EBF Recommendation on the use of DBS in regulated Bioanalysis

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on behalf of the EBF

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Presentation outline

- DBS versus Micro sampling
- EBF & DBS – the story so far...
- Plasma versus blood
- Results from the EBF DBS - Micro Sampling Consortium
  - Internal Standard addition
  - Hematocrit
  - Spot homogeneity
  - Aging of samples
- Conclusions & Recommendations
- Future Perspective

DBS versus Micro sampling

All volumes smaller than 20-50 ul (depending on who you talk with) belong to the ‘micro sampling’ family

Dried Matrix spots

DBS (Dried Blood Spots)

Dried Plasma Spots

Dried “X” Spots

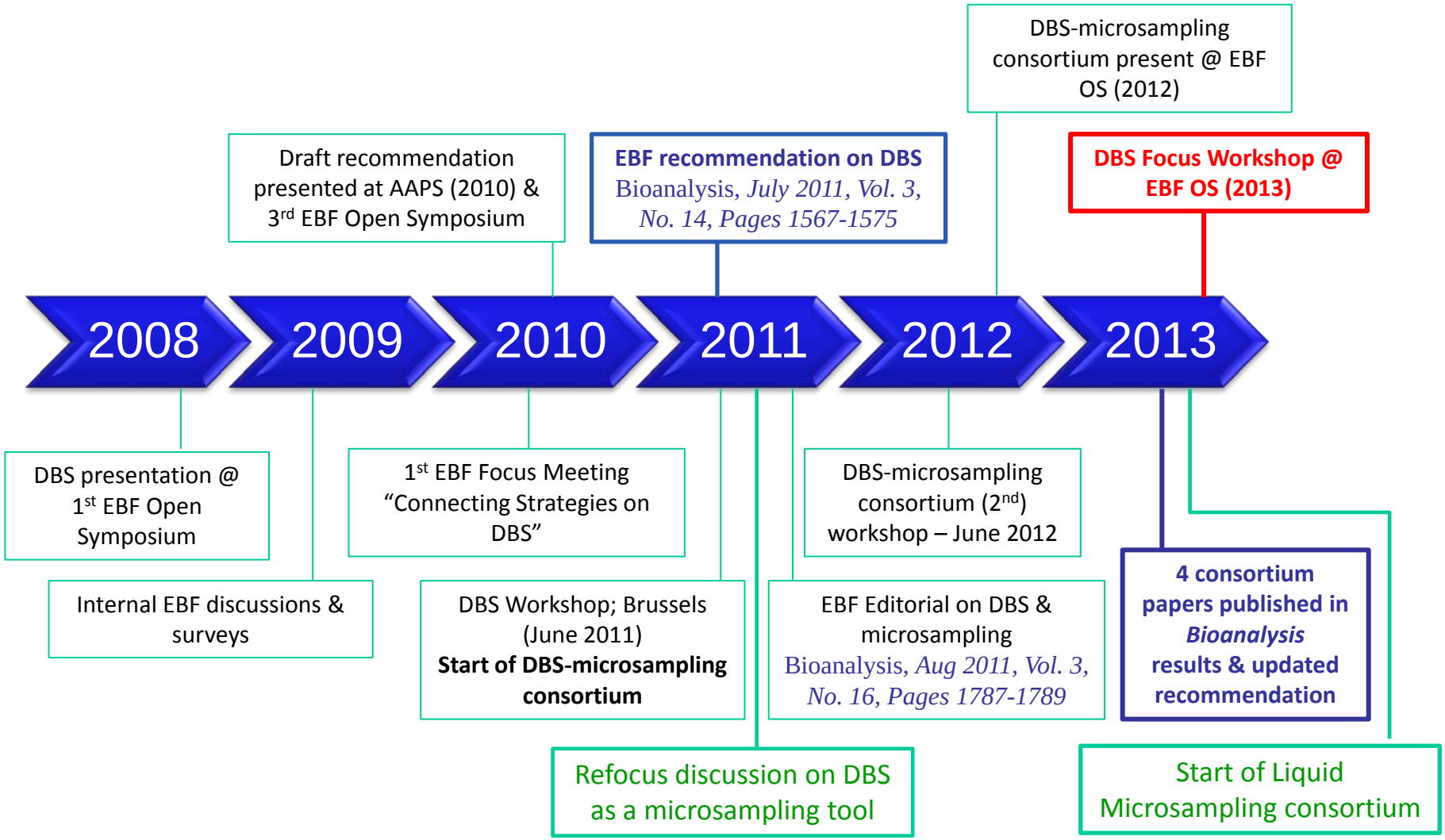
Small liquid samples

CMS (Capillary Micro Sampling)

Other small volume device

Nano tools like lab-on-a-chip, ……

EBF & DBS – the story so far...



Plasma versus blood

- A downstream consequence of DBS: PK document in blood compounds instead of industry standard plasma/serum.
- BA experience with plasma assays >>> whole blood assays.
  - How to compare assays?
    1. DBS assay ↔ whole blood assays
    2. DBS assay ↔ DPS assay
    3. DBS assay ↔ Liquid plasma assays
  - Comparison ‘1’ or ‘2’ are suggested interim comparisons to understand assay differences
- PK experience plasma data >>> whole blood PK
  - How to compare PK?
    - PK responsibility

Plasma versus blood

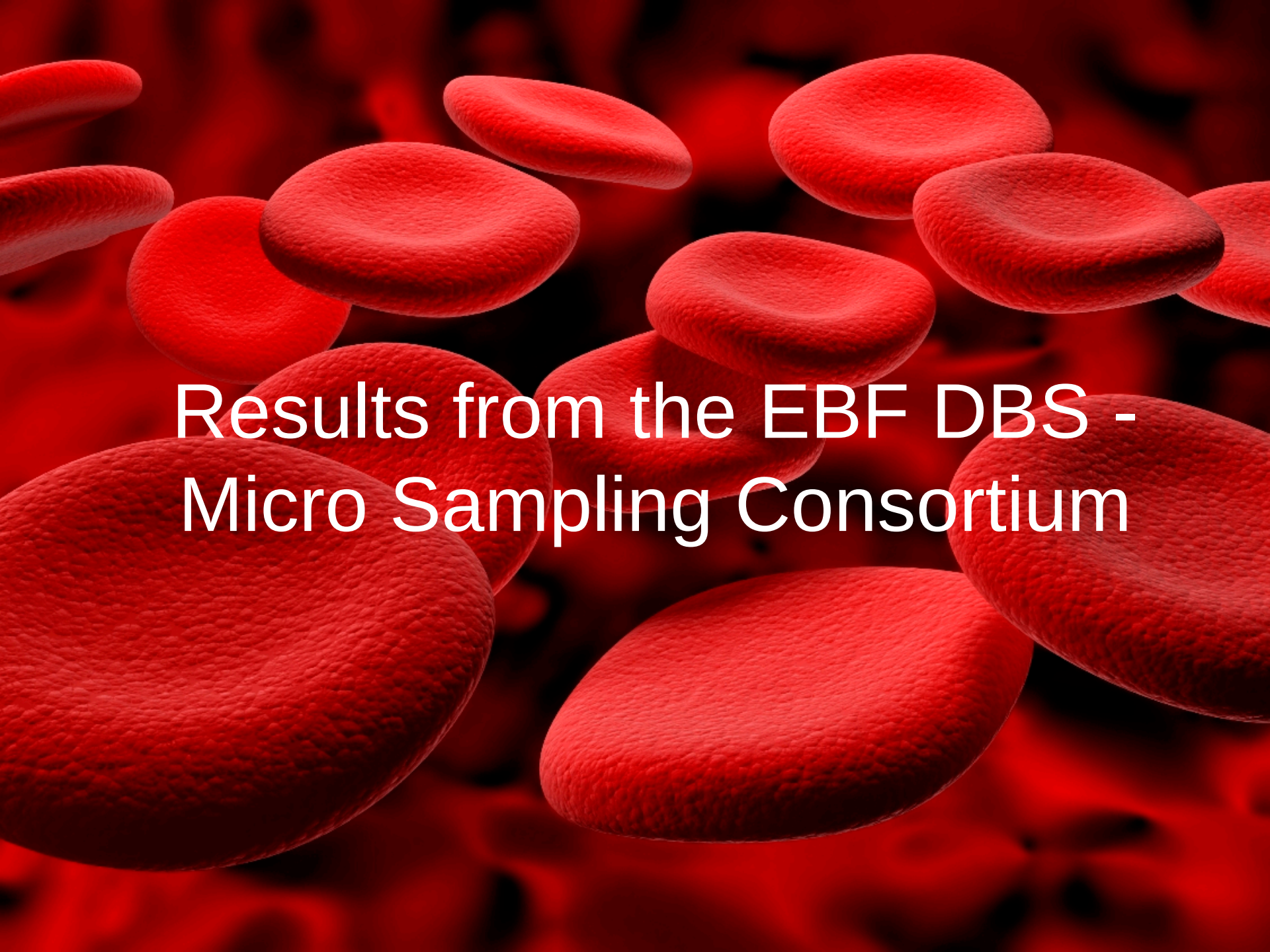
$$\alpha = \beta + \varphi$$

With:

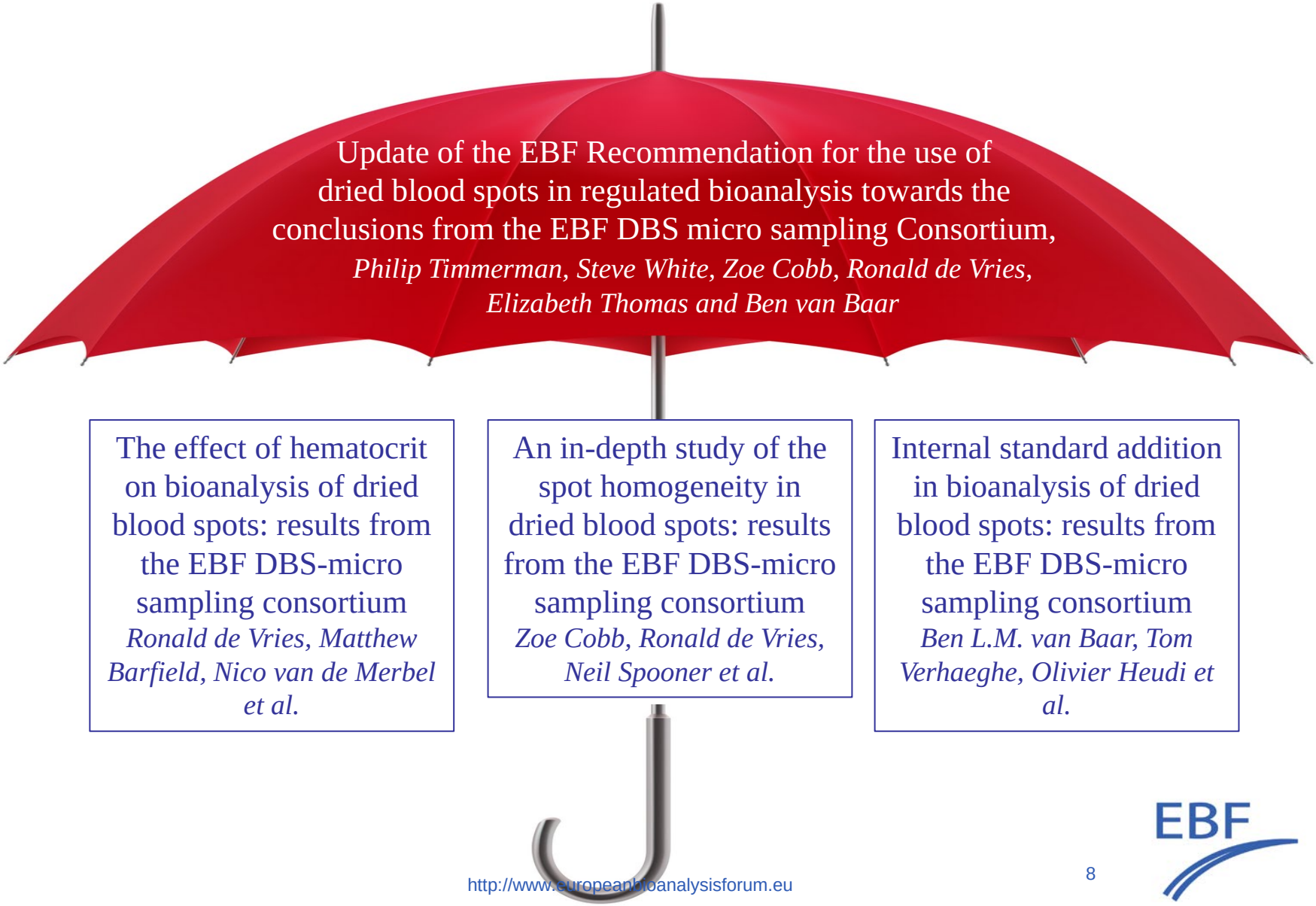
α being Effect of hematocrit changes on blood concentration

β being PK consequences of hematocrit changes

φ being BA consequences of hematocrit changes


Results from the EBF DBS -
Micro Sampling Consortium

Recently published recommendation paper and supporting scientific papers



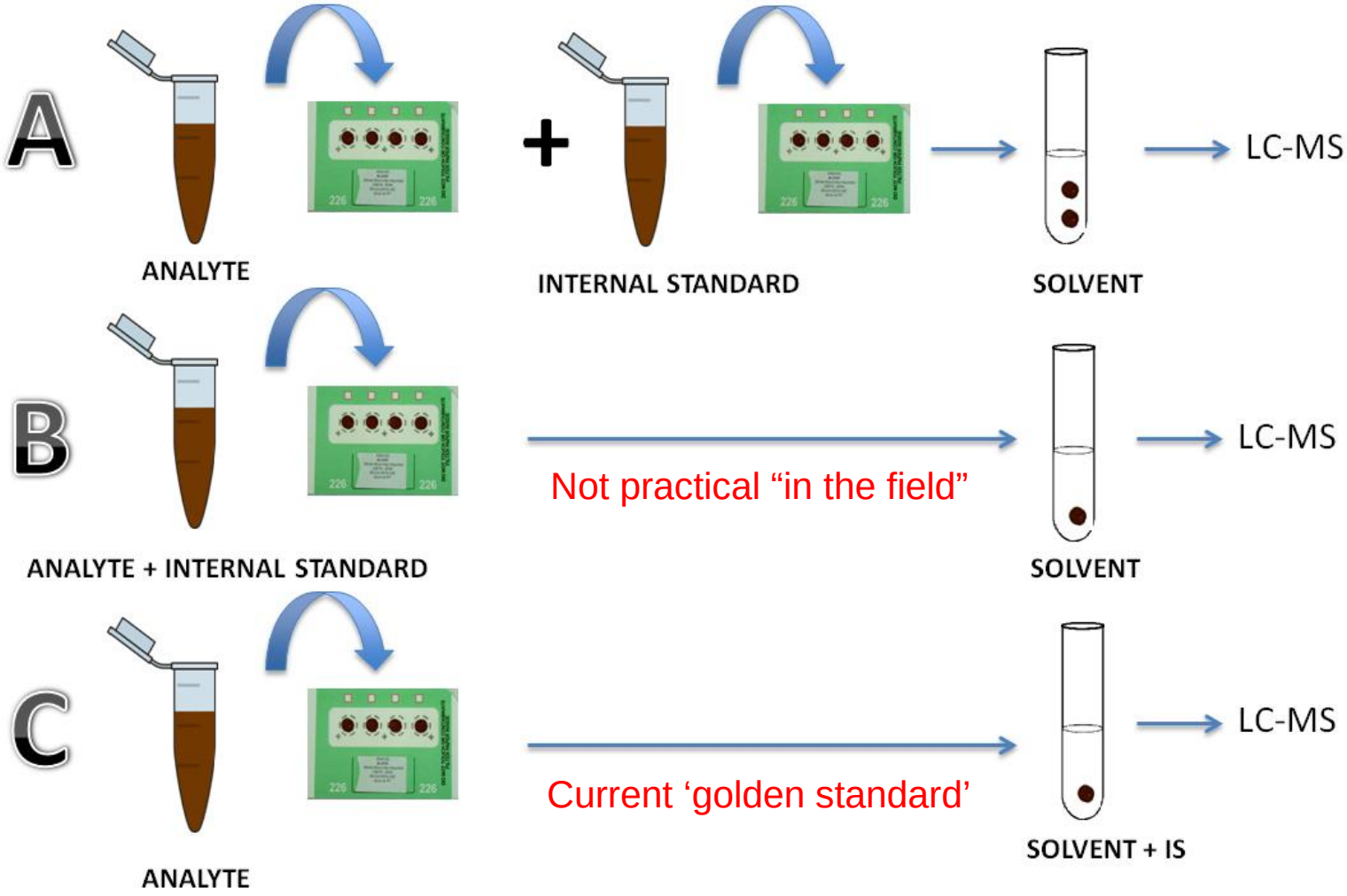
The effect of hematocrit on bioanalysis of dried blood spots: results from the EBF DBS-micro sampling consortium
Ronald de Vries, Matthew Barfield, Nico van de Merbel et al.

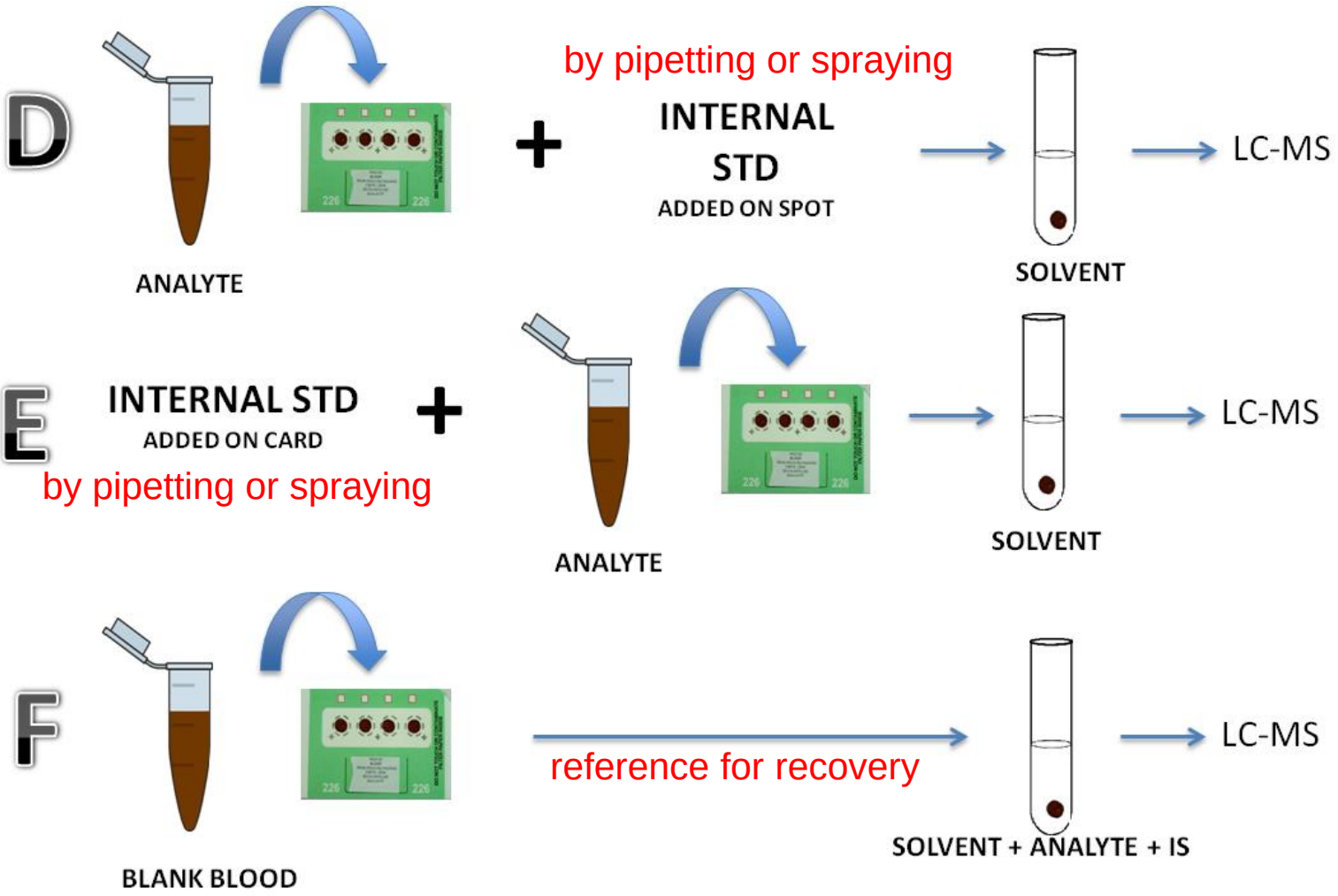
An in-depth study of the spot homogeneity in dried blood spots: results from the EBF DBS-micro sampling consortium
Zoe Cobb, Ronald de Vries, Neil Spooner et al.

Internal standard addition in bioanalysis of dried blood spots: results from the EBF DBS-micro sampling consortium
Ben L.M. van Baar, Tom Verhaeghe, Olivier Heudi et al.

Internal Standard Addition

6 processes tested...



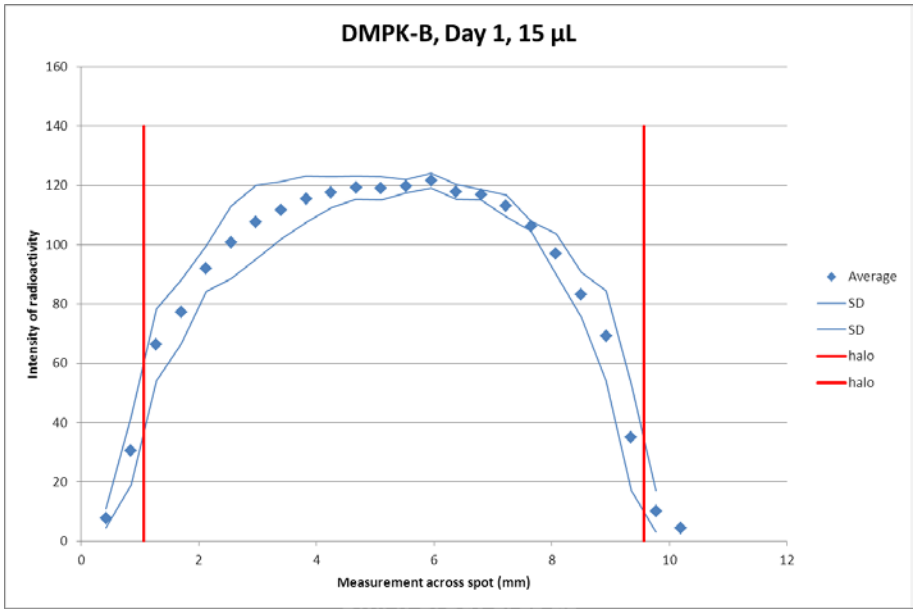

D
ANALYTE
by pipetting or spraying
INTERNAL
STD
ADDED ON SPOT
SOLVENT
LC-MS
E
INTERNAL STD
ADDED ON CARD
by pipetting or spraying
ANALYTE
SOLVENT
LC-MS
F
BLANK BLOOD
reference for recovery
SOLVENT + ANALYTE + IS
LC-MS

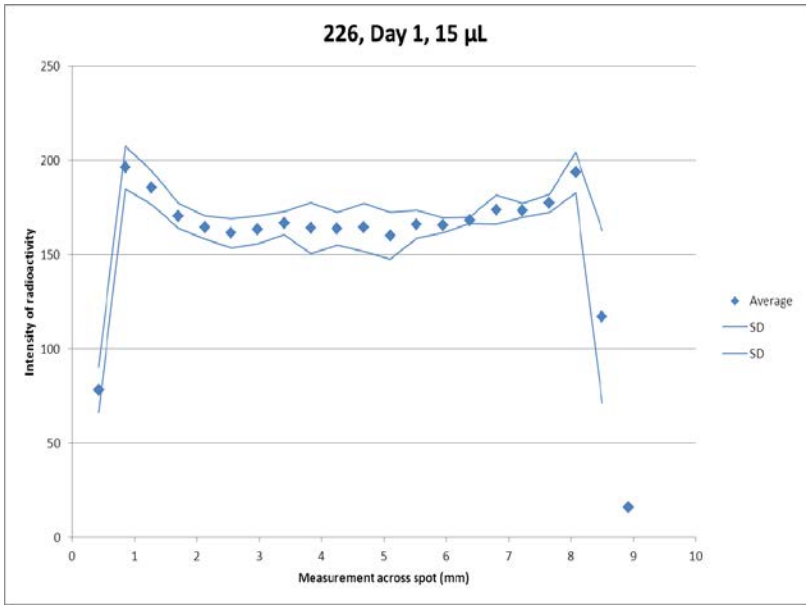
Conclusions and recommendations IS application

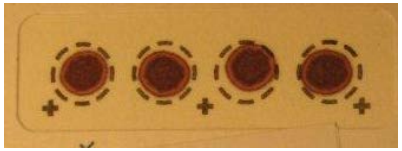
- application of IS to the DBS samples requires prudence
- Current ‘golden standard’ (C) aligns more with external standardization
- All 6 procedures had their analytical and procedural advantages or disadvantages
  - B: this method mimics IS best, but impractical
  - ACDEF: None of these methods gave satisfactory results.
- compound dependant impact on accuracy / precision.
  - focussed experiments should be executed in MDEV & MVAL to document the IS behaviour

Homogeneity – an example

- Radioactive compound (Lacosamide ^{14}C) spiked to mouse blood on Day 1 and (15 and 30 µL) spotted on Day 1, 2, 3, 5, 8, 10 and 15.
- Radioactivity measured (every 0.425 mm) across spot.
- Haematocrit native, not measured.







Data from other cards available (DMPK-A and Bond Elut)

Conclusions and recommendations Spot homogeneity

- Spot homogeneity is influenced by card type, analyte and hematocrit and may impact A&P to fall outside acceptance criteria for regulated BA.
- Proposed way out: taking the complete spot:
  - will require the (pre)clinical lab to sample and spot an accurate blood volume. This will add complexity to any (pre)clinical study → need for a simple reliable accurate spotting device.
  - introduces a challenge to perform reanalysis, ISR or metabolite work

Conclusions and recommendations on Hematocrit

- Our experiments show that hematocrit changes remain the single most important parameter defining compound behaviour and DBS assay performance.
- Data of EBF teams confirm industry data: the overall impact of clinical relevant hematocrit changes on spot formation, spot homogeneity, accuracy and precision and recovery in both fresh and aged spots is significant.
- In addition, these effects are compound dependent, which makes documenting and managing them an integral part of the method establishment of a compound and not of the use of a card type or the technique in general.
- Since we understand the issue, it is possible to accurately document these effects on assay performance as part of assay validation (although potentially resource intensive).

Effects of ageing

- current experience in industry is still relatively limited with respect to storing DBS cards for a significant time after sampling (6 months or more).
- Our data show impact of stored /aged spots under controlled circumstances, and invite for more investigation on the impact of prolonged storage, including the effects temperature and relative humidity. E.g. phase III trials where storage conditions surpass 6 months and limited experience with DBS-card storage variability and compliance.
- Also, and not investigated by the EBF, we need to acknowledge that ageing may introduce superimposable effects which may be difficult to separate. Failed long term stability experiments in DBS may be caused by compound degradation, changes in extraction recovery upon storage or a combination.


Conclusions &
Recommendations

Conclusions & Recommendations - 1

- EBF feels that DBS is currently not a competitive tool for routine general use in regulated bioanalysis
- Additional experiments are required to validate a DBS assay towards acceptance criteria for regulated BA
  - impractical or costly compared to validation of liquid assays
  - requires a careful balance of the potential advantages like patient comfort, sample handling or 3R outweigh investment or can stimulate discussion on required acceptance criteria
- Most, if not all of the challenges have an assignable cause and are scientifically manageable
- With appropriate documentation, the issues should not preclude DBS use where there is no viable alternative

Conclusions & Recommendations - 2

- A pivotal downstream consequence of DBS is that PK/TK/PD will be evaluated in blood
  - What kind of strategy of bridging PK data is needed?
- General comment: we should be carefully when assigning acceptance criteria for new technologies.
  - Adopting acceptance criteria from chromatographic (small molecule) assays for DBS may have been premature.
  - Widening acceptance criteria, provided this does not affect patient safety may be appropriate.
  - Wider acceptance criteria would not remove the need of additional validation experiments, but could simplify or remove some experiments or consequences.

Future Perspective

- EBF considers DBS as a developing tool and awaits further innovations to better balance the advantages of the technique versus its current limitations.
- We should be careful not to copy any conclusion on DBS to other micro sampling techniques yielding a ‘liquid’ plasma, serum or blood sample.
- Learnings from DBS should stimulate continued critical scientific thinking on how to embrace other techniques for the benefit of the patient.

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Questions?