

# Checklist for the use of capillary microsampling in regulatory studies

B. Feyen<sup>1</sup>, H. Stieltjes<sup>1</sup>, L. de Zwart<sup>1</sup>, L. Dillen<sup>1</sup>, S. Patel<sup>2</sup>, K. Diloreto<sup>2</sup>, S. Bryant<sup>2</sup>

<sup>1</sup> Janssen Research & Development, a Division of Janssen Pharmaceutica NV, Beerse, Belgium

<sup>2</sup> Janssen Research & Development, LLC, 1400 McKean Road, Spring house, PA19477, United States

email: bfeyen@its.jnj.com

## INTRODUCTION

Many companies have been using different microsampling techniques for toxicokinetic profiling which is an essential part in preclinical safety studies. One of the major benefits of microsampling is the elimination or reduction of satellite animals. Microsampling in discovery, dose range finding and PK studies is already widely applied, but often extension of the use in regulatory studies remains limited. The major barriers for implementing microsampling in regulatory studies can relate to bioanalytical aspects and also to the removal of satellite animals. The potential impact of microsampling on functional and clinical pathology endpoints in main study animals can be a concern. Therefore to facilitate acceptance, a checklist for the use of capillary microsampling in main study animals of regulatory studies was implemented at Janssen. This checklist contains 4 main questions.

## CHECKLIST

### Bioanalytical aspects

The main question is whether a small assay volume is feasible. Several questions have to be taken into account when evaluating this aspect:

- What is the plasma/serum volume required for each assay?
- Is there potential for reanalysis?
- Are there bioanalytical issues such as sensitivity, adsorption/recovery, stability etc?
- Is there a requirement for incurred sample analysis (ISR)?
- Is there a requirement for metabolite analysis?

### Metabolite evaluation aspects

For each project/study, the requirement for metabolite evaluation can be identified. If needed, microsampling often still allows metabolite evaluation in the diluted samples. For potential metabolite evaluations with microsampling in a regulatory toxicity study the following aspects need consideration:

- Current techniques allow for metabolite evaluations in diluted plasma.
- Sufficient sample remains available.
- Pooling of samples can be taken into consideration when necessary.
- Additional microsamples can be sampled.

### Blood volume

When sampling from main study animals, the total volume of blood taken should not impact toxicological endpoints. Therefore the following aspects should be taken into account when designing a study:

- Number of time points to be sampled in 24h
  - Frequency of sampling
  - Volume sampled/timepoint
  - Sampling design (serial or composite design)
- For rats recommendations are described in literature (Ref.1)
- When sampling <200µl in 24h, microsampling can be performed in main study animals as there is no impact on toxicological endpoints
  - When sampling > 200µl in 24h, it is recommended to add satellite rats

Also for juvenile rats, recommendations are described in literature (Ref. 2, 3)

### Toxicological aspects

When sampling from main study animals, the toxicological profile of the compound evaluated should be considered including all evaluations in the study design:

- Compounds with the haematopoietic system as a target organ
- Compounds with cardiovascular effects
- Compounds with a fast Cmax associated with CNS observations
- Inclusion of biomarker evaluation
- Built in Irwin evaluation
- Built in micronucleus evaluation

## DISCUSSION

- All of the checklist items mentioned above should be discussed before microsampling is selected as the sampling technique for TK sampling in a regulatory study. Sampling designs can be proposed to mitigate some concerns encountered in the checklist.
- Bioanalytical issues such as stability or sensitivity problems, non capillary microsampling can offer an alternative. When metabolite evaluation is warranted, microsampling does in general not hinder metabolite evaluation as sufficient diluted sample remains available. In cases where there is a concern, an adaptation of the sampling design is generally sufficient. In Figure 2, a sampling design is presented where 5 animals per timepoint are sampled but only 3 are analysed. The 2 remaining samples can be kept for additional metabolite evaluation.
- Blood volume limits should be considered as well. When sampling 32µl/timepoint in a standard study design with 6 timepoints, the recommended volume of 200µl in adult rats is never exceeded, regardless of your sampling design. In our dose range finding studies, a serial design (Fig. 1) is applied to obtain as much information as possible for a broad dose range and to link Tox and TK parameters in individual animals. In regulatory studies, a composite sampling design is applied. Two examples are shown in Fig 2 and 3.
- Toxicological aspects are also important to consider. When including Irwin evaluation or micronucleus evaluation into a regulatory study, a potential approach is to use half of the animals for the additional evaluations and perform TK evaluation in the other half. If the total blood volume is below 200µl/24 h, no impact on the micronucleus evaluation is expected and a composite sampling design as presented in Figure 2 or 3 can be considered as well.
- In the discussion above, just a few examples are mentioned on how to anticipate to possible issues. The checklist should be consulted for each compound/project to propose a sound decision with respect to sampling in main study animals.

Figure 1: Serial sampling design

Animal No.	Sampling time point					
	#1	#2	#3	#4	#5	#6
1	x	x	x	x	x	x
2	x	x	x	x	x	x
3	x	x	x	x	x	x
4	x	x	x	x	x	x
5	x	x	x	x	x	x
	N=5	N=5	N=5	N=3=5	N=5	N=5

Figure 2: Composite sampling design with n=5

Animal No.	Sampling time point					
	#1	#2	#3	#4	#5	#6
1	x			x	x	
2	x			x		x
3		x		x	x	
4		x	x			x
5	x		x	x		
6	x		x		x	
7	x		x			x
8		x			x	x
9		x	x		x	
10		x		x		x
	N=5	N=5	N=5	N=5	N=5	N=5

Figure 3: composite sampling design with n=3

Animal No.	Sampling time point					
	#1	#2	#3	#4	#5	(#6)
1	x			x		
2	x			x		
3	x				x	
4		x			x	
5		x			x	
6		x				(x)
7			x			(x)
8			x			(x)
9		x				
10				x		
	N=3	N=3	N=3	N=3	N=3	N=3

## CONCLUSION

Unlike the decision tree proposed by NC3r which assists to determine whether a study is amendable to microsampling and which approach is the most relevant, the checklist items proposed drive the decision for microsampling in main study animals or not. In our experience, a no-go decision for microsampling is rather the exception. However, adaptations of the sampling procedures, including non capillary microsampling as an option, or an adaptation of the study design can be the outcome when some items of the checklist are of concern.

## REFERENCES

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