Biomarker calibration standards in ligand binding assays: Feedback from JBF

Yoshiaki Ohtsu
on behalf of the Japan Bioanalysis Forum (JBF)
JBF biomarker task force members and JBF steering committee members shared and discussed the following articles:


Mostly understandable

Today, feedback and questions will be presented.
Selection from commercially available proteins

JBF agrees with EBF and Cowan et al.:
- Smaller lot-to-lot variability
- Greater information availability

JBF additionally suggests:
- Compare experimentally proteins from multiple vendors
- If it is a kit, replace the reference standard
- Make calibration samples, quantify commercially available serum/plasma, and compare the measured values with literature data

Question:
What if the protein is only available from a single supplier?
Selection of vendors

JBF agrees with EBF

Long term supply

JBF additionally suggests

Internal experience

Literature: long history of business activity

More information on their products

Determination methods for the protein conc.

Full length or partial

Photo of electrophoresis

Manufacture process (purification from biological matrix, recombinant, cell lines etc.)

Stability test (method and acceptance criteria)

Additional points by Cowan et al. may be unnecessary.
EBF wrote
“35% of all responders stated that they would use a calibration standard past the expiry date”

JBF’s position
- OK in preliminary experiments
- Best to refrain in assay qualification/validation and sample analysis
JBF prefers not to use “certificate of analysis” for biomarkers.

EBF wrote

Minimum for CoA: unique name, nominal concentration, manufacturer, and a lot number.
Other useful information: retest date, source and identity of calibration standard, (e.g. cell line, amino acid sequence, buffer or auxiliary reagents)

JBF additionally suggests

Biosafety information (Cartagena Protocol)
Storage condition
They don’t have to be on data sheet,
but should be communicated to the user in advance

Cowan et al. suggested
For treatment decision making, CoA plus additional internal characterization.
Synthesis in house or at CRO lab

The EBF article did not discuss this matter very much. Cowan et al. discussed it in detail.

JBF:
- Has extensive experience
- Just started cross-industry discussion
- Especially interested in:
  - Identification and concentration determination immediately after synthesis
  - Stability
  - Lot-to-lot management
## Determination of lot-to-lot variability (1/2)

<table>
<thead>
<tr>
<th>EBF</th>
<th>JBF</th>
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<tbody>
<tr>
<td></td>
<td>Overall, all approaches are fine.</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>Less likely to be used due to absence of an international standard</td>
</tr>
<tr>
<td>- Analyze new lot calibration samples, new lot mid-range samples, original lot mid-range samples in multiple replicates in 1-3 runs. (original lot should be highly reliable; international standard)</td>
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<tr>
<td>- Compare results from mid-range samples</td>
<td></td>
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<tr>
<td><strong>B</strong></td>
<td>Most common in daily work in Japan</td>
</tr>
<tr>
<td>- Measure QC samples (3 conc., n=3) with new and original lot calibration samples</td>
<td></td>
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<tr>
<td>- Compare the sample analysis results</td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>- Not common</td>
</tr>
<tr>
<td>- More common in chrom. methods than LBA.</td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>Useful, but less likely to be used due to absence of suitable samples.</td>
</tr>
<tr>
<td>- Measure &gt;30 samples with new and original lot calibration samples</td>
<td></td>
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<tr>
<td>- Compare the sample analysis results</td>
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Cowan et al. suggested
For treatment decision making, the evaluation should be repeated multiple times by multiple operators over several days.

JBF’s current thinking:
May not be practical for routine use
May be effective if there is a assay-specific concern
**Assessment of lot-to-lot variability across runs (1/3)**

Mainly about trend analysis of QC samples
QC samples can be “spiked QC” and “matrix QC”.

<table>
<thead>
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<tbody>
<tr>
<td>1</td>
<td>Simple graph drawing</td>
</tr>
<tr>
<td>2</td>
<td>1 with predefined acceptance criteria (e.g. +/- 20% from mean or nominal concentration)</td>
</tr>
<tr>
<td>3</td>
<td>1 with statistical tools such as Levey-Jennings</td>
</tr>
<tr>
<td>4</td>
<td>Data distribution, median, and range (details are Algeciras-Schimnich et al.)</td>
</tr>
</tbody>
</table>

Cowan et al. introduced “commutability”.

Deming residual statistical approach

Use multiple study samples
Common practice in clinical chemistry
Demonstrate interchangeability across multiple lots

JBF current thinking

Not familiar with this

A hurdle would be limited availability of the appropriate study samples
Parameters other than those from QC samples and study samples

EBF wrote: Record, document, and monitor

Signal response of the zero analyte or blank
Maximal response of the calibration curve
Slope of the calibration curve

JBF agrees that these parameters are useful
JBF’s interpretation is to

Record the parameters in raw data
Not document the parameters in reports
Briefly evaluate the parameters

Question: How extensively should you monitor the parameters?
## Choice of approach regarding lot-to-lot variability (1/2)

<table>
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<tr>
<td>1. Use several lots without any a priori comparison</td>
<td>Sometimes (unfortunately) taken</td>
</tr>
<tr>
<td>2. Have one lot to cover a study, within expiry date</td>
<td>Very often taken</td>
</tr>
<tr>
<td>3. Have one large lot to cover multiple studies, ignoring expiry date</td>
<td>Not preferred</td>
</tr>
<tr>
<td>4. Use several lots with normalization to the previous lot</td>
<td>Useful (see next slide)</td>
</tr>
<tr>
<td>5. Use several lots with normalization to the international standard</td>
<td>Useful but not used often as there is no international standard.</td>
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</table>

**Question regarding “Approach No.3”:**

How do you demonstrate that an expired reference standard is reliable? Definition by SOP? Should you conduct stability tests after use?
Choice of approach regarding lot-to-lot variability (2/2)

JBF is comfortable with Approach No. 4 “Use several lots with normalization to the previous lot”.

However,
- Correction can be a cause of human error.
- Depending on the reference standards, lot-to-lot differences are relatively small.

Proposal
If the lot-to-lot differences are within a pre-defined acceptance criterion, normalization is not required.
Request to reference standard vendors

EBF
- Full characterization and stability
- Open communication on analytical method
- Smaller lot-to-lot variability
- Timely communication regarding lot changes

Cowan et al.
- Open communication on
  - Analytical method
  - Protein used to determine the reference standard concentration

JBF
- Agrees with EBF and Cowan et al.
- Additionally requests the provision of reliable conc. by selecting an appropriate method for determination and purification
Conclusion

- JBF agree with most of the EBF’s recommendations.
- Feedback and questions from the JBF were presented.
- JBF would very much appreciate it if EBF could act as a partner and/or collaborator to promote good practice on biomarker assays to industries/authorities.
Acknowledgment

- Other members of the JBF biomarker task force
  - Harue Igarashi
  - Masaaki Kakehi
  - Takahiro Nakamura
  - Rui Ohashi
  - Yutaka Yasuda

- JBF steering committee members

- EBF
Coming up is the 10th Commemorative Symposium

Good Venue: PACIFICO Yokohama (Yokohama, Kanagawa, Japan)

Good Program:
http://bioanalysisforum.jp/en

Good Attendees: YOU!
Recent articles on biomarker assays from Japan
