

The logo for the European Bioanalysis Forum (EBF) is located in the top right corner of the slide. It consists of the letters 'EBF' in a white, sans-serif font. Below the letters is a white, curved line that starts under the 'E' and ends under the 'F', resembling a stylized arc or a partial circle. To the right of this arc, the words 'European Bioanalysis Forum' are written in a smaller, white, sans-serif font, stacked vertically.

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DBS Dilution Sub-team

Summary Nov 2011

presented by Susanne Globig

Team members (alphabetical order)

- Aziz Filali-Ansary (Sanofi-Aventis)
- Stephanie Fischmann (Abbott)
- Susanne Globig (Actelion)
- Sally Hannam (Icon)
- Warren Keene (AstraZeneca)
- Markus Kohlmann (Sanofi-Aventis)
- Elizabeth Thomas (Icon)
- Phillip Turpin (Covance)

Aim and scope:

Aim: To investigate and summarise ways to perform dilutions for DBS samples

1. Off-line approaches:

- a) Matrix match dilution
- b) Solvent dilution
- c) MS based approaches

2. On-line approaches

- a) Internal std based
- b) MS based approaches

1. Off-line approaches

a) Matrix match dilution

- Ensures matrix effects relatively constant

- Matrix blank + IS
 - Most common approach used by team members
 - Drawback: labor intensive, not easy to automate

- Variant: Dilute with extracted blank matrix (no IS)
 - Extract sample with a more concentrated IS
 - Dilute with the blank extract

1. Off-line approaches

a) Matrix match dilution, cntd

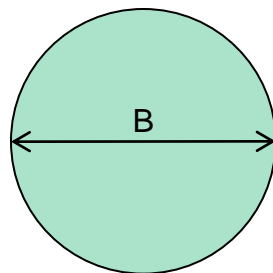


Halo (doughnut) dilution

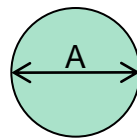
- Dilution of a smaller punch with blank extraction
- Less commonly used by team members but routinely used in some laboratories
- Can be semi-automated on certain instruments
- Dilution factors possible (up to x80 tested)
- Hematocrit values might alter halo results?
- Example of process

Halo (doughnut) dilution

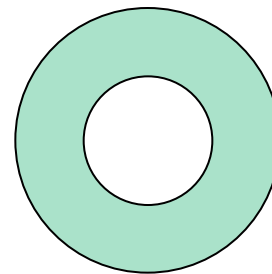
- » Assay punch (Diameter B)
- » Punch blood spots requiring dilution with a smaller core (Diameter A)
- » To maintain a consistent level of matrix in each sample, prior to extraction add a core (diameter B) from a control matrix sample which has had a core (diameter A) removed.



Assay punch



Diluted sample
punched with
smaller diameter



Control matrix sample
with core removed

$$\text{dilution factor} = \frac{(\text{core diameter } B)^2}{(\text{core diameter } A)^2}$$

1. Off-line approaches

b) Dilution with solvent

examples for 10-fold dilution

- Extract with x10 more concentrated IS, dilute x10 with solvent
Same injection volume used for entire batch.
- As above, inject x10 less for dilution samples.
- Extract all samples with the same concentrated IS solvent, dilute the extract x10 after, and track the dilution by the response of the IS

All 3 approaches may be biased by matrix effects
(less of an issue for stable isotope labelled IS)

Dilutions up to x100 have been reported.

1. Off-line approaches

c) LC-MS approaches:

- Multiple assay ranges
 - Used to support preclinical and clinical studies where dose escalation requires quantification across a broad range of concentrations.
 - Not a preferred approach as need to perform two validations.
- Isotope peak detection
 - Selection of a less abundant isotope peak for dilution samples generates a reduction in signal

1. Off-line approaches

c) LC-MS approaches, cntd:

- Alternative mass transition and MS condition
 - Use less sensitive mass transition or MS condition for the dilution samples
 - Results suggest up to a 100,000 fold linear range can be achieved on a MS/MS instrument.
- Injection of different volumes
 - Only possible if the assay does not use an IS

2. On-line approaches to DBS dilution

a) Instruments still being developed/ tested

- Too early to fully explore
- Some instruments use more or less IS in the loop
- IS applied to the spots

b) LC-MS approaches: Isotope peak detection and alternative MS conditions can be applied in the same manner as discussed for off-line work

Next steps

- Off-line DBS dilution experiments planned for the next couple of months...
 - Isotope peak detection
 - Alternative mass transition
- Publication of ideas and findings related to DBS dilutions

Conclusions

- Many different approaches to DBS dilution possible
- Different companies have different preferences
- Regardless of which approach is used for a specific assay, that procedure should be confirmed as suitable during validation (precision and accuracy within usual 15% limits)