Workshop on ICH M10

ISR in practice

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Introduction to Round table G2

5. Incurred sample reanalysis (ISR)

- The performance of study samples may differ from that of the calibration standards and QCs used during method validation, which are prepared by spiking blank matrix. Differences in protein binding, back-conversion of known and unknown metabolites, sample inhomogeneity, concomitant medications or biological components unique to the study samples may affect measured concentrations of the analyte in study samples. ISR is intended to verify the reliability of the reported sample analyte concentrations.

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ISR should be performed at least in the following situations:

- For nonclinical studies within the scope of this guideline, ISR should, in general, be performed at least once per species.
- All pivotal comparative BA/BE studies
- First clinical trial in subjects
- Pivotal early patient trial(s), once per patient population
- First or pivotal trial in patients with impaired hepatic and/or renal function

ISR is conducted by repeating the analysis of a subset of samples from a given study in separate (i.e., different to the original) runs on different days using the same bioanalytical method.

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- The extent of ISR depends upon the analyte and the study samples and should be based upon an in- depth understanding of the analytical method and analyte. However, as a minimum, if the total number of study samples is less than or equal to 1000, then 10% of the samples should be reanalysed; if the total number of samples is greater than 1000, then 10% of the first 1000 samples (100) plus 5% of the number of samples that exceed 1000 samples should be assessed. Objective criteria for choosing the subset of study samples for ISR should be predefined in the protocol, study plan or an SOP. While the subjects/animals should be picked as randomly as possible from the dosed study population, adequate coverage of the concentration profile is important. Therefore, it is recommended that the samples for ISR be chosen around the maximum concentration (Cmax) and some in the elimination phase. Additionally, the samples chosen should be representative of the whole study.
- Samples should not be pooled, as pooling may limit anomalous findings. ISR samples and QCs should be processed and analysed in the same manner as in the original analysis. ISR should be performed within the stability window of the analyte, but not on the same day as the original analysis.

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The percent difference between the initial concentration and the concentration measured during the repeat analysis should be calculated in relation to their mean value using the following equation:

- For chromatographic methods, the percent difference should be within ±20% for at least 2/3 of the repeats. For LBAs, the percent difference should be within ± 30% for at least 2/3 of the repeats.
- If the overall ISR results fail the acceptance criteria, an investigation should be conducted and the causes remediated. There should be an SOP that directs how investigations are triggered and conducted. If an investigation does not identify the cause of the failure, the potential impact of an ISR failure on study validity should also be provided in the Bioanalytical Report. If ISR meets the acceptance criteria yet shows large or systemic differences between results for multiple samples, this may indicate analytical issues and it is advisable to investigate this further.
- Examples of trends that are of concern may include:
  - All ISR samples from one subject fail
  - All ISR samples from one run fail
  - All aspects of ISR evaluations should be documented to allow reconstruction of the study and any investigations. Individual samples that are quite different from the original value (e.g., > 50%, “flyers”) should not trigger reanalysis of the original sample and do not need to be investigated. ISR sample data should not replace the original study sample data.

ISR Summary

Requirement	ICH M10	FDA	EMA
Studies in scope	TK species FIH Pivotal BA/BE (Pivotal) FIP (Pivotal) hepatic/renal	All Pivotal for labelling or approval: TK/species All BE, pivotal PK and PD	TK/species BE, FIH, FIP, hepatic/renal
When to execute	Not on the same day as the original analysis	In separate runs	In separate run, at different days
How many samples	10% first 1000	10% first 1000	10% first 1000
	5% > 1000	5% > 1000	5% > 1000
Which samples	Near C_{max} and elimination phase - representative for the whole study	Near C_{max} and elimination phase	Near C_{max} and elimination phase
Acceptance Criteria	2/3 (67%) within 20% (LC/MS), 30% (LBA)	2/3 (67%) within 20% (LC/MS), 30% (LBA)	2/3 (67%) within 20% (LC/MS), 30% (LBA)
If it fails…	SOP based investigation	SOP based investigation	Investigation

Pre-meeting survey

	question	y	n
Q1	Have you changed your process for ISR since ICH M10?	6	40
Q2	Are you performing ISR for more studies than required as per ICH M10?	17	21
Q3	if so, why?		
Q4	if so, for which studies		
free text			

Summary on ICH M10 related to ISR (EBF Nov 2022 Workshop)

- **Recommended** to use ICH M10 to reset your compass and not overdo ISR – not all studies are in scope for ISR, they never were and still aren't
- Understand pivotal as where the primary endpoint of the clinical trial is related to PK.
- Know your project and understand the purpose of each and every study you support.
- Be clear how to distinguish between ISR conduct per guidance requirements and how to used ISR to mitigate business risks for pivotal studies.
- For guidance on for how, when and where to conduct and document investigation for failed ISR, and for selection of samples for ISR, see Bioanalysis, (2009) 1(6) 1049-1056

Feedback from the HA

- FDA 5-day inspection (Aug 2023) for 3 BE studies.
- Hybrid Immuno-affinity LC-MS/MS assay validated in accordance with FDA/EMA BMV (with acceptance criteria ± 20%).
- Plasma samples analysed in accordance with FDA/EMA BMV (with acceptance criteria ± 20%).
- ISR performed in accordance with FDA/EMA BMV (with acceptance criteria ± 30%) and passed.

- Request from HA to provide ancillary summary statistics (Mean, Median, Min, Max, 25th percentile and 75th percentile)
- Provided requested statistics based on ISR % bias (our assumption).
- Provided requested statistics based on ISR concentration (later clarified by FDA).

Feedback from the HA

Statistical Evaluation of ISR % Bias	
Mean	-3.9
Standard Deviation	17.1
Median	-4.3
Minimum	-121.4
Maximum	53.9
25th Percentile	-14.6
75th Percentile	6.6

Statistical Evaluation of ISR Original Concentration (pg/mL)	
Mean	657
Standard Deviation	238
Median	642
Minimum	152
Maximum	1370
25th Percentile	492
75th Percentile	793

Statistical Evaluation of ISR Repeat Concentration (pg/mL)	
Mean	634
Standard Deviation	236
Median	618
Minimum	133
Maximum	1280
25th Percentile	466
75th Percentile	776

- 453 ISR samples analysed with 93.4% within ± 30%

- Summary statistics of % Bias does allow identification of trends.
- Summary statistics of Original and Repeat concentration adds no apparent value.
- No further feedback from FDA.

So, let's discuss (and recommendation)

Why do we continue to do more? It's not for free…

Have we ever seen a failed ISR in a study not in scope of the guideline jeopardising patient safety?

Not in scope meaning, only below is in scope…

- For nonclinical studies within the scope of this guideline, ISR should, in general, be performed at least once per species.
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Side discussion on ‘pivotal studies

See slide deck: *G01FB-C. Katja - Michaela - pivotal studies.pptx*

Raw data from the pre-meeting survey comments

- In the next slides we provide the unredacted details from 56 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 56 files

On Q1: Have you changed your process for ISR since ICH M10?

- N, No new studies/projects started since the implementation of ICH M10
- Investigation would be required in instance of barely passing ISR Yes, single outliers should not be reanalysed or investigated.
- Y - start investigation when variability is seen between runs while acceptance criteria are met
- Y (used as process control to monitor assay performance in study sample analysis)
- ISR was strong already before
- y re reporting, ISR in now included in the BDR, previously only in the MVR.
- N - For one project, we only performed ISR once for rat Tox studies
- N (it was already in line with ICH M10)
- Y. no more reassays on ISR.

On Q2: Are you performing ISR for more studies than required as per ICH M10?

Yes = 17	No = 21

- Depending on sponsor requirements No, unless requested by clients
- y, PK and PD, but in the future only PK
- y, run ISR for each clin study
- Y - Potentially our company is applying the product in a pipeline approach and currently we perform ISR per indication.
- possibly yes in the past
- If customer ask for it

On Q3: If so, why…

- sponsor request x 8
- Really nice, process control of the study sample analysis.
- in process control
- Choosing to apply ISR in all Clinical Studies unless our Sponsor asks us not to.
- Risk is then owned by the Sponsor.
- just in case.
- difficult to keep track of all clinical studies in terms of patient population, dosing regimes etc so easier to conduct on all rather than miss one accidentally
- Better to do more and be safe
- Automation and lean procedures means it is nota lot of extra effort
- If you fail an ISR having more data allows statistical analysis and make it less risky
- included as standard for all sample analysis studies
- get reliable clinical PK data
- in study QC
- Unclarity in the guideline (first clinical trial in subject vs once per patient population)
- to minimize question
- unclear scope of some studies, lack of communication with sponsor
- each study is unique
- We always ran ISRs in all studies
- on Sponsor's request
- For pilot and Biomarker studies to check method performance (if needed)
- To cover different patient populations
- because we run cancer studies and the population is not equal in each study
- General expectations of Sponsors, team members.

On Q4: if so, for which studies?

- Most regulatory studies
- As defined by client clinical studies, most likely due to insufficient clearness if a study is "pivotal"
- clinical trails with outsourced BA
- All Clinical Trials x 4
- All PK and TK studies
- all studies
- 10% of samples in all studies.
- not only once per patient population or species
- PK with low sample numbers and/or occasion frequency
- All Pivotal trials per indications other preclinical , other clinical studies
- ISR are included in each new study
- Various Clinical phase only
- other preclinical , other clinical studies
- Urine assays

On Q5: Free text

- Definition of pivotal/comparative is crucial for this discussion.
- If ISR meets the overall acceptance criteria, but shows large/systematic differences between results (e.g. all samples of a subject or a run or a BA phase period fail), this should also be investigated