Biomarker calibration standards in ligand binding assays: Feedback from JBF

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on behalf of the Japan Bioanalysis Forum (JBF)

Background

- JBF biomarker task force members and JBF steering committee members shared and discussed the following articles:
  - EBF: Kunz, U., *et al.* (2017). Addressing the challenges of biomarker calibration standards in ligand-binding assays: a European Bioanalysis Forum perspective. *Bioanalysis*, *9*(19), 1493-1508.
  - US: Cowan, K. J., *et al.* (2017). Recommendations for selection and characterization of protein biomarker assay calibrator material. *AAPS journal*, *19*(6), 1550-1563.
- 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.

Expiry date

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

Information from the vendors

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)

EBF		JBF
		Overall, all approaches are fine.
A	- 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) - Compare results from mid-range samples	Less likely to be used due to absence of an international standard
B	- Measure QC samples (3 conc., n=3) with new and original lot calibration samples - Compare the sample analysis results	Most common in daily work in Japan
C	- Determine samples made from new lot (whole curve range) using calibration samples made from original lot	- Not common - More common in chrom. methods than LBA.
D	- Measure >30 samples with new and original lot calibration samples - Compare the sample analysis results	Useful, but less likely to be used due to absence of suitable samples.

Determination of lot-to-lot variability (2/2)

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”.

EBF		JBF
1	Simple graph drawing	Very often use this approach for matrix QC
2	1 with predefined acceptance criteria (e.g. +/- 20% from mean or nominal concentration)	Very often use this approach for spiked QC
3	1 with statistical tools such as Levey-Jennings	Not experience
4	Data distribution, median, and range (details are Algeciras-Schimnich et al.)	Not experience

Algeciras-Schimnich A, Bruns DE, Boyd JC *et al. Clin. Chem.* 59(8), 1187–1194 (2013).

Assessment of lot-to-lot variability across runs (2/3)

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

Assessment of lot-to-lot variability across runs (3/3)

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)

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

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

Next JBF Symposium (12-14 Feb 2019)

Coming up is the 10th Commemorative Symposium

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

http://www.pacifico.co.jp/visitor/calendar/tabid/231/pdid/69290/Default.aspx

Good Program:

http://bioanalysisforum.jp/en

Good Attendees: YOU!

For your information

Recent articles on biomarker assays from Japan

- Saito, Y., *et al*. (2018). Current situation on biomarker validation in Japan. *Bioanalysis, 10(12),* 901-903.
- Wakamatsu, A., *et al.* (2018). Proposed selection strategy of surrogate matrix to quantify endogenous substances by Japan Bioanalysis Forum DG2015-15. *Bioanalysis, 10(17),* 1349-1360.