Analytical perspective on developing a DBS assay for daily routine.

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Scope of the lecture:
The lecture will discuss the different analytical aspects that are important during the development and (pre)validation of a dried blood sample-based method for routine use, designed to quantitate small molecules using liquid chromatography tandem mass spectrometry.

Learning objectives:
1. How to ensure a dried blood sample-based method is fit for purpose?
2. Which analytical considerations should be made during method development?
3. How to perform the analytical validation of a dried blood sample-based method (with particular emphasis on the non-traditional validation parameters)?

Extended abstract:
The interest in dried blood spots and other dried blood samples (e.g. volumetric absorptive sampling or VAMS) for therapeutic drug monitoring has drastically increased during the last 10 to 15 years. This is due to the many advantages that are associated with this sampling strategy. More particularly, it is an easy and minimally invasive way of obtaining a representative sample, which allows to transport and store samples under ambient conditions. This enables patients to collect a sample themselves at any given time and to send it to a laboratory for analysis. Furthermore, dried blood samples only require a very small volume of blood and tend to improve the analyte's stability.

To obtain a dried blood sample-based method which is suitable for routine use, some important analytical considerations will have to be made, already from early method development on. Although this is often overlooked, it is of the utmost importance that every aspect of a dried blood sample-based method should be fit for purpose: i.e. the way samples are collected, transported, stored and analyzed should be compatible with the setting in which the method will have to be applied. Therefore, every decision made regarding the set-up of the method will depend on the context in which the method eventually will have to be applied. The parameters that require special consideration include: the collection method, the substrate, the sample volume, the drying and storage process, the punch size, the method of internal standard incorporation, the type of blood used, and the procedure to prepare calibrators and quality control samples. An overview of the different options for the set-up of a dried blood sample-based method is depicted in the flowchart in figure 1. Additionally, the importance of proper stress testing of the method will be highlighted, as it allows potential issues to be detected at a relatively early stage and it increases the chances of a successful method validation.

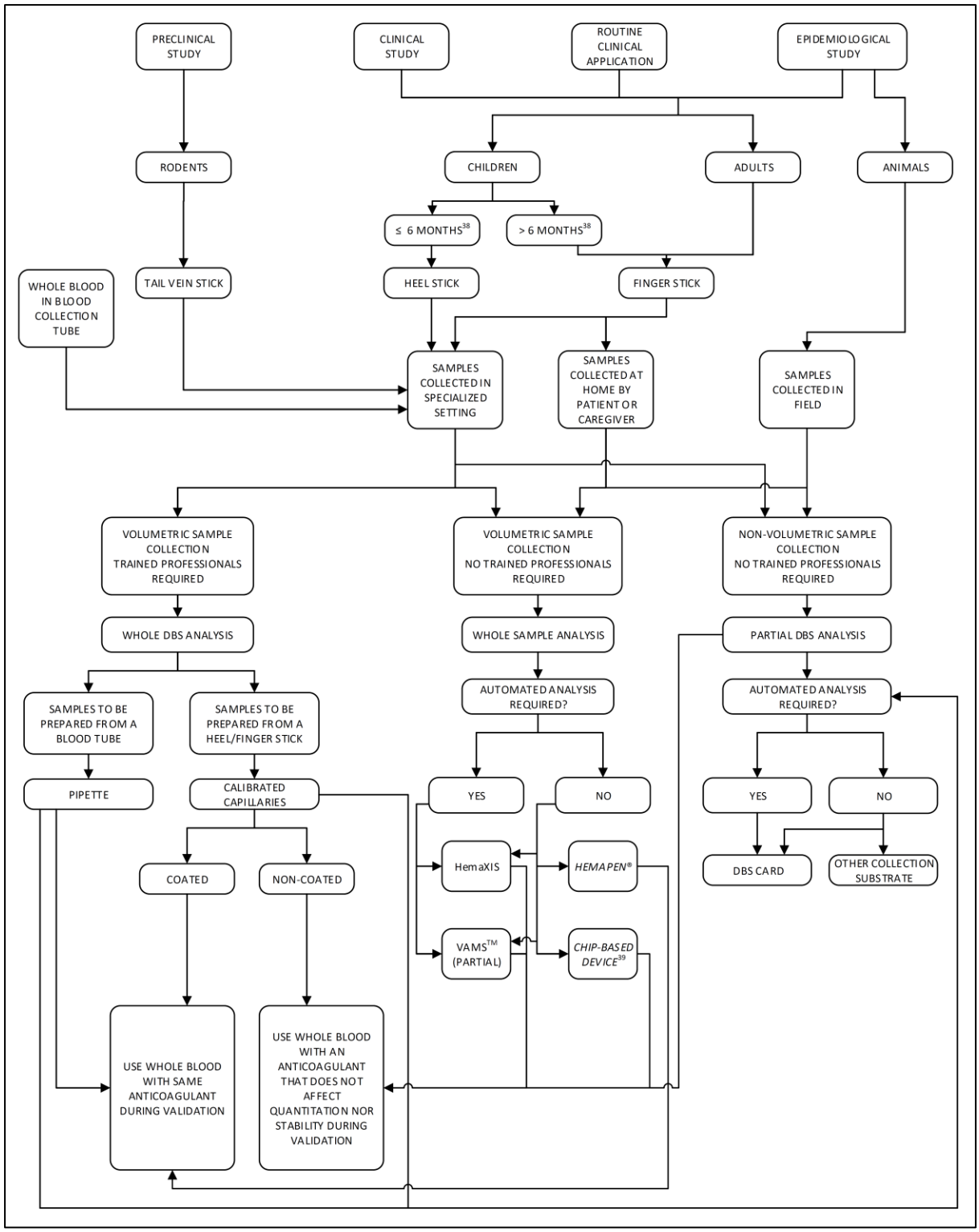

Figure. 1: Flowchart depicting different options for the set-up of a dried blood sample-based method

Currently, there is no guideline available for the validation of dried blood sample-based bioanalytical methods. Obviously, guidelines set up for the validation of traditional biological matrices such as blood, plasma and urine can be used as a basis. However, not all of the experiments included in those guidelines are relevant for dried blood sample-based methods, and other experiments will need some refinement. Moreover, the analysis of dried blood samples can be influenced by some additional variables such as the hematocrit of the blood used to generate the samples (i.e. hematocrit effect), the volume of the samples (i.e. volume effect) and the location in which a punch is made from a dried blood sample (i.e. volcano effect). Although the latter parameters are not incorporated in traditional validation guidelines, it is essential to evaluate the influence of these variables on the analytical results during the analytical validation of a dried blood sample-based method, to ensure the method is capable of delivering valid results throughout the entire target population. An overview of the validation parameters that require additional evaluation and concrete advise on how to evaluate them, is given in table 1.

Table 1: Overview of the analytical validation parameters that require additional evaluation in dried blood sample-based methods, and how to assess them

Validation parameter	**Evaluation**	**Statistical test/ Acceptance criterion**
Recovery, matrix effect, process efficiency	Evaluate at 2 QC levels using 6 different matrices, at at least 3 Hct levels, at least in singulo.	Should be constant, precise and reproducible, both between matrices and Hct values (%RSD $\leq$ 15%).
Volume effect	Evaluate at 2 QC levels and at least at 3 Hct levels and 3 DBS volumes	One-way ANOVA with bonferroni post-hoc analysis ($p \leq 0.05$). Backcalculated values $\leq$15 % of medium DBS volume.
Hematocrit effect	Evaluate at 2 QC levels, and at least at 3 Hct levels.	One-way ANOVA with bonferroni post-hoc analysis ($p \leq 0.05$). Backcalculated values $\leq$15 % of medium Hct values.
Volcano effect	Compare central and peripheral measurements. Evaluate at 2 QC levels and at least at 3 Hct levels and one DBS volume (typically, the highest).	Paired t-test ($p \leq 0.05$) Backcalculated 'peripheral' values $\leq$15% of 'central' values