Supporting Tests for Non-capillary Microsampling of plasma in rat and mice in-vivo studies: small volumes, containers - it all matters!

H. Stieltjes[1], L. Dillen[1], L. Diels[1], A. Vroman[1], C. Sas[2], L. Sips[1], T. Verhaeghe[1]

[1]Preclinical Development and Safety, Regulated Bioanalysis, Janssen Research & Development, Beerse, Belgium

[2]Preclinical Development and Safety, Toxicology, Janssen Research & Development, Beerse, Belgium
email: hstieltj@its.jnj.com

ABSTRACT

Different microsampling techniques have created great interest from toxicokinetic and pharmacokinetic scientists. They offer the potential to reduce sample volumes for exposure assessment in rodents and, consequently, serial profiles can be obtained. In GLP tox studies, microsampling can remove the use of satellite animals. Although the full potential to reduce the sample volume would be obtained with blood sampling and analysis, the general preference is to keep plasma as the matrix of choice. The current industry trend is to collect a small blood sample in capillaries and after centrifugation transfer plasma to a second, smaller capillary. At Janssen, however, we choose to sample a small volume of blood (60 µL) into an adapted recipient to generate an accurate volume of 10 or 20 µL plasma in a more classical way.

For current GLP studies these procedures were validated before implementation. Accuracy and reproducibility of the pipetting step was safeguarded through cross-training of the in-vivo staff with the bioanalysis staff. Two observations were made during the validation of the procedures.

The pipetting technique, and type of pipette are critical to obtain accurate results.

In addition, it was observed that for some compounds the recipient and the anti-coagulant influence the recovery. Results clearly showed that volume, temperature and time all can have an impact on the recovery of the analyte from the blood collection tube. No relation with adsorption or instability could be made. Other collection devices did not show similar losses.

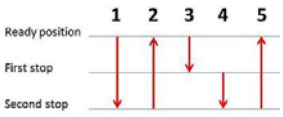
METHODS

Figure 1. Traditional sampling with adapted device for small volumes (Microvette CB 300)

Note: Volume shown above is a 300 µL sample.

Because taking plasma from the microvette will not allow for pre-wetting of the pipet tip, pipetting in reverse mode was selected. This provided the highest accuracy of pipetting compared ot other techniques, including positive displacement pipettes.

Figure 2 Reverse Pipetting with an air column Pipet



The bioanalytical facility prepared a Test Quality Control sample spiked with a reference compound at 200 ng/mL in human plasma.

- This Test QC is aliquoted in 50 or 60 µL volumes into microvette tubes (this is the blood volume that will be taken, so plasma level in the tube will be at the actual height of an in-vivo situation), and stored in a freezer.
- all assigned technicians (both in vivo and bioanalytical technicians) prepare 6 replicates. 20 µL plasma is transferred from the microvette in a micronic tube and further diluted with 180 µL 2% BSA
- the Test QC is also included during the sample analysis without transfer into the microvette.

RESULTS 1

Table 1: Results pipetting 10 µL plasma from 50 µL volume (20 ng/mL cpd 1) in a microvette

	Evaluation 1				Evaluation 2	
	BA staff 1	in vivo staff 1	in vivo staff 2	in vivo staff 3	BA staff 1	in vivo staff 4
Mean conc. (ng/mL)	16.4	15.6	15.9	15.6	15.5	15.1
accuracy (%)	**82.0**	**78.1**	**79.6**	**78.2**	**77.3**	**75.6**
%CV	2.9	2.9	4.1	2.7	6.3	1.2
n	6	6	6	6	6	6
% difference to BA staff		-4.9	-3.0	-4.8		-2.3

All in-vivo staff passed the test. Although microvette pipette QC samples showed a reduced accuracy, the same source QC sample was within acceptance criteria to validate the batch.

RESULTS 2

Table 2: Recovery (% to ref) from plasma and blood in microvette tubes as a function of matrix volume and collection volume (cocktail spiked)

					cpd1		cpd2		cpd3	
recipient	matrix	µL in	µL out	pipet	techn1	techn2	techn1	techn2	techn1	techn2
micronic = reference	plasma	60	20	standard	100	100	100	100	100	100
microvette K2 EDTA	plasma	60	20	standard	**88**	92	**89**	**88**	98	102
microvette K2 EDTA	plasma	200	20	standard	93	96	95	91	97	104
micronic = reference	plasma	60	10	electronic	100		100		100	
microvette K2 EDTA	plasma	60	10	electronic	**85**		**84**		101	
microvette K2 EDTA	plasma	200	10	electronic	91		**89**		100	
micronic = reference	blood	200	20	standard	100	100	100	100	100	100
microvette K2 EDTA	blood	60	20	standard	**74**	**77**	**85**	**84**	97	98
microvette K2 EDTA	blood	200	20	standard	**66**	**90**	**89**	91	100	101
micronic = reference	blood	200	10	electronic	100		100		100	
microvette K2 EDTA	blood	60	10	electronic	**78**		**82**		96	
microvette K2 EDTA	blood	200	10	electronic	**87**		92		98	

Microvette containers show reduced recoveries for 2 out of 3 compounds evaluated. Recovery issues are more pronounced at lower volumes. Blood or plasma matrix in microvette does not change the conclusion. This was consistent across 2 different technicians. For compound 3 no recovery issues were identified.

Table 3: Recovery (% to ref) from microvette tubes as a function of anti-coagulant, collection volume and time

100 ng/mL spiked to EDTA plasma				cpd1		cpd2		cpd3		cpd4	
recipient	matrix	µL in	µL out	t=0h	t=1.5h	t=0h	t=1.5h	t=0h	t=1.5h	t=0h	t=1.5h
micronic = reference	plasma	60	10	100	90	100	**79**	100	102	100	**77**
microvette K2 EDTA	plasma	60	10	91	**77**	**87**	**70**	96	96	**88**	**77**
microvette Li Hep	plasma	60	10	95	91	**83**	**69**	96	93	**69**	**49**
micronic = reference	plasma	200	10	100	98	100	97	100	91	100	97
microvette K2 EDTA	plasma	200	10	93	91	98	**79**	101	94	**87**	**72**
microvette Li Hep	plasma	200	10	95	92	96	**84**	103	95	**79**	**64**
100 ng/mL spiked to heparin plasma				**cpd1**		**cpd2**		**cpd3**		**cpd4**	
recipient	matrix	µL in	µL out	t=0h	t=1.5h	t=0h	t=1.5h	t=0h	t=1.5h	t=0h	t=1.5h
micronic = reference	plasma	60	10	100	100	100	102	100	96	100	92
microvette K2 EDTA	plasma	60	10	90	**85**	97	**81**	96	104	**88**	**68**
microvette Li Hep	plasma	60	10	95	94	90	**83**	94	92	**70**	**49**
micronic = reference	plasma	200	10	100	102	100	105	100	107	100	102
microvette K2 EDTA	plasma	200	10	97	92	100	**88**	104	105	92	**78**
microvette Li Hep	plasma	200	10	98	96	94	**87**	104	104	**82**	**62**

Microvette containers show reduced recoveries for 3 out of 4 compounds. Recovery issues are more pronounced with Li-heparine in the microvette for compound 4. Time dependency was noticed. Also, the degree of filling was important. For compound 3 no recovery issues were observed.

Table 4: Recovery (%ref) in different containers as function of collection temperature and time

100 ng/mL spiked to EDTA plasma				cpd1		cpd2		cpd3		cpd4	
recipient	µL in	µL out	condition	t=0h	t=2h	t=0h	t=2h	t=0h	t=2h	t=0h	t=2h
Falcon = reference	7000	10	**ice**	100		100		100		100	
LoBind (0.5 mL)	60	10	**ice**	101	98	100	100	102	95	102	100
LoBind (1.5 mL)	60	10	**ice**	99	98	96	101	100	102	98	100
Glass1 (insert)	60	10	**ice**	99	99	97	98	103	99	101	98
Glass 2 (tube)	60	10	**ice**	98	98	95	96	98	96	97	97
micronic	60	10	**ice**	98	98	96	95	90	98	97	98
microvette K2 EDTA	60	10	**ice**	96	**87**	97	90	111	103	94	93
microvette Li Hep	60	10	**ice**	96	97	92	**84**	97	102	**89**	**73**
Falcon = reference	60	10	**RT**	100		100		100		100	
LoBind (0.5 mL)	60	10	**RT**	100	103	107	101	103	103	101	101
LoBind (1.5 mL)	60	10	**RT**	101	102	101	98	102	103	102	99
Glass1 (insert)	60	10	**RT**	96	101	98	98	97	102	96	101
Glass 2 (tube)	60	10	**RT**	102	101	100	102	104	103	97	100
micronic	60	10	**RT**	100	100	101	101	104	102	97	104
microvette K2 EDTA	60	10	**RT**	92	**78**	**88**	**79**	99	106	90	**82**
microvette Li Hep	60	10	**RT**	99	95	93	**82**	97	102	**79**	**54**

The recovery issues observed in the Microvette containers were confirmed. Other recipients do not show the same recovery problems. The use of melting ice reduced the recovery loss.

Table 5: Information on containers evaluated

Tube description	Supplier	Reference
Microvette CB 300 K2EDTA	SARSTEDT	16.444
Microvette CB 300 LiHep	SARSTEDT	16.443
Micro Tube 1.3 ml K3EDTA	SARSTEDT	41.1504.005
Multivette 600 K3EDTA	SARSTEDT	15.1671
Multivette 600 LiHep	SARSTEDT	15.1673
Microvette 500 K3EDTA	SARSTEDT	20.1341
Microvette 500 LiHep	SARSTEDT	20.1345
BD Microtainer 500 K2EDTA	Becton Dickinson	365975
VACUETTE 1 ml K3EDTA	Greiner Bio-One	454034
VACUETTE 2 ml LiHep	Greiner Bio-One	454089
BD Vacutainer 3 ml K2EDTA	Becton Dickinson	8082243
BD Vacutainer 10 ml LiHep	Becton Dickinson	8083925
LoBind Tube 0.5 ml	Eppendorf	0030 108.094
LoBind Tube 1.5 ml	Eppendorf	0030 108.116
Epjes 1.5 ml	Eppendorf	0030 125.150
Microtube 2 ml, PP brown	SARSTEDT	72.609.003
Microtube 1.5 ml, PP	Greiner Bio-One	717201
Tube 12x55 Non sterile PPN	VWR	VWR1216-1164
Micronic		
Nunc cryotube 3.6 ml	Nunc	366524

As we had never tested recovery in various blood sampling devices and plasma recipients, we evaluated all in-house available types, correcting the added volume for the size of the container.

Table 6: Recovery in different recipients and in function of collection temperature

K2EDTA plasma rat			cpd1		cpd2		cpd3		cpd4	
Store at RT 100 ng/mL	µL in	µL out	% ref t=0h	% ref t=2h	% ref t=0h	% ref t=2h	% ref t=0h	% ref t=2h	% ref t=0h	% ref t=2h
Microvette CB 300 K2EDTA	60	10	101	81	96	**76**	108	101	96	**72**
Microvette CB 300 LiHep	60	10	98	92	95	**74**	104	104	86	**48**
Micro Tube 1.3 ml K3EDTA	200	10	102	105	101	97	104	107	102	106
Multivette 600 K3EDTA	100	10	100	102	93	96	102	98	97	102
Multivette 600 LiHep	100	10	103	102	96	92	102	103	102	100
Microvette 500 K3EDTA	100	10	101	103	99	96	103	104	99	102
Microvette 500 LiHep	100	10	100	102	98	100	104	97	102	100
BD Microtainer 500 K2EDTA	100	10	101	103	100	100	99	107	98	99
VACUETTE 1 ml K3EDTA	500	10	102	101	102	95	105	107	101	97
VACUETTE 2 ml LiHep	500	10	102	103	97	96	102	100	102	101
BD Vacutainer 3 ml K2EDTA	500	10	101	102	98	96	107	107	98	100
BD Vacutainer 10 ml LiHep	500	10	101	100	94	89	105	98	103	98
LoBind Tube 0.5 ml	60	10	103	104	101	104	99	107	101	98
LoBind Tube 1.5 ml	60	10	103	102	101	103	98	97	100	101
Epjes 1.5 ml	60	10	103	104	96	97	104	103	100	102
Microtube 2 ml, PP brown	60	10	106	105	104	100	102	104	101	103
Microtube 1.5 ml, PP	60	10	102	102	102	95	102	102	100	102
Tube 12x55 Non sterile PPN	60	10	104	104	98	99	103	100	103	102
Micronic	60	10	102	99	99	100	104	102	102	96
Nunc cryotube 3.6 ml	60	10	103	106	100	98	105	105	102	100
Glas (insert)	60	10	101	101	99	102	101	100	101	99
Glas (tube)	60	10	100	103	91	99	99	99	98	104
Ref. (Falcon buis)	60	10	102	98	101	99	102	98	101	99
Microtube 2 ml, PP brown	500	10	103	106	101	100	105	102	103	106
Microtube 1.5 ml, PP	500	10	105	104	99	108	101	104	101	103
Tube 12x55 Non sterile PPN	500	10	103	102	94	97	102	103	99	99

The recovery issues with the Microvette containers were again confirmed. No other type of blood collection device, nor any recipient did show this recovery problems. There are no significant differences in anti-coagulant content between the tubes.

CONCLUSIONS

- Microvette CB300 blood sampling devices have the potential to cause recovery issues which are clearly compound dependent.
- The observed effects do not seem to be related to adsorption potential of compound (compound 3 has shown adsorption issues in method development, while compound 2 has not)
- Also anti-coagulant, time and collection temperature have an impact on the observed effect.
- No other blood collection device or plasma recipient showed these recovery issues
- The root cause is still unknown.

This study was conducted by Janssen Research and Development

janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson