

Opportunities for REDUCTION and REFINEMENT: Use of Dried Blood Spots for Generation of Toxicokinetic Data



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What are Dried Blood Spots (DBS) ?

Before a new medicine is given to people, an assessment of its safety in animals must be made; any harmful effects (toxicity) are evaluated and related to the amount of medicine circulating in the blood of the animal (toxicokinetics). A safety margin can then be estimated by comparing the circulating blood concentrations of medicine in the animal at doses causing adverse effects with concentrations predicted to be effective in treating disease in humans.

Traditionally, toxicokinetics have been performed using plasma, which is easy to handle and transport, compared to whole blood; technology is now available to enable the use of dried blood spots (DBS) instead. The blood is spotted onto cards, which are then allowed to dry at room temperature before storing and shipping.



Dry blood spotting has been around for over 40 years and is an easy way of collecting, shipping & storing blood samples. It has been widely used for new born screening and clinical trials in remote areas, where equipment such as centrifuges and freezers may not be readily available.

Before now DBS has not been widely utilised to measure drug levels during the new medicine development. This was primarily due to analytical limitations as each DBS sample is smaller than traditional plasma samples; therefore contains less material for accurate detection and quantification.

Advantages

Much smaller volumes of blood are required offering exciting opportunities for the 3Rs in safety studies - particularly refinement through potentially removing the need for local or whole body warming to facilitate blood sampling .

Scientifically, we may now be able to look at toxicity and toxicokinetics in the same animals, which before was impossible.

Reduction in cost may also be possible in terms of shipping and storage.

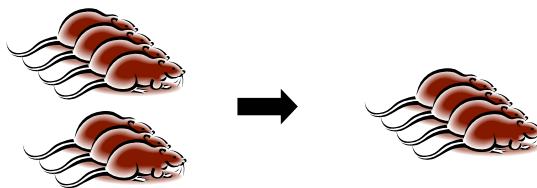
Clinically, DBS will facilitate paediatric studies and will also enable clinical studies to be performed in developing countries.

Experience thus far within GSK has shown that drugs may be more stable on the card than in frozen plasma.

Potential 3Rs Benefits

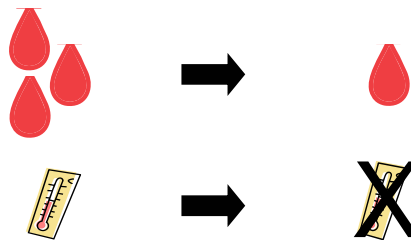
Reduction

Collect toxicokinetic samples from toxicology rats to **remove requirement for extra animals** on early safety studies, meaning **up to 36% animal reduction**.



Refinement

In the rat, reduce blood volume requirement from **nearly 1.5mL to 0.5mL**, which **removes need for warming prior to sampling** (usually needed to increase vasodilation and blood flow to facilitate sampling).



Regulatory Perspective (FDA, MHRA etc)

ICH S3A says"The quantification of systemic exposure provides an assessment of the burden on the test species and assists in the interpretation of similarities and differences in toxicity across species, dose groups and sexes. The exposure might be represented by plasma (serum or blood) concentrations or the AUCs of parent compound and/or metabolite(s)."

.....The choice of analyte and the matrix to be assayed (biological fluids or tissue) should be stated and possible interference by endogenous components in each type of sample (from each species) should be investigated. Plasma, serum or whole blood are normally the matrices of choice for toxicokinetic studies"

DBS Methods and Validation

DBS technology allows minimisation of blood volumes.

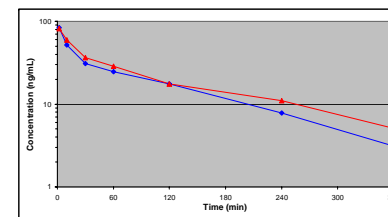
Approximately 3 x 0.015 mL drops of whole blood are spotted onto the card, Samples, calibration standards and quality controls are all spotted and allowed to dry in the same way.

The card is dried for approximately 2 hours at room temperature.

Cards are subsequently shipped and stored in sealable bags containing desiccant (to keep cards completely dry).

Analysis is conducted by punching a small circular disc from the centre of the spot either manually or using automation. The punched DBS is then mixed in organic solvent containing an internal standard to remove the drug into the liquid. This can then be quantified using standard analysis (high performance liquid chromatography; HPLC, mass spectrometry; MS).

A number of different types of drugs were evaluated, both on the bench in the laboratory (ie without using animals) and also from animal studies, one example of which is shown below.



Blood:water & DBS

References

Barfield et al (2008). Application of dried blood spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies. *J.Chromatogr.B*; **870**, 32-37.

ICH Topics and Guidelines: Safety Guidelines
<http://www.ich.org/cache/compo/276-254-1.html>

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