chemical and physical means, it may be necessary to express quantities of activity in biological units of potency, each defined by an authoritative, designated reference standard.

Units of biological potency defined by the World Health Organization (WHO) for International Biological Standards and International Biological Reference Preparations are termed International Units (IU). Monographs refer to the units defined by USP Reference Standards as “USP Units.” For biological products, units of potency are defined by the corresponding U.S. Standard established by FDA, whether or not International Units or USP Units have been defined.
1.8 per disaccharide unit. It has a potency of not less than 90 and not more than 125 Anti-Factor \(X_a\) International Units (IU) per mg, and not less than 20.0 and not more than 35.0 Anti-Factor \(II_a\) IU per mg, calculated on
Product-Specific Potency Assays

- Can be called out in a Monograph or General Chapter
- Monograph requirements supersede Chapter Requirements
- A potency test or at least bioidentity test based on a functional assay is required for most biologics and biotechnology-derived medicines licensed in the US market. Some smaller proteins/peptides have only HPLC-based Assays to determine potency (e.g., vasopressin, oxytocin, leuprolide, etc.)
- In many cases the potency test has a dedicated RS associated with it, typically labeled “USP xxx RS for Assay” or “USP xxx RS for Bioassay”
Case Study – Heparin Potency

Key Issue: Heparin


Contacts

- **Scientific Liaison:** Anita Szajek (aey@usp.org or +1-301-816-8325)
- **Reference Standards Technical Support:** RS Technical Service (rtech@usp.org or +1-301-816-8129)
- **Reference Standards Ordering:** USP Customer Service (custsvc@usp.org or +1-800-227-8772)
- **Media:** Laura Provan (lnp@usp.org or +1-301-816-8268)

Updates

- **Heparin Sodium Monograph Comment Period Extended (30–Nov–2012)**
- **Heparin Labeling Revisions (05–Nov–2012)**
  
  The labeling sections of the currently official Heparin Sodium Injection and Heparin Lock Flush Solution monographs in USP–NF are being revised to ensure that labels comply with General Chapter <1> Injections. General Chapter <1> requires that the label reflect strength per total volume as the primary expression of strength, followed in close proximity by strength per milliliter (mL). An example of an expression of product strength as articulated in General Chapter <1> would be “30,000 USP Units/30 mL (1000 USP Units/mL).”
Case Study: Heparin Potency

- Anti Factor IIa chromogenic assay replaced the sheep plasma clotting assay for potency assignment in stage 2 revision of Heparin Sodium monograph, official since October 1st, 2009
- Minor revision to PF 33 (2) test incorporating recommendations from the Advisory Panel
- Validation study was conducted on revised PF 33 (2) test
- Incorporation of an anti-factor Xa/ anti-factor IIa ratio specification: NLT 0.9 and NMT 1.1
- New Acceptance criteria: The potency of Heparin Sodium, calculated on the dried basis, is NLT 180 USP Heparin Units in each mg.
- USP harmonizes units with IU when *USP Heparin Sodium for Assays RS*, Lot F, was introduced in July 2009
Anticoagulant Actions of Heparin

Contact system:
- HMWK, PK, F XII
- F XIa, Kallikrein
- F X, F IX
- F VIII, F VIIIa
- F IX, F IXa

Cellular injury:
- Tissue Factor (TF)
- TFPI

Thrombin (F IIa)
- Prothrombin (F II)
- F X, F Xa
- F V, F Va
- Activated Protein C

Fibrinogen
- Fibrin monomer
- Fibrin multimer
- Factor XIII
- Factor XIIa

Crosslinked fibrin

Unfractionated Heparin
LMW Heparin
Heparan sulphate

Heparin Co factor II

Antithrombin
Anticoagulant Activities of Sulphated Polysaccharides

Contact system:
- HMWK, PK, F XII
- F XIa, Kallikrein
- F XI, F Xla
- F IX, F IXa
- F VIIIa, F VIII
- Activated Protein C
- Protein S
- Protein C + Thrombomodulin
- Crosslinked fibrin

Cellular injury:
- Tissue Factor (TF)
- F VIIa, F VII
- Prothrombin (F II)
- F V, F Va
- Thrombin (F IIa)
- Fibrinogen → Fibrin monomer → Fibrin multimer
- Factor Xllla, Factor XIII
- Dermatan sulphate
- Oversulphated chondroitin sulphate
- Chitosan sulphate
- TFPI

Unfractionated Heparin
- LMW Heparin
- Heparan sulphate
- Oversulphated chondroitin sulphate
- Dextran sulphate
- Antithrombin
- Antithrombin Activities of Sulphated Polysaccharides

Global Expertise | Trusted Standards | Improved Health

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Not specific for heparin; will detect any anticoagulants that prolong clotting times

- EP and USP (pre-October 2009) assays, use sheep plasma as substrate; highly influenced by other sulphated polysaccharides

- APTT using human plasma as substrate; less influenced by other sulphated polysaccharides than sheep plasma assays

- Others e.g. thrombin time
Specific Activity Assays for Heparin

- Mostly chromogenic substrate assays:
  - Antithrombin dependent anti-Xa and anti-IIa assay, will work for heparin, LMW heparin and heparan sulphate; not influenced by other polysaccharides that potentiate heparin co-factor II
  - Heparin co-factor II dependent anti-IIa assay, will work for heparin, LMW heparin and other polysaccharides that potentiate heparin co-factor II
  - Anti-Xa and anti-IIa assays that use plasma as a source of antithrombin. Depending on the protocol, anti-IIa activity can be influenced by the presence of other polysaccharides
Issues with Clot–Based Assays

- UFH can be neutralized by plasma proteins such as platelet factor 4 (PF4)
- Neutralization by PF4 is largely molecular weight dependent.
- PF4 is released into plasma from activated platelet
- Substrate sheep plasmas used in the USP and EP anticoagulant assays have varying levels of PF4 as confirmed by van Dedem et al
- Because of the differences in specific activities and molecular weight profiles of the reference standards and test samples, PF4 may have a differential effect on the anticoagulant activities of the standards and tests
- The introduction of the reference standards with specific activity similar to the clinical products have helped to reduce this problem.

However, this will rely on the reference standards being similar to test materials and this may not always be the case.
Changes in Specific Activities of Heparin

- Typical specific activities of unfractionated heparin from the ‘80s or earlier were around 150 U/mg while those produced in the ‘90s or later are typically close to 200 U/mg.

- The international, EP and USP standards and especially the USP standards (Lot K series) issued in the early 90s have much lower specific activities – around 150 U/mg.

- Molecular weight distributions of these standards were also different to the “modern” clinical products.

- This meant that the assay of these clinical UFH against these standards was getting away from the important principle of bioassays - LIKE against LIKE – leading to inaccurate estimation of potency.
Impurities such as dermatan can potentially influence the anticoagulant potency of UFH estimated by plasma based assays:

– Compete for PF4 and other heparin binding proteins

– Potentiate inactivation of thrombin by heparin co-factor II
Antithrombin Dependent Chromogenic Assays

**Anti-IIa assay**

**Anti-Xa assay**

Simple enzymatic assays!
Anti-Factor IIa Assays by USP Method: Intra-Laboratory Variation (%GCV)

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<th>Lab</th>
<th>T</th>
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Range = 1.4 – 29.1 %; 27/52 < 5%; 44/52 < 7%; 46/52 < 10%

Data from collaborative study to value assign 6th International Standard for Unfractionated Heparin
Reference Standard was issued as *Lot F, Heparin Standard for Assays* – this allowed USP to have the still current potency standard and the new standard available simultaneously for industry transition.

Reference Standard has been calibrated relative to the 5th International Standard for UFH.

Assignment of potency value is based on the proposed chromogenic Anti-factor IIa assay.

Standard has been available as of July 22, 2009.
Transition Challenges – Heparin

Transition from a non-specific to a highly specific test

Old compendial test and reference standard were not linked to an International Standard (IS) and USP potency unit over time (+30 years) had drifted away from the IU by approximately 10%

Product is still dosed in units – any adjustment to the potency has immediate practitioner and patient impact

Fast-tracked introduction of the new assay and reference standard during a public health crisis – introduction of new test and standard required close coordination with FDA and industry
Harmonization – Why is it not so easy?

- Diverging and emerging regulatory requirements:
  - Insulins and other peptides – bioidentity test requirement is US only
  - Biosimilars: different approaches in different regions

- Diverging scientific opinions regarding the adherence to metrological principles for biological materials and tests
  - Role of uncertainty measurement

- Logistics:
  - Endotoxin: fully harmonized limit standard used by USP, JP, EP and WHO:
    - Batch size of current RS 75 000 vials
    - Development time: >6 years
    - Standard depletes at different rates in different parts of the world
Harmonization – Why is it not so easy?

Access:
- Availability of material(s) from manufacturers globally
- Availability of information in support of these materials
- Global network of competent laboratories to perform associated testing and experts to evaluate data based on agreed-upon principles/criteria

Despite all of these barriers, harmonization efforts are critical to the advancement of public health and USP continues to be a dedicated supporter of ongoing international efforts:
- Insulin IS initiative
- Strong support for all WHO ECBS standardization programs
- Bi- or multilateral harmonization of standards with other pharmacopeias wherever possible
Thank You