Disclaimer

This presentation was compiled by representatives from industry and regulators and represents current best practices. Other than the described approaches can also be acceptable. The content is not binding and should be used for training purposes only.

Contributors:

Barry Cherney  Amgen Inc.
Dieter Schmalzing  Genentech
Tony Mire-Sluis  Amgen Inc.
Nancy Kirschbaum  CBER, FDA
Juhong Liu  CDER, FDA
Matthew Borer  Eli Lilly
Markus Blümel  Novartis
Anne Munk Jespersen  Novo Nordisk
Scope

- Preparation, qualification and control of a manufacturer’s in-house reference standard for an active pharmaceutical ingredient (API)
- Current industry “best practices” for therapeutic proteins, e.g. monoclonal antibodies, recombinant proteins or blood products
- Product development and commercial manufacturing
- Out of scope are standards for:
  - Product- or process-related impurities, such as host cell protein mixtures or isolated product-related variants
  - Sub-visible particles
  - Critical reagents, assay controls to establish system suitability or control routine assay performance, etc.
Outline

➤ Scope and Definitions
➤ Different types of reference standards
➤ **Best practices** on
  1) Selection of representative standard
  2) Preparation / Filling of the reference standard
  3) Analysis of *interim reference standard* (Early Dev.) and its replacement
  4) Analysis of *primary reference standard* and its replacement
  5) Analysis of *working standards* / secondary reference
  6) Continuous Trending
➤ References and Outlook
Definitions

- **in-house (interim) Reference Standard**
  Appropriately characterized material prepared from representative clinical or production lot(s) for Quality Control purposes during Development-stage of a product

- **in-house Primary Reference Standard**
  Appropriately characterized material prepared from representative clinical or production lot(s) preferably reserved for calibration of Secondary Standards

- **in-house Secondary Reference Standard**
  [also referred to as: Working Standard]
  Material prepared to routinely control of product lots for Quality Control purposes, such as biological assays and physicochemical testing. It is always calibrated against the in-house primary reference standard or (if available) a primary reference standard

**Note:** The expression ‘(Primary) Reference Standard’ is reserved for international or national standards (ICH Q6B)
References used in routine Quality Control analytics

**Development phase**

- Early technical development
- GLP-Tox or early clinical trials
- Phase 3 or pivotal trials

**Commercialization**

- Working standard batch 1
- Working standard batch n

**Scenario A:**

- External reference standard is not available

- References used in routine Quality Control analytics
- Primary reference (used to release working standards)

**Scenario B:**

- External reference standard is available

- Calibrate in-house working standards against external reference standard
Key-Processes in Reference Standard Life Cycle

Selection of representative material

Preparation of interim or primary reference

Analytical testing and Release

Storage and Distribution

Monitoring and Trending or Re-testing

Preparation of a working standard

End-user in analytical lab

Reference standards from external supplier

Replacement of reference standard
1) Selection of representative material

- Drug substance (DS) is usually suitable for use as a reference standard also for the drug product. (In exceptional cases both DS and DP reference standards may be needed)

- The first interim reference standard is derived from either tox material or an early GMP run

- Quality attributes of the reference standard should be ‘representative of production and clinical materials’ (ICH Q6B)

- Ideally, the purity profile should represent expected impurities and product-related variants to allow comparison against the reference. → Requirements may be: ‘no new impurities’, ‘similar elution time’, etc

- A potency reference standard (within a particular bioassay) must have the same biological activity as the test sample (i.e. shows parallelism). → It should be free from substances that may interfere with the assay. → Links to the material used in the “pivotal” clinical trials are essential
2) Preparation / Filling of the reference standard

- The selected drug substance material may be pooled from multiple lots, formulated, diluted (e.g. with placebo) or concentrated to a concentration best suitable to support all relevant analytical assays and to ensure prolonged stability of the reference standard.

- The reference standard does not need to be filled under GMP, but under conditions controlled as well as possible to ensure optimum quality and integrity.

- Sufficient quantities of a Primary Reference standard should be manufactured to maintain consistent product history, i.e. to minimize the need to have to replace the primary reference.

- Storage containers and temperature do not have to be identical to the primary packaging of neither DS nor DP; (e.g. storage in glass ampoules or PP Vials below -60°C)

- Select overprotective packaging and storage temperature (next slide)
2) Preparation / Filling of the reference standard

➤ Select **overprotective** packaging and storage temperature; some feasible examples are provided below:

**Solution presentation typically mandatory**

<table>
<thead>
<tr>
<th>Poly-cryovials</th>
<th>Cryo-compatible glass ampoules</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Re-closable</td>
<td>- Hermetic seal</td>
</tr>
<tr>
<td>- No special filling equipment</td>
<td>- Inert atmosphere</td>
</tr>
<tr>
<td>- Compatibility testing</td>
<td>- Fewer issues with adsorption/extractables</td>
</tr>
<tr>
<td>- CO₂ ingress</td>
<td>- Less expensive</td>
</tr>
<tr>
<td>- adsorption</td>
<td></td>
</tr>
<tr>
<td>- extractables</td>
<td>- Single use</td>
</tr>
<tr>
<td></td>
<td>- Special filling equipment</td>
</tr>
</tbody>
</table>

compiled by Matt Borer, Eli Lilly
3a) Analysis of *interim* reference standard (Early Dev.)

- The first (interim) reference standard is compared to specifications in place at that time; reporting results is usually sufficient.
- Accurate determination is essential for potency and protein content/concentration, when these parameters will be determined in the test sample by direct comparison to the reference standard.
- Select additional characterization tests (which are typically not performed for batch release of clinical material) to demonstrate that the reference is *fit for purpose*.
- Potency assignment strategy for the first (interim) reference depends on nature of the product and availability of an International Standard (or Pharmacopoeia Reference Standard):
  - If International Standard (IS) or Pharmacopoeia Reference Standard is available and appropriate:
    - In-house reference standard is calibrated according to IS units.
  - Without an external Reference Standard the first reference standard is assigned a value of 100% relative potency or an arbitrary absolute value (e.g. 100 units/ml).
3b) Replacement of interim reference standard

- Replacement of interim reference standard *may* be required after major process changes with an effect on critical quality attributes.
- Replacement of reference standard **is not necessary** if a process improvement alters the impurity profile **without** an impact on potency nor the ability to compare to the impurity profile of the existing reference.
- Minimizing drift is the most essential consideration when moving from one reference standard to another.
- The relative potency testing must be designed to discern real potency differences between old and new reference standard or to demonstrate equivalence within accepted/acceptable assay variability.
- Other performance signals beyond the actual potency result (such as slopes, asymptotes etc.) should be tracked to illustrate if there are subtle differences between standards.
- Take into consideration the need for multiple bioassays including *in vitro* tests of ancillary functions.
4a) Analysis of primary reference standard

- The rationale for the selection of qualification tests and the acceptance criteria (if any) must be detailed in a qualification report.

- Qualification tests generally include: potency, protein content/concentration, molecular mass, primary/(secondary/tertiary) structure, disulfide structure, carbohydrate structure, impurity profile, and (as applicable) thermal stability, *in-vitro* functional tests.

- Tests may be added or removed during development dependent upon scientific judgment or the latest state-of-the-art technology.

- The intended use of the reference standard influences selection of qualification tests and requirements:
  - A potency reference standard that is *not used* for physicochemical assays may not need the same level of physicochemical characterization (but documented evidence of molecular integrity should be provided).

- At time of marketing application: Acceptance criteria established for relevant tests for future reference standard qualifications (Reference standard qualification and maintenance program).
4a) Analysis of primary reference standard

- Results from reference qualification must be compared to data obtained from clinically qualified or commercial material to demonstrate suitability of the reference.

- For critical quantitative product attributes such as potency, there should be pre-determined agreement among replicate results to assign a final value (e.g. CV ≤ 5%, or using a tolerance interval approach).
  - The replicate number for a final result depends on intended use, assay performance, method capability, and the stage of development. When feasible, the reported result should be based on sufficient statistical power to ensure high degree of confidence.

- An accurate estimation of the potency of the primary reference standard is a critical component in maintaining a consistent product linkage for potency throughout the development lifecycle (maintain the unit size!).

- The reference standard must be evaluated to ensure it is a homogeneous preparation (i.e. minimal vial-to-vial variation).
4a) Analysis of *primary* reference standard

- Assess the availability and suitability of an international standard for use in qualifying your standards and if there isn’t one, consider working with a public standards organization to create one.

- If no International Standard is available declare an in-house primary reference standard; Potency assignment strategy for the primary reference depends on the nature of the product.

- First primary reference standard is often assigned a value of 100% relative potency or an arbitrary absolute value (e.g. 100 units/ml)

- Investigate, when the average potency falls outside the expected interval:
  - Question the suitability of the standard
  - Consider a larger number of replicate measurements
  - Consider to assign the value obtained (eg: 107%), when other potency parameters demonstrate that the result is acceptable and “a correction factor” will not significantly amplify errors in the estimate of the true value, e.g. 50% would multiply errors by 2 fold.
4b) Replacement of primary reference standard

- Ensure to keep sufficient stock of the first primary reference standard (your *gold* standard) for calibration purposes during the whole product life cycle.

- The number of primary reference standard replacements should be kept to a minimum in order to ensure that the historical link to the clinical material is maintained without disruption.

- Keeping the primary reference standard (if stability is ensured) as the calibrator each time another working standard is developed; compare $A \rightarrow B$, $A \rightarrow C$, $A \rightarrow D$ and not $A \rightarrow B \rightarrow C \rightarrow D$.

- The sample size should be such that there is sufficient statistical power to demonstrate equivalency in potency between the new (primary) reference standard and the former reference or minimally to obtain an accurate estimate of the “true” value.
5) Analysis of working standards (secondary reference)

- Qualification of a **working standard** requires less characterization data, as compared to the qualification of a primary reference standard (which should be characterized with the full range of assays).

- Minimally, testing should demonstrate that the standard is *representative* of the product and that it is fit for purpose for its intended use.

- Potency determined for a working standard measured vs. the primary reference should be within a pre-defined window, e.g. 95 – 105 U/ml:
  - If International Standard (IS) or Pharmacopoeia Reference Standard is available and appropriate: Working standard is calibrated according to IS units.
  - Without an external Reference Standard the working standard should be calibrated against the in-house primary reference standard (same units!)

- The working standard could be assigned 100% relative potency, if:
  - derived from the same source as the primary reference standard.
  - potency measured versus the primary reference standard is within a pre-defined window (e.g. 95.0 – 105.0%).
6) Continuous Trending

- All trended parameters that are derived from the reference standard should be evaluated for a shift in the assay results, especially following the implementation of a new reference standard lot.

- If stringent selection and qualification practices are executed, the impact to trended parameters that are directly influenced by the reference standard can be minimized however not completely eliminated due to measurement uncertainty.

- If a shift in a trended parameter occurs directly following the implementation of a new standard, the consequence to the program must be determined.

- Control routine performance of quantitative assays with a product control preparation to mitigate against systematic assay bias or subtle drift (i.e. due to instability or degradation of the reference standard).

- Periodic confirmation of reference standard stability against the international standard (if available; frequency is based on experience).
References and Outlook

➢ ICH Q6B “Specifications: Test Procedures and acceptance criteria for Biotechnological/Biological Products”

