ISR in every clinical study –
What have we done? ....What have we learned?
...Where do we go from here?

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Incurred Sample Reanalysis in guidance I

- Incurred sample reanalysis (ISR) is a regulatory requirement in bioanalytical method validation - to demonstrate the reproducibility of the measured study sample concentration levels.

- It is assessed by repeating the analysis of a subset of samples from a given study in separate runs on different days to support the precision and accuracy measurements established with spiked QCs using the same bioanalytical method.

- For at least 67% (2/3) of the repeats the percent difference between the initial and repeat concentration should be within 20% of their mean.
Draft FDA Guidance for Industry:
“ISR is expected for all in vivo human BE studies and all pivotal PK or PD studies.”

Guidance for Industry
Bioanalytical Method Validation

EMA Guideline on bioanalytical method validation:
“ISR should be done at least in the following situations: all pivotal bioequivalence trials, first clinical trial in subjects, first patient trial, first trial in patients with impaired hepatic and/or renal function.”
Clinical Bioanalysis Alliance introduction
The AstraZeneca and Covance Laboratories Clinical Bioanalysis Alliance was launched in 2011

- 5 year strategic alliance
- Supported all small molecule (LC/MS) clinical bioanalysis (Ph I-Ph IV)
- Global Covance policies and templates were applied
- Roles, responsibilities and standard processes were determined as part of the implementation
- Covance Project Managers represented bioanalysis within the internal AstraZeneca study teams
- AstraZeneca bioanalytical scientist were not involved in study activities but rather focused on working strategically at the project level
What have we done?

Standard processes agreed for incurred sample reanalysis:

- ISR to be included in every clinical study

Motivated at the time by:

- It can be challenging to know what studies may be “pivotal” as development progresses
- Efficiency gain in that the AstraZeneca/Covance teams should not be required to determine whether ISR is needed on a study by study basis
- Removes any inconsistencies / subjective variability between the different team members in deciding when to conduct ISR
- ISR data provides an additional process/quality check parameter
What have we done?

At the end of the first 5-year cycle of the alliance we have data collected from the:

>120 different projects (development compounds) supported
>130 methods developed
>310 methods validated
>550 clinical studies supported with sample analysis
>350,000 samples analysed

Unique ISR data set has been generated
What have we learned?

In addition to the data set an ISR questionnaire was sent out to all Covance PMs and AstraZeneca BioA scientist

- Establish the number of "true" ISR failures and ISR investigations, to differentiate poor method performance from human/technical error
- Take a deep dive into the true ISR failures – understand when, where and why ISR fails and any implication on study and/or patient safety

ISR Questionnaire format

<table>
<thead>
<tr>
<th>Project</th>
<th>Assay platform</th>
<th>Assay matrix</th>
<th>Study in which ISR failed</th>
<th>Observations from the ISR data</th>
<th>RCA reason for ISR failure</th>
<th>Previous ISR experience with the assay</th>
<th>Adapted BioA approach</th>
<th>Implication on study level</th>
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What have we learned? – ISR overview

Out of 550 clinical studies conducted over 5 years true ISR failures have been experienced in 10 studies (2%) 

Additional ISR "failures" have been experienced due to technical issues or human errors (1%) and ISR investigations have been triggered by trending results (1%)
What have we learned? – Study type

8 of 10 true ISR failures were experienced in first time in man (FTIM) study or first time in use (FTiU) of the assay.

- 80% of studies with ISR failing upon first time use of the assay (often FTIM study).
- 10% of studies with ISR failing when using assay for sample analysis in new patient population.
- 10% of studies with ISR failing after long time use of assay.

1 ISR failure was experienced in new patient population (10%) and only 1 of the ISR failures was experienced after long term use of the assay (10%).
What have we learned? – Matrix type

In relation to the number of studies conducted in each matrix

ISR fails more often in urine matrix and other more complex matrices (such as CSF, PUF, dialysate) compared to plasma
What have we learned? – ISR failure categories

Metabolites often play an important role in the root cause of ISR failures …

- Selectivity
- Sample preparation procedure
- Thawing/mixing
- Overall variability

…being unstable, back converting or having an impact on overall method performance …
What have we learned? – Study impact

In a majority (70%) of the studies impacted by ISR failure data has been reported as is only with a note to file about the investigation conducted.

ISR failures in FTIM study where plasma PK data is requested for dose escalation decision most likely to have great study impact
LC-MS method for quantification of a small molecule in plasma (range 2-2000nmol/L) developed for FTIM study

- The initial method was protein precipitation based.
- Issues with matrix effects in individual plasma lots during development triggered additional method development

Addition of β-Mercaptoethanol at 1% v/v combined with protein precipitation reduced the variability and a full validation was performed successfully.
ISR failure 1 – Initial observations

ISR assessments were conducted continuously during cohort 1-4

- Limited ISR data from cohort 1 did not indicate any reproducibility issues – 92% were within the 20% acceptance criteria

- In the next ISR run with samples from cohort 1-3 most of the low concentration terminal end samples assessed were out of acceptance – still considered a partial data set and overall ISR acceptance criteria met (72% within the 20% acceptance criteria)

- As data from cohort 4 was included and all ISR samples from the terminal phase were outside acceptance criteria ISR failed overall acceptance criteria (65% within the 20%)
The ISR samples not meeting the acceptance criteria:

- All show a positive bias compared to the original data
- A great majority were from the terminal end (collected 48-120h after dose) and had low sample concentration levels

- All sample analysis put on-hold
- Further dose escalation in FTIM study put on-hold
during ongoing ISR investigation
ISR failure 1 – Investigation data I

Could the great variability be related to the previous binding issues in different individuals experienced during method development?

In incurred sample pools with similar concentrations and sample collection time created, investigation on 3 different extraction days

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<th>EXTRACTION DAY 1</th>
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<th>EXTRACTION DAY 3</th>
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<td>1% BME</td>
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Samples where ISR variability observed (collected 48-120h after dose)

Samples where ISR variability not observed (collected 0.5-12h after dose)

The impact of increased concentration of β-mercaptoethanol (5%) investigated.
Higher sample concentrations observed at 1% BME – issue not related to binding issues observed during development

Issue related to the samples’ collection time (48 -120 hours after dose)

Samples collected 0.5 to 12 hours after dose - results are consistent from day 1 to day 3 using either 1 or 5% BME

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<td>1% BME</td>
<td>5% BME</td>
<td>%CV</td>
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<td>10.5</td>
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<td>POOL 12</td>
<td>1470</td>
<td>1510</td>
<td>1.9</td>
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The presence of an unidentified component (possible metabolite since it can be correlated with the sample collection time) suspected to cause the variability in the incurred sample results.

Increasing the amount of β-mercaptoethanol in the method may increase the reproducibility of the results, but concentrations are lower and results are not consistent

- Current assay withdrawn
- A new assay developed and validated using pools of incurred samples.
- New assay used to reanalyze all samples in FTIM study and ISR assessed with 97% pass rate
- PK back up samples valuable for metabolite investigation
Reanalysis of all study samples confirmed that both the overall exposure (AUC) and the half-life ($t_{1/2}$) had been overestimated by about 10%.

Failing ISR had a significant impact on study timelines and BioA workload but without any risk for patient safety.
ISR failure 2 – ongoing small molecule program

LC-MS combination method for quantification of two small molecules in plasma to support ongoing clinical program.

No stability issues had been noticed during method validation in spiked plasma samples.

**Initial Observations**

- ISR for one of the analytes in ongoing PK study was not met. Overall pass rate was only 41%.
- All ISR samples that did not meet the acceptance criteria had negative bias compared to the original concentrations.
- Samples that had been thawed 3 times (3F/T cycles) had even lower bias compared to samples with 2 F/T cycles – suspect stability issue!
- ISR for second analyte have a 100% overall pass rate - no performance issue?
ISR failure 2 – root cause and approach

Scientific rather than performance cause - indication that study samples are unstable upon repeated freeze/thaw

Method OK for initial analysis using samples that have not been thawed?

Agreed investigational approach by cross functional team

• Repeat already performed ISR and continue ISR assessments with current method using back-up samples that had never been thawed (in a tiered approach)
  • 156 out of 162 samples did meet the acceptance criteria when ISR was repeated on back-up samples - overall pass rate 96%!

Method OK to use for analysis of study samples without multiple F/T
Where do we go from here?

- First time use of an assay
- First time use of an assay in a new patient population

ISR data provides valuable insight into the assay’s fit for purpose and the reliability of the reported sample concentrations.

- No reason to do ISR assessment beyond regulatory requirements

- Known or unknown metabolite(s) often plays an important role in the ISR failure

All previous BioA/DMPK experiences are of great value and should be taken into consideration when preparing for clinical assay development
Acknowledgement

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