Feedback on EBF survey on Incurred Sample Stability (ISS)

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on behalf of EBF team (TT-02)

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Background

- 2008 - Discussions within EBF: ISS vs ISR
- First EBF- Survey on Incurred Sample Stability 2009
- Second EBF-Survey on Incurred Sample Stability 2011

Why consider ISS analysis?

- Incurred Sample Stability (ISS) is not a regulatory requirement

  however,

- It may be difficult in situations to distinguish between **I**ncurred **S**ample **R**eproducibility (ISR) demonstrated by Incurred Sample reanalysis - and **I**ncurred **S**ample **Stability** (ISS)

Why consider ISS analysis?

Regulatory

- "For compounds with potentially labile metabolites, the stability of analyte in matrix from dosed subjects (or species) should be confirmed."

  FDA guidance on bioanalytical method validation, May 2001

- "Additional validation may include investigation of samples from dosed subjects."

  FDA guidance on bioanalytical method validation, May 2001

- "Study samples may be used in addition to QC samples, but the exclusive use of study samples is not considered sufficient as the nominal concentrations of those samples is not known."

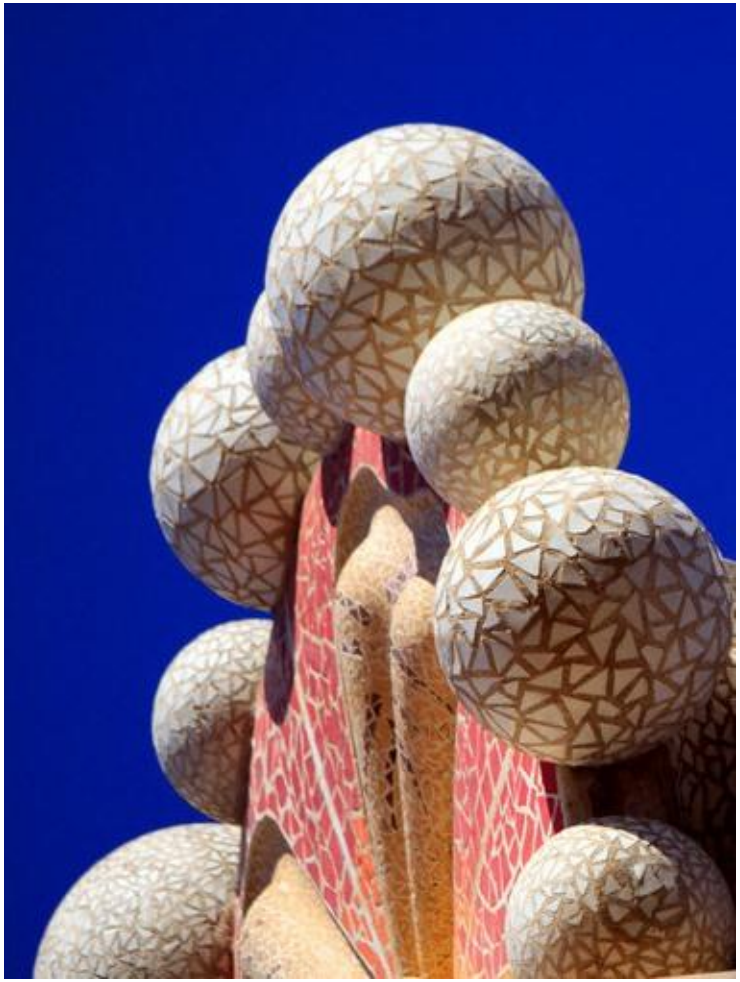
  EMA guideline on bioanalytical method validation, February 2012

Why consider ISS analysis?

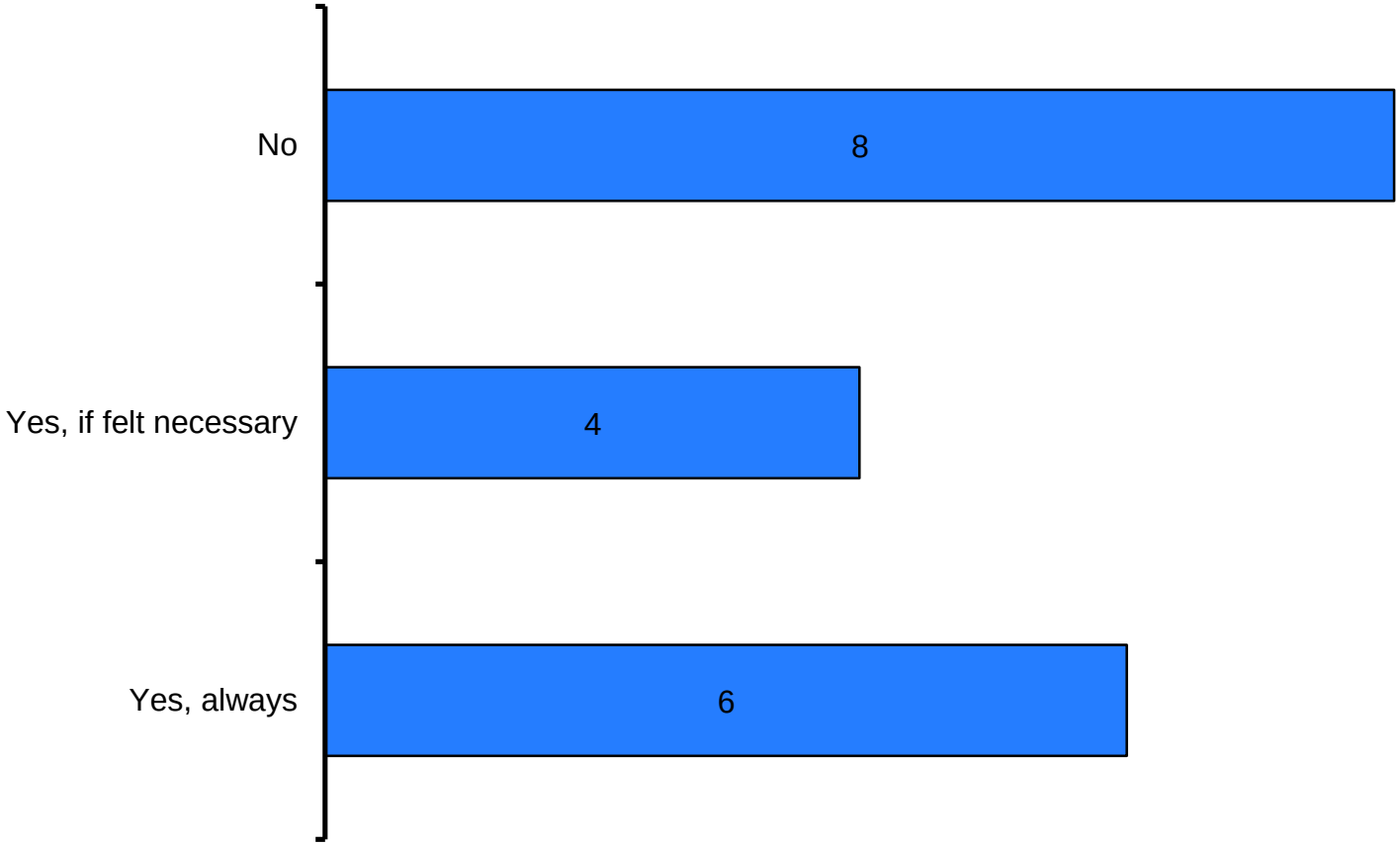
Scientific

- Back conversion of unknown conjugates – directly on parent or metabolites
- Acyl Glucuronides
- labile analytes / metabolites
- Enzymatic degradation of analyte(s)
- Species differences

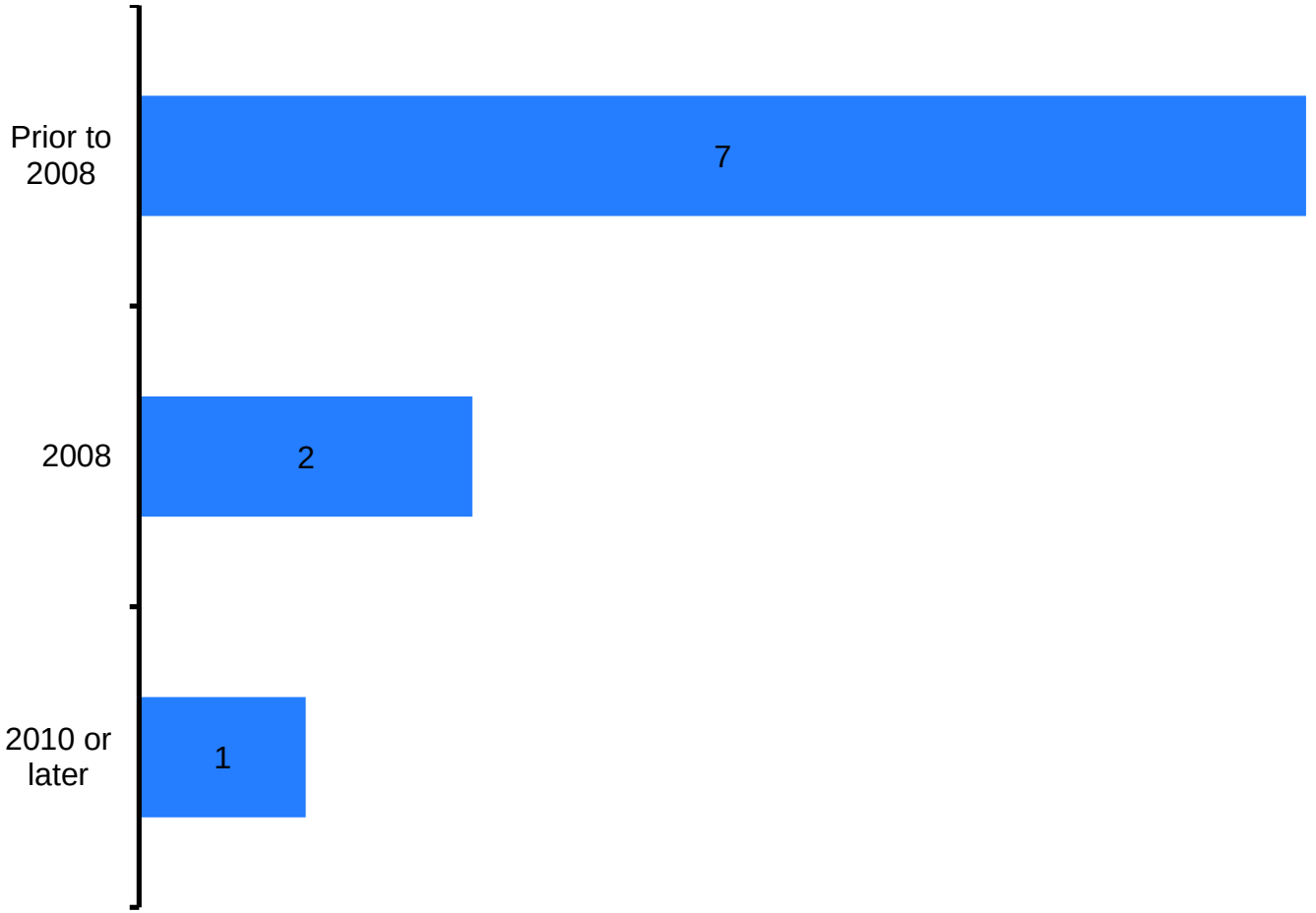
EBF-Survey on Incurred Sample Stability



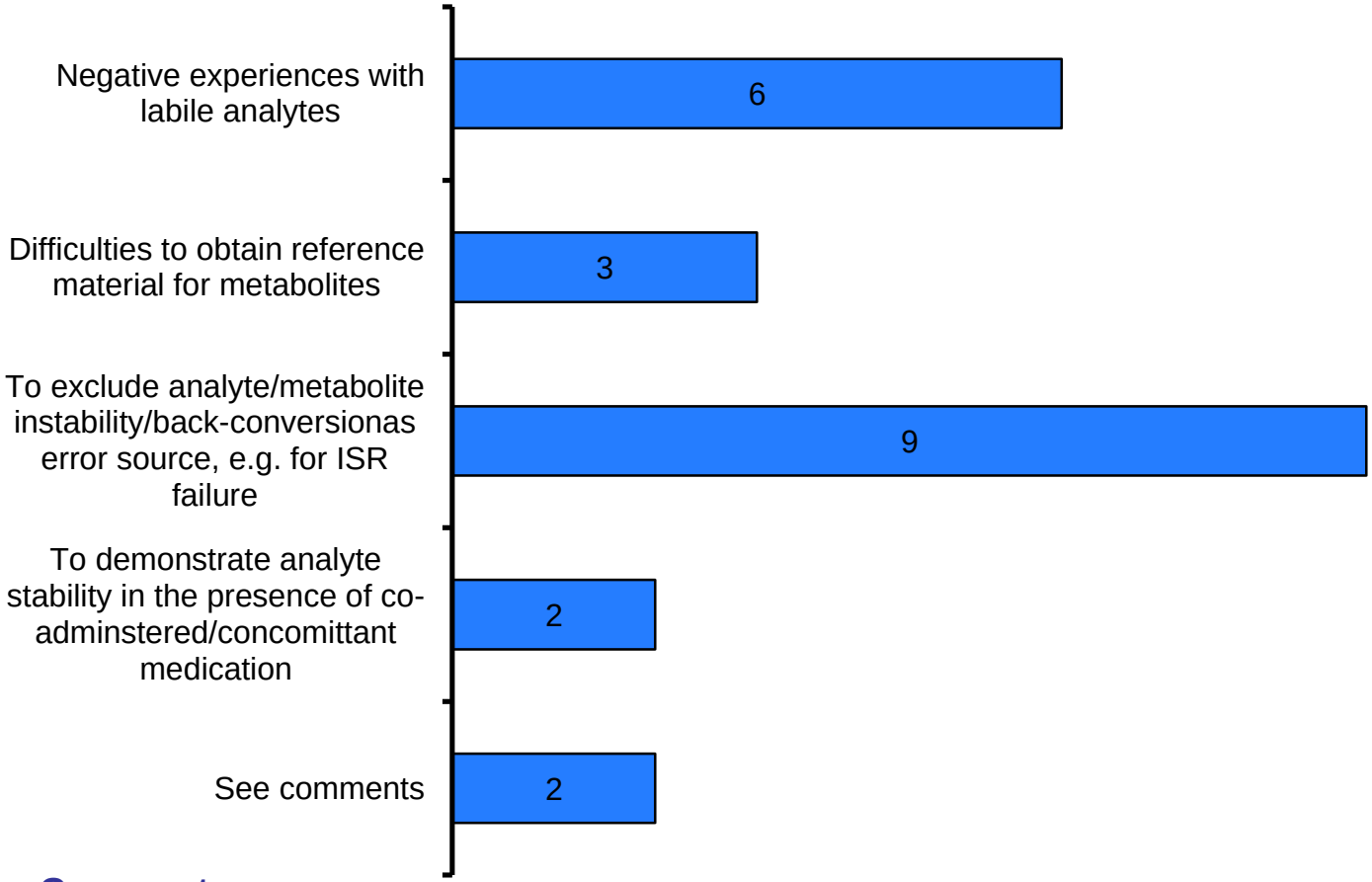
Is ISS performed in your bioanalytical laboratories?



When did you implement ISS?



What is your rationale for testing ISS (up to 3 voting's)?

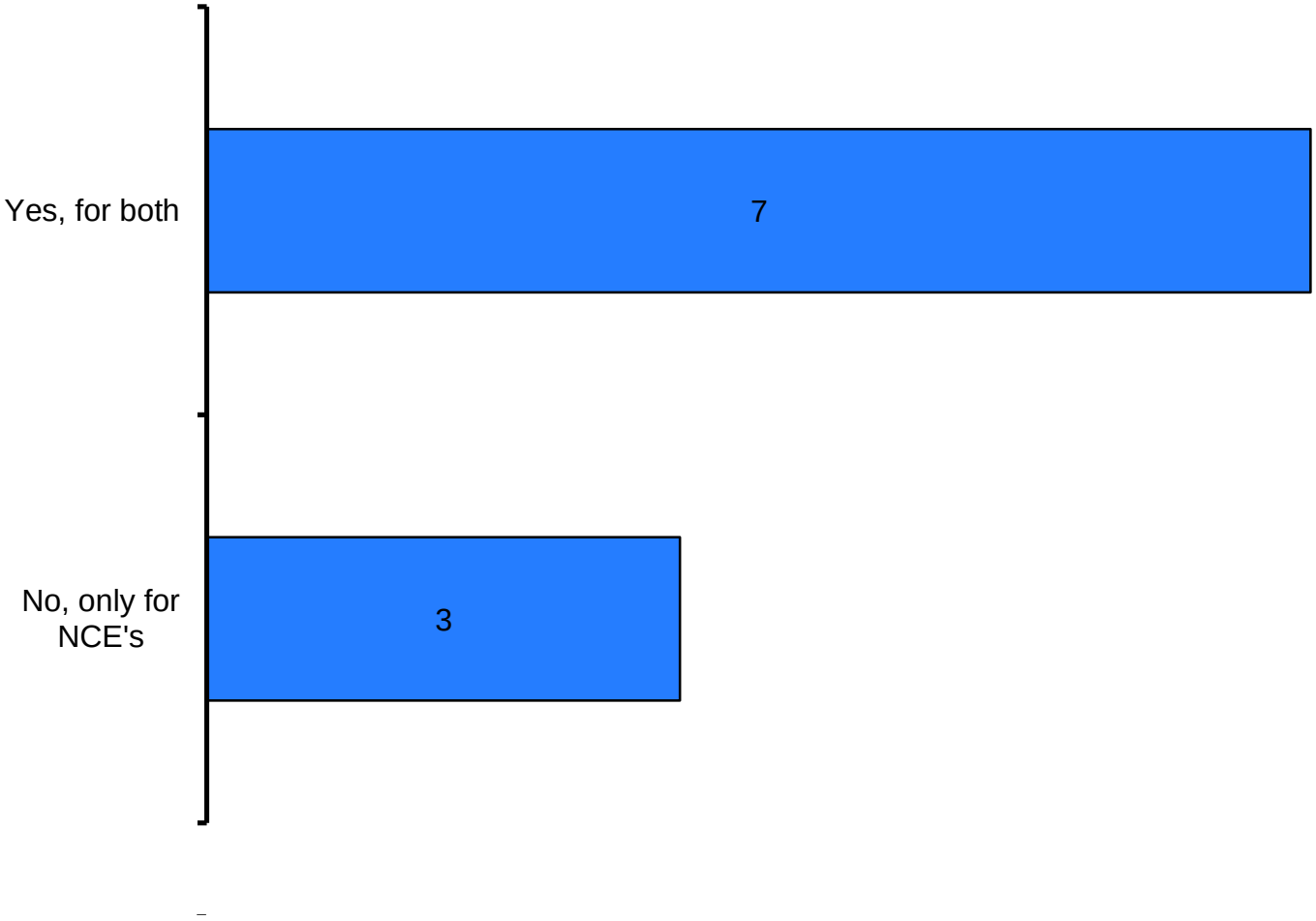


Comments:

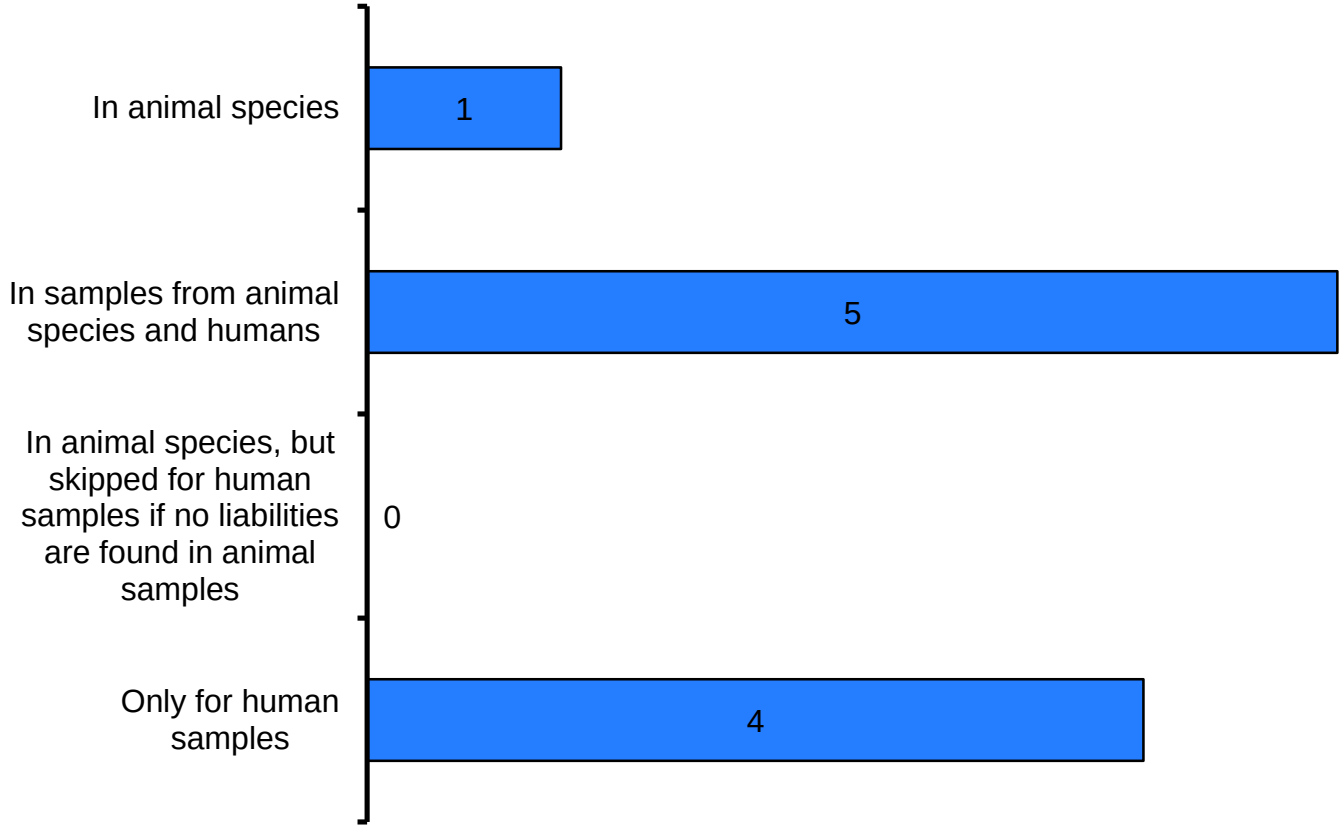
To know what is the maximum time available between the collection of samples and the assay

To investigate if ISS can support and supply LTS demonstrated by spiked samples

Would you consider ISS for NCE's and NBE's?



Do you investigate the Stability of Incurred Samples?

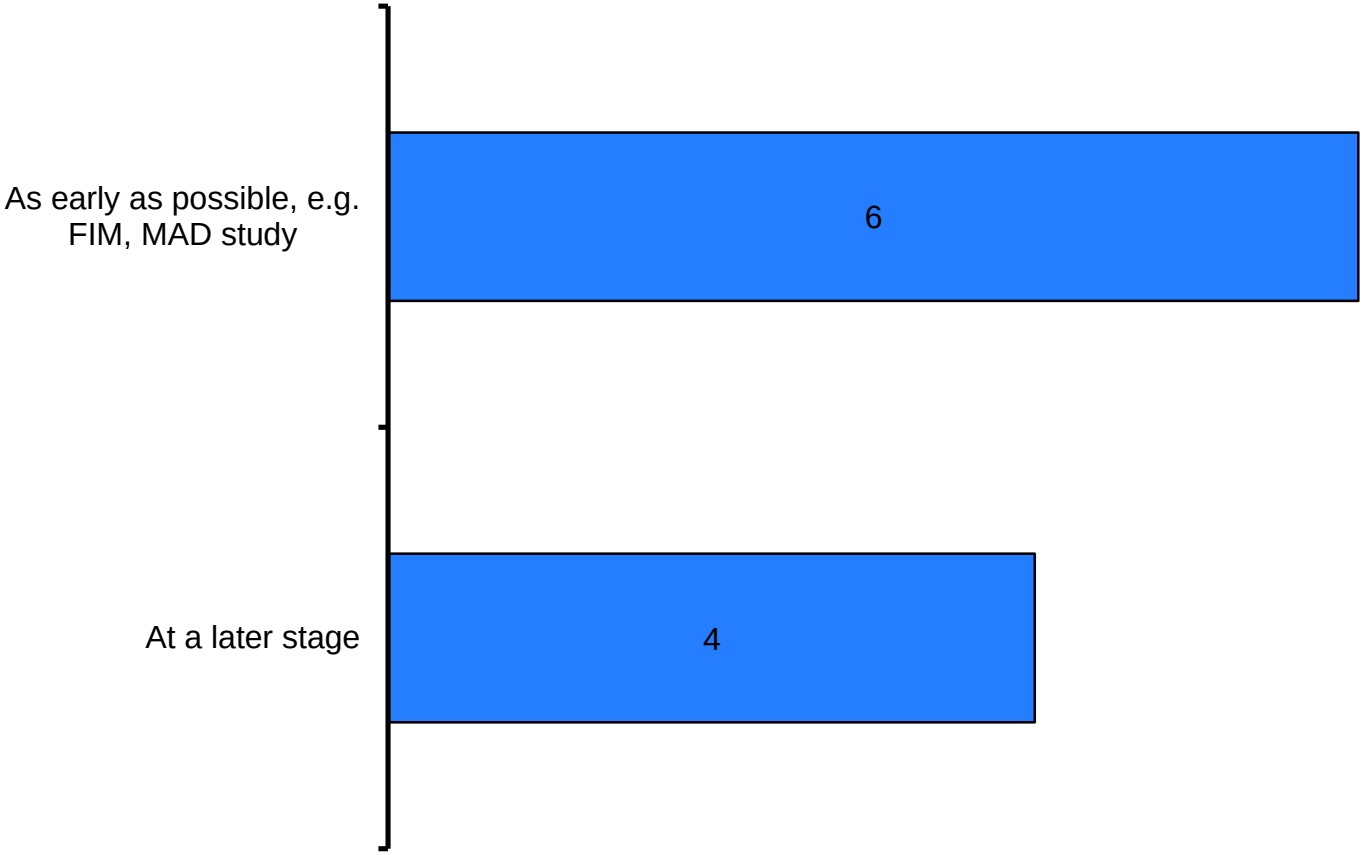


Comments:

In human samples; in animals species only if we have hints of instability

Human always, animal on a case by case situation (e.g. labile metabolites, long term tox with running samples at end of study)

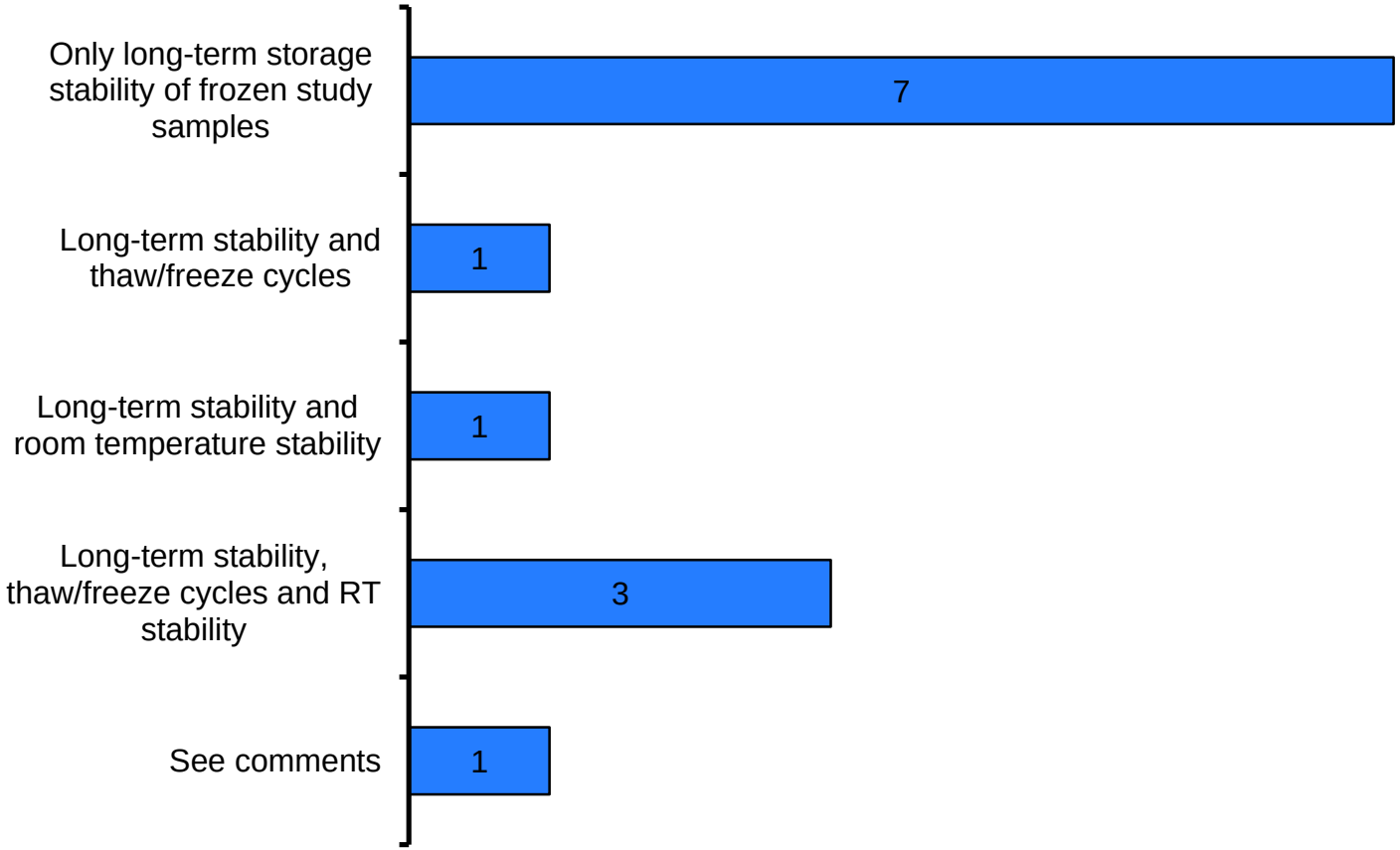
ISS in human samples is conducted?



Comments:

At a later stage we use the samples from the first couple of human trials to prepare pools for ISS. Aim is to have LTS data available before phase III

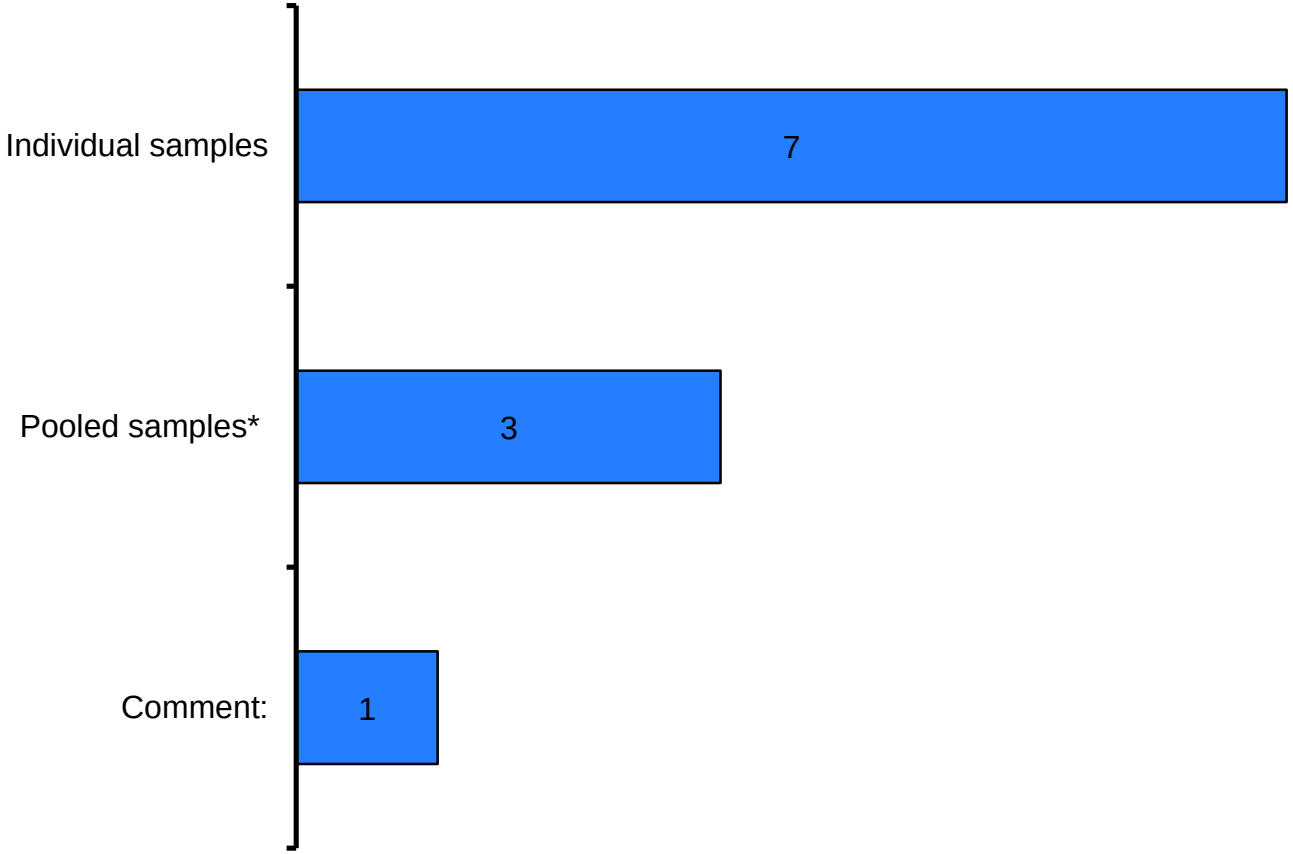
Which experiments do you include in ISS?



Comments:

Case by case. Long term storage (freezer) always, freeze-thaw and room temp on a case by case basis. If nasty (very) unstable metabolites then we include RT and FT

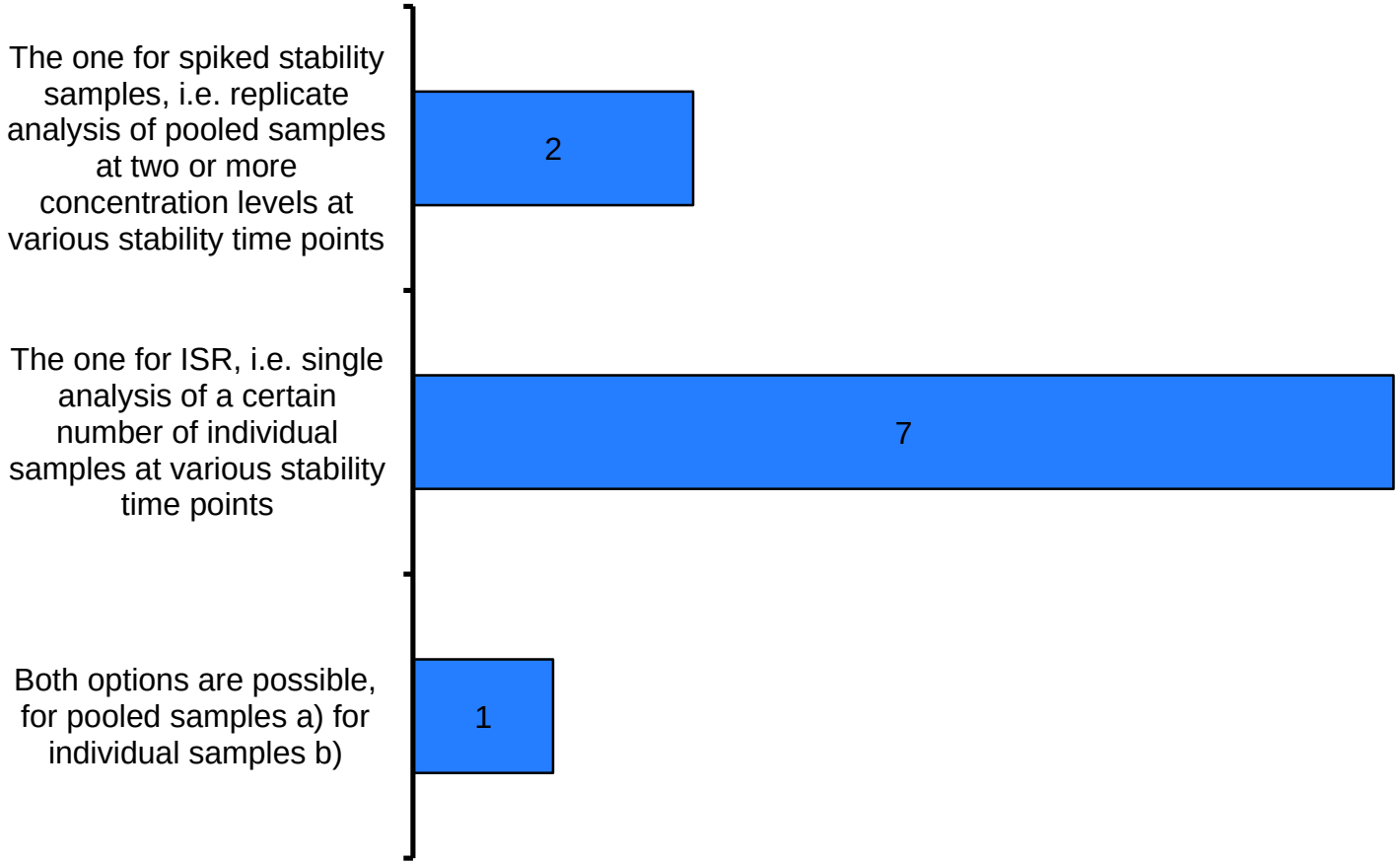
Do you prefer to use individual study or pooled samples?



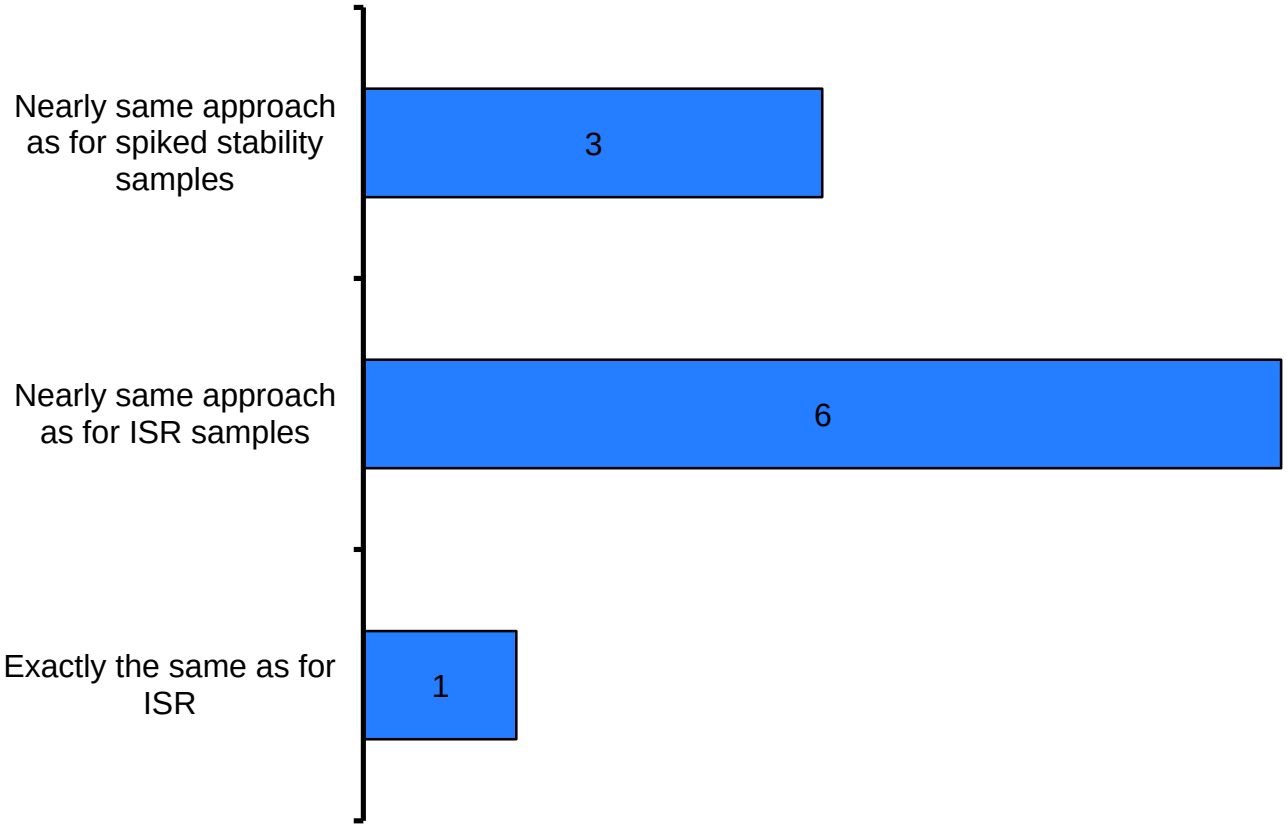
Comments:

**) These would require an established reference concentration at t = 0*
Occasionally also individual e.g. if at a moment in time is the only way to obtain LTS data for x months/years. Individuals are also used to get an impression on how ISS may look when we start a protocol led ISS study.

Your experimental design of ISS is similar to?



Which acceptance criteria do you apply for ISS?



Comments:
If at least 6 stability time points are available, data should be analyzed by regression analysis. The limit of stability will be estimated as the last time (within the range of observed time-points) at which the 90% two-sided lower confidence interval for estimated stability from time 0 h (t0) is contained within the specification interval (85%, 115%)

General Comments (condensed):

- In nearly 100% of all cases, the ISS (up to 2 years of storage) could be shown.
- We do not perform ISS as part of our standard validation process. It is a case by case decision mainly driven by the evaluation of the stability of parent compound and metabolites.
- 2 recent examples with antibody drug conjugate (ADC): No issue of stability with spiked frozen QCs up to 2 years. With incurred frozen samples, increase of ADC concentrations with time compared to those measured at time 0 (same stability points of QCs and incurred samples analyzed in the same run). No obvious explanation
- Note, Acyl Glucuronides are only stable when hell freezes over.

General Comments (condensed), cntd:

- ISS is well established in my company. We perform it in the long term tox rat and large species mainly, also in both normal and patient samples. Generally, for stable samples we see no difference from in vitro stability. For unstable compounds, sample stabilizing procedures must be in place before embarking on ISS.

- We have experienced in a few cases that ISR/ ISS conducted in FIM provided information's on human specific conjugates (direct N- , O-gluc or acyl gluc conjugates) or a different distribution between conjugates and aglycones in human than in animal toxicity species at a stage in the development where we did not yet have any human AME data or human screening data. Here IRS showed poor reproducibility that after investigation was explained by ISS - instability that was not observed in spiked samples. After optimization of stabilization of extract, ISS was confirmed, IRS was confirmed and study continued.

Conclusion:

- Currently no uniform approach within EBF member companies on if, how and when to conduct ISS.
- However, the team consider ISS to be an imortant parameter that should be adressed to some level, depending on a scientific case by case evaluation.
- Team/EBF plans to continue the discussion in preparation of an EBF recommendation

Acknowledgement

Thanks to

- All EBF members for input to the survey

- EBF Topic Team 2

  - Silke Lüdtke, Boehringer-Ingelheim *(Chair)*
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  - Morten Kall, H. Lundbeck