Workshop on ICH M10

C1 - Hybrid assays & ICH M10

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(table moderators: Luca Ferrari / Kamil Sklodowski)

14 November 2023 – Barcelona, Spain

From the Guideline

- Nothing….

3. CHROMATOGRAPHY

3.1 Reference Standards
3.2 Validation
3.2.1 Selectivity
3.2.2 Specificity
3.2.3 Matrix Effect
3.2.4 Calibration Curve and Range
3.2.5 Accuracy and Precision

4. LIGAND BINDING ASSAYS

4.1 Key Reagents
4.1.1 Reference Standard
4.1.2 Critical Reagents
4.2 Validation
4.2.1 Specificity
4.2.2 Selectivity
4.2.3 Calibration Curve and Range

Which requirements do we consider for hybrid assays?

Pre-meeting survey (26 responses)

	the question	Yes	No	Sometimes
Q1	Do you feel the ICH M10 provides clarity on how to validate hybrid assays?	2	21	1
Q2	Do you use 4-6-15 as acceptance criteria for hybrid assays? Use : Yes- No – Sometimes	9	3	8
Q3	Do you use 4-6-20 as acceptance criteria for hybrid assays? Use : Yes- No - Sometimes	5	1	10
Q4	What is driving the acceptance criteria you use?	Multiple answers		
Q5	Do you determine the Hook effect during validation? Use: Yes- No- Sometimes	3	12	4
Q6	Do you determine the analyte recovery during validation? Use: Yes- No- Sometimes	14	4	

Feedback from the round tables

Questions for the round table discussion

32% of participants had knowledge or experience in hybrid assay validation

1. Do you believe that additional guidance on the topic of hybrid assays would help? If yes, to cover which areas?

2. Analyte recovery for hybrid assays: the assessment is mandatory for all methods that employ sample extraction. Should this be determined at the protein level, at the peptide level, or at both?

3. Hook Effect for hybrid assays: do you think this assessment is applicable? If yes, how would you conduct the experiment?

4. Number of independent analytical runs: 3 for Chrom, 6 for LB. How many do you think are needed and why?

Question 1:
Do you believe that additional guidance on the topic of hybrid assays would help? If yes, to cover which areas?

Comments:

60% - No fixed statements needed - consider assay/analyte complexity and scientific judgment to select the criteria to adopt

40% - a little more guidance needed because of the fear of not meeting the regulatory expectations

Recommendation: a position paper (from the EBF?) would help clarify the complexity of the assays and decide how best to validate them

Question 2:

Analyte recovery for hybrid assays: the assessment is mandatory for all methods that employ sample extraction. Should this be determined at the protein level, at the peptide level, or at both?

Comments:

60% Overall recovery (combining recovery at protein + peptide level)

20% at peptide level only

0% at protein level only

Recommendation: recovery should ideally be assessed using a holistic approach (protein level + peptide level, including digestion efficiency if applicable).

Question 3:
Hook Effect for hybrid assays: do you think this assessment is applicable? If yes, how would you conduct the experiment?

Comments:
100% – to be determined

Recommendation: ‘saturation effect’ should be considered as a requirement rather than the LBA defined ‘Hook Effect’ which does not seem to be applicable. There is 100% agreement on this, despite the initial survey results (because of lack of understanding of what the problem is)

Question 4:
Number of independent analytical runs: 3 for Chrom, 6 for LB. How many do you think are needed and why??

Comments:
50% thinks 3 independent analytical runs are needed
33%: more than 3 independent analytical runs
17%: hard to decide upfront

Recommendation: Majority (not absolute!) agrees with the requirement of chrom. assays

Raw data from the pre-meeting survey comments

- In the next slides we provide the unredacted details from 26 survey files reaching us prior to the deadline.

- Surveys that have arrived after the deadline could not be included anymore, for logistic reasons. Please speak up if your comment wasn't already captured in the other 26 files

On Q1: Do you feel the ICH M10 provides clarity on how to validate hybrid assays?

- Currently NA for us
- Sometimes
- No experience with Hybrid yet
- Clear as mud, a degree of freedom/interpretation is good.
- Not so clear
- But we do not use hybrid assays
- no, there's nothing related to hybrid assay
- Not completely clear.
- no, there's nothing related to hybrid assay

Yes	No
2	21

Lack of clarity is evident, additional guidance would help

On Q2: Do you use 4-6-15 as accept. criteria for hybrid assays?

- Currently N/A for us
- Sometimes x 8
- Depending on qualifications vs validation.
- Qualification have no predetermined acceptance criteria.
- Teams need to know the standard. In practice all meets stringent criteria.
- Not done hybrid assays yet

Yes	No	Sometimes
9	3	8

On Q3: Do you use 4-6-20 as accept. criteria for hybrid assays?

- Currently NA for us
- Sometimes x 10
- Only if 4-6-15 is demonstrated not to be achievable
- Would most likely use LBA criteria
- not done hybrid assays yet
- Y, for LLOQ 25

Yes	No	Sometimes
5	1	10

Both acceptance criteria are equally considered

On Q4: What is driving the acceptance criteria you use?

- Currently NA for us
- Availability of Internal Standards
- EBF and other publications
- Context of use
- Commentary from FDA
- **The complexity of the compound**
- Aligning with M10
- performance of the assay
- **internal standard and pre-validation experiments**
- if during development is observed that the criteria of 15% is not meet the criteria will increased to 20%
- **Availability on labelled protein as ISTD**
- method development
- compound, purpose
- The presence or not of immunocapture step in the assays. - Depending on the level of validation for biomarkers
- IS used, performance of the method in method development (pre-validation batches)
- The availability of internal standard
- IS used, performance of the method in method development (pre-validation batches)
- Generally, LBA approach and results of proof of concept
- The instrument platform. If using LC-MS then 15% if using LBA then 20%

Various criteria are considered:

- **Availability of SIL ISTDs**
- **Pre-validation experiments**
- **Industry and regulatory feedback**

On Q5: Do you determine the Hook effect during validation?

- N
- If IP Used
- Sometimes
- Sometimes
- Would do it in method development
- Y, dilution linearity as for LBA approach,
- Sometimes
- Y (but limitations apply because of the impossibility to analyse undiluted ULOQ samples)

Yes	No	Sometimes
3	12	4

Disagreement on this requirement

On Q6: Do you determine the analyte recovery during validation?

- Currently NA for us
- Sometimes x2
- Not really required as long as it is consistent
- If not consistent A/P would not work
- Y for small molecules. - Sometimes - for hybrid assays depending on timelines (or decided case by case)
- YES for LCMS.
- Y, immunocapture and digestion efficiency
- Y but limitations apply (we determine the IAC recovery + recovery at peptide level assuming 100% digestion efficiency). Total recovery not possible to determine. Intact vs digested form
- Now assessed during assay development
- We moved recovery to Method development after implementation of ICH M10
- Hybrid assays not mentioned in ICH M10
- no internal example yet
- Question on recovery: Recovery spiking of the pure solution into the blank extracted sample. How much alteration of the matrix is allowed? Sometimes we struggle to get the same end solution. Can this be adjusted outside of the approved extraction method only for recovery purposes?
- no hybrid assays

Yes	No	Sometimes
14	4	-

There is agreement on this requirement

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