# Recommendations for regulated biomarker analysis using LBA kits

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on behalf of **JBF DG2017-34**

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# Background

- Biomarker analysis is important for drug development and to facilitate PK/PD modeling.
- Numerous kits Ligand Binding Assay (LBA) kits are available for biomarker analysis from multiple sources worldwide.
- Certain LBA kits (for research use only) may potentially have critical problems for regulated biomarker analysis.
- Several questions were received regarding commercial LBA kits during the 8$^{th}$ JBF symposium held on February 8–9, 2017.
- This is the first presentation from the JBF Discussion Group featuring LBA kits.

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# Case Study 1: Kit Accessories*

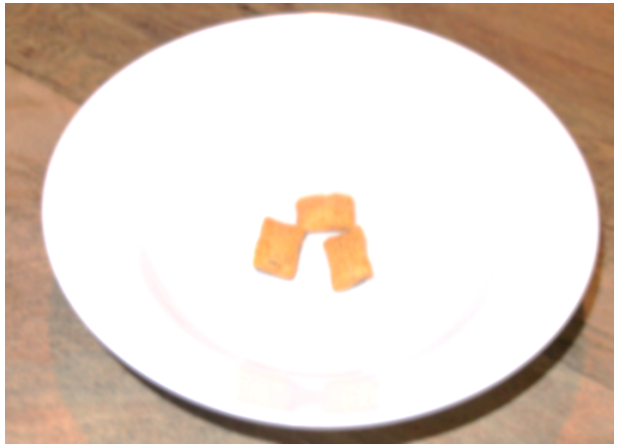
*Reference Standard, Quality Control (QC), and Buffer.

## What is the problem?

- Insufficient volume of reference standard to prepare validation samples.
- Insufficient volume of wash buffer to utilize a plate washer.
- Uncertainty of the dilution buffer formula makes it difficult to prepare more.

Ideal

Real



# Case Study 1: Kit Accessories (Cont.)

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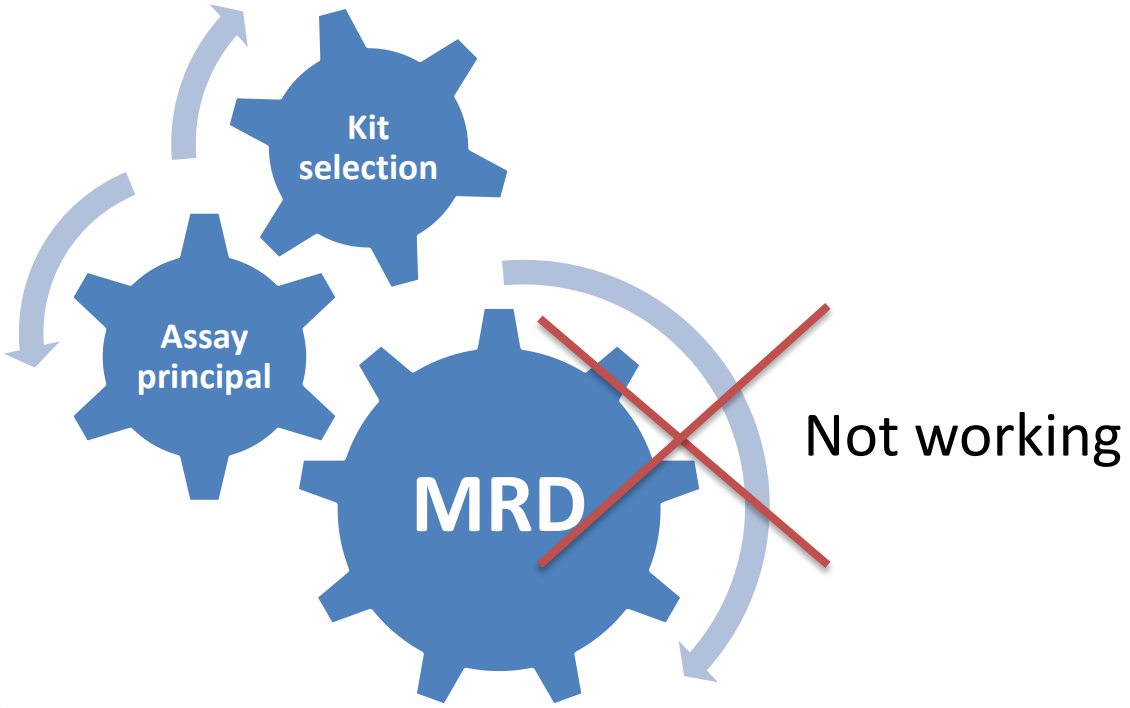
1) Check if reference standard and buffer in the kit can be purchased from the same vendor as independent reagents.
   a) If yes, purchase these at different times.
   b) If no, purchase extra kits to acquire the additional reference standard and the buffer. Or consider purchasing from a different vendor or preparing in-house.

2) Use kit QC should only to confirm the kit's reactivity or for batch QC only during the early phase of methods development.

# Case 2: Matrix Interference

What is the problem?

No recommended minimum required dilution (MRD)/
Poor recovery with even recommended MRD



# Case 2: Matrix Interference (Cont.)

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1) Optimize MRD
2) Optimize dilution buffer / blocking buffer
   (+ add Tween20 or HBR in the buffer)
3) Change dilution buffer / blocking buffer
4) Change the dynamic range / change the kit itself

# Case 3: Lot-to-lot Variation

## Case study

- [Case] Because of a different lot, the background signal increased and the overall signal-to-noise ratio of the calibration curve decreased with the decreased shape of the calibration curve.
- [Action] The assay method required changing the dynamic range per lot, and additional validation was performed using the calibration range that can be quantified using the lot.

Ideal

Real

# Case 3: Lot-to-lot Variation (Cont.)

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Lot-to-lot variation is frequent and may affect multiple phases (methods development and validation as well as sample analysis). We consider options to solve this problem as follows:

1) Secure an amount of a single lot sufficient to complete the entire analysis

2) Perform bridging assays between multiple lots

# Selection Guide for LBA Kits

What will be the checkpoints?

- Does this kit satisfy your analytical purpose?
- What is the performance of the specific brand?
- How quickly does the manufacturer deliver?
- Does the Cartagena Protocol regulate any components of the kit?
- Strip type or full-plate type?
- Are the kit components sold separately?

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Choose a reliable kit by going through these check points

# Evaluation of Kit Performance

How would you evaluate performance?

1) Confirmation of endogenous levels (6–10 individuals should suffice)
2) MRD (Selectivity or Parallelism)
3) Reproducibility

| Endogenous level: Low | Endogenous level: High |
|---|---|
| Selectivity determines the MRD. | Parallelism determines the MRD. |
| Use an individual with a low endogenous level. | Use an individual with a high endogenous level. |
| Reproducibility will be evaluated using the actual matrix spiked with the reference standard. | Reproducibility will be evaluated using the actual and surrogate matrices spiked with the reference standard. |

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Make sure the assay performance before the validation

# Case Studies of Multiplexing

- Popular kits are available from MSD, Luminex, and BD.
- Difficulty using multiple lots to analyze the same subject/patient (calibration curve concentrations will be different per lot and per analyte): Using a single lot is much preferred
- How many analytes are appropriate?: 4–7-plex is preferred
- Should data rejection be performed per analyte or per plate?: Both approaches are justified if described sufficiently by the SOP.
- What acceptance criteria should be used?
  - ❖ Only duplicate %CV
  - ❖ Accuracy ± 30%, Precision <30%

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No clear consensus is available. Most important is using a single lot to analyze the same subject/patient.

# Summary (Recommendations)

- Analytical methods described in kit instructions may be optimized for regulated biomarker analysis.
- Prior consideration is recommended for kit accessories, matrix interference, and lot-to-lot variation.
- Selection guide for LBA kit
- Evaluation of kit performance
- Specific multiplexing issues are identified. Further discussions are required

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1) Understand the major issues with kits
2) Try to select the appropriate kit from oceans of kits
3) Evaluate assay performance before validation

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|---|---|
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- Green = Pharmaceutical company
- Gray = CRO