L3 GBC assay formats team

Overview of team and current status

L3: Assay formats

Team members:

Team lead

Sherri Dudal EU
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Other members

- Daniel Baltrukonis NA
- John Smeraglia EU
- Karolina Osterlund EU
- Katherine McKay EU
- Mahesh Kumar APAC
- Yoshitaka Taniguchi APAC
- Alison Joyce NA
- Rebecca Crisino NA
- Jihong Yang NA
- Jaya Goyal NA

In scope

- Assay platforms for LBAs – Gyros, MSD, Biacore, AlphaLISA, Delfia, Singulex, Luminex, Immuno-PCR, ELISA (384), Cell-based assays, FACS, RIA
- Acceptance criteria for these methods for both validation and sample analysis
- How to set up the assays – placement of standards and QCs in these new formats
- Pros and cons of using these formats
- Multiplexing with these formats and the criteria required

Interdependencies with other teams

- **A11 Biomarkers:** The measurement of many biomarkers depends on the use of various platforms.
- **L1 Large molecule specific run acceptance:** acceptance criteria for new methods/platforms versus ELISA 96 well plate

Out of scope

- **L2: set-up of a balanced design for 96 well ELISA**
- **L4: stability of critical reagents**
- **L5: any automation activities linked to the platform**

Strategy

The guidelines and practices described by FDA, EMA, JBF, APAC were combined into one working document. China follows the guidelines that are currently in place so no adjustment was needed. The ANVISA guidelines are very small-molecule oriented and thus do not differ from current recommendations.

The team was divided into work groups to focus on the different platforms and bring information and discussion points to the overall team.

- Each team has been formed to ease time differences and is grouped according to expertise with a particular platform.
- The platform issues, criteria and pros and cons have been presented and discussed within the team and these will now be presented to colleagues at the workplace and in forum discussion groups to obtain more feedback.
- The work groups were organized as follows:

Platform	Leader	Team member	Team member
Gyros	Karolina (EU)	Sherri (EU)	Alison (NA-E)
Cell-based assays	Daniel (NA-E)	Yoshitaka (APAC-Japan)	Jaya (NA-E)
RIA	Mahesh (APAC-India)	Daniel (NA-E)	
384-well format	John (EU)	Karolina (EU)	
Alpha-ELISA/Delfia	**Rebecca (NA-E)**	Jaya (NA-E)	John (EU)
Singulex	Alison (NA-E)	Rebecca (NA-E)	Mahesh (APAC-India)
Biacore	Sherri (EU)	Jihong (NA-W)	Alison (NA-E)
MSD multiplex	Katherine (EU)	Yoshitaka (APAC-Japan)	Karolina (EU)
Luminex multiplex	Jihong (NA-W)	Katherine (EU)	Jaya (NA-E)
Immuno-PCR	Jaya (NA-E)	Jihong (NA-W)	

Current status

- Understanding and comparing the different platforms has been completed.
- Both best practices and pros and cons of each platform has been brought forward
- Consensus on the acceptance criteria, assay operations, and special criteria.
- Further feedback required for:
  - Sample analysis in series
  - Multiplexing
  - Platform specific effects such as carry over, hot spots
  - How to treat large dynamic ranges
  - When can singlet analysis be put in place
  - System suitability controls

These additional areas are currently being discussed in the various work groups to be brought forward for team discussion.

A best practice document is currently being drafted and any major recommendations will be brought forward through GBC if appropriate.

Comparison of assay criteria relative to ELISA

Platform	Format	Dynamic range	Matrix effects	Hook effect (unavoidable)	Sensitivity (compared to average ELISA)	Neat Sample volume
ELISA (reference platform)	Plate	2 logs	medium	No	20 ng/mL	10-30 µl
MSD	Plate	**3-4 logs**	low	No	+	10-30 µl
Biacore	Series	2 logs	low	No	-	10-20 µl
RIA	Series	2-3 logs	low	No	+	**50-100 µl**
AlphaLISA	Plate	2-3 logs	**high**	**Yes**	+	**5-10 µl**
DELFIA	Plate	2 logs	low	No	+	10-20 µl
Gyrolab	Series	**3-4 logs**	low	No	+	**4-8 µl**
Singulex Erenna	Plate/ Series	**3-4 logs**	low	No	++	**50-100 µl**
Immuno-PCR	Series	**3-4 logs**	low	No	++	**5-10 µl**
Luminex	Plate	2-3 logs	**medium**	No	+	10-50 µl
Cell-based Assays	Plate	2 logs	**high**	No	-	**50-100 µl**

Comparison of pros and cons of platforms

Platform	Labeled reagents	Time required (equiv 96w)	Carry over , hotspots or cross-talk	Automated sample handling	Multiplexing possible	Vendor-specific reagents	Watson LIMS interface
ELISA (reference platform)	Yes	6 hours	No	No	No	No	Yes
MSD	Yes	**4 hours**	Yes (cross-talk)	No	**Yes**	Yes	Yes
Biacore	**No**	6 hours	Yes (carry over)	**Partial**	**Yes**	Yes	**No**
RIA	Yes	6 hours	No	No	No	No	Yes
AlphaLISA	Yes	**2 hours**	No	No	No	Yes	Yes
DELFIA	Yes	6 hours	Yes (hotspots)	No	**Yes**	Yes	Yes
Gyrolab	Yes	**2 hours**	Yes (carry over)	**Partial**	No	Yes	Yes
Singulex	Yes	6 hours	No	No	No	Yes	Yes
Immuno-PCR	Yes	8 hours	No	No	**Yes**	Yes	Yes
Luminex	Yes	6-8 hours	Yes (cross-talk)	No	**Yes**	Yes	Yes
Cell-based Assays	Yes	**Days**	Yes (cell contam.)	No	No	No	**No**

Platforms with special issues

Samples run in series versus on plates

- Calibration standards run first and QCs interspersed throughout the run
- Frequency of passing QC series determined during validation
- Suggested to be every 20-50 samples
- Platforms affected: RIA, Biacore, Gyrolab, Singulex, & Immuno-PCR

Carry over, hotspots and cross-talk effects

- Requires determination during method development
- Ideally, adjust conditions to eliminate effects.
- Platforms affected : Gyrolab (non-disposable tips), Biacore, Delfia, MSD, & Luminex
- Gyrolab or Biacore (carry-over): May require QCs to be placed more often if in series and another Calibration standard series after a certain number of samples
- Delfia (hotspots): run triplicate samples or use a white plate to run duplicates
- Luminex (cross-talk): change bead combinations to avoid cross-talk
- MSD (cross-talk): diminish the number of spots in the well or change antibodies
- Cell-based (cell contamination) : contamination of adjacent wells can be avoided through technique or placement of duplicates.

Platforms with special issues

Multiplexing

Validation criteria

- Each analyte is treated separately in terms of validation
- Effects of mixing different analytes is determined for recovery and precision
- Assay acceptance and data report: analyte specific
- Again, cross-talk must be avoided

Sample analysis criteria

- When one analyte fails, run must be repeated.
- Partial sample run failure: accept samples within criteria

Large dynamic range

- Increase number of calibration standards with a minimum of 3 per log of dynamic range
- May require more anchoring points for curve fit
- 2.5 QCs per log for validation and 1.5 QCs per log for sample analysis to keep the same ratio as with ELISA with the minimum number in accordance to the current guidelines
- Platforms concerned if typically give a 3-4 log dynamic range

Cell-based: assay criteria

Special aspects of validation:

1. Controlling cell passages, plating and activity from experiment to experiment
   - Visual or marker characteristics to validate use of cells
   - Strict procedure for handling, plating and carrying out tests required once critical factors are defined
   - F/T stability must be demonstrated; maximum number of cell aliquots prepared after one passage
   - Effects of changing serum lots and other critical reagents must be shown.

2. Functional test requirements
   - Control standard required to apply to all experiments to show expected functional effect
   - High level of variability expected
   - Treatment group can be run in parallel and normalized with control group
   - Effects of individual sera may be high leading to difficulties of robustness

3. Further discussions ongoing....

FACS: assay criteria—adjust with feedback from cell-based assays team

Special aspects of validation:

1. Controlling preparation, staining and analysis from experiment to experiment
   - Cells must be coming from a validated procedure for cell handling
   - Fixing and staining of the cells must be done according to a validated protocol and tested for robustness to identify any variables leading to changes in staining intensity
   - The necessary controls for controlling the antibody staining (primary and secondary antibodies), a non-stained control for gating purposes, an isotype control, and a positive staining control if possible to control between experiments.
   - The FACs instrument must be validated and calibrated on a regular basis following an SOP
   - Effects of changing serum lots and other critical reagents must be shown.

2. Further discussions ongoing....

L3: Current Status & Future Discussion

Accomplishments to date:

- Understanding and comparison of the different platforms,
- Best practices, potential platform issues, and pros & cons of each platform has been brought forward.
- Consensus on the acceptance criteria, assay operations, and special criteria have been achieved.

Actively seeking feedback on:

- Sample analysis in series (calibrators and quality control placement)?
- Multiplexing?
- Handling instrument failures mid-read?
- System suitability controls (monthly instrument calibrations)?
- Platform cross-validation (how is it conducted)?
- Carry-over (mitigation strategies)?

Outstanding work to be completed:

- A best practice document is currently being drafted.

Thank you for your attention!