aaps National Biotechnology CONFERENCE
MAY 9-12, 2022
ANAHEIM MARRIOTT | ANAHEIM, CA

Feasibility of Supporting LBA PK Analysis using Dried Blood Sample Collection Technique: Volumetric Absorptive Microsampling (VAMS)

May 12, 2022

Dong Hun (Don) Lee

MERCK
Be well

Session Description and Objectives

- Volumetric absorptive microsampling (VAMS) is a relatively new technique that permits patient- centric sampling both in and out of the clinic.
- This methodology has been successfully implemented for small molecule PK studies via LC/MS.
- Can we support LBA PK analysis using dried blood samples?
- A Merck mAb therapeutic (mAb A) was used as a model compound for this study.
- The VAMS Mitra tips, reliably collect 10, 20, or 30 µL of blood. Tips are dried and stored and transported at ambient conditions.
- How we extract mAb drug from dried blood?
- How dried blood sample assay differ from serum-based assay?
- Can we provide VAMS PK assay as reliable as serum PK assay?
- For calibration curve, can we use curve in surrogate matrix solution instead of VAMS tips?

Biography and Contact Information

- Don has 16 years of experience in LBA based bioanalysis of biotherapeutics including PK, ADA, TE, cell-based NAb assays.

- Highlights of his Merck career include contributions to three FDA approved Merck drugs: Keytruda (pembrolizumab), Zinplava (bezlotoxumab), and Bridion (Sugammadex).

Contact information

dong.hun.lee@merck.com

Materials and Methods

VAMS Mitra Tips

- The Mitra® tips, based on Volumetric Absorptive Microsampling (VAMS™) technology: 10 µL Tip.
- Once the sample is collected, the Mitra tips were dried and stored in storage bags with desiccants at ambient temperature.

Extraction Method Optimization

- Buffer selection (1% BSA/PBST)
- Shaker/Mixer (Eppendorf Thermomixer C)
- Sonication (No improvement)

Final Extraction: 10µL VAMS tips in 500µL 1% BSA/PBST on Thermomixer C for 1 hour.

Mitra tips in deep well plate in 1%BSA/PBST

Agitation on Thermomixer C (Eppendorf)

Table 1. Optimal agitation time for extraction

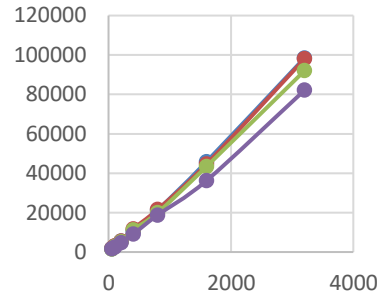
mAb A (ng/mL)	ECL Response			
	Post Extraction Spike*	2Hr Shaker	1Hr Shaker	0.5Hr Shaker
7000	388760	366786	361999	324947
2333	118868	116217	116377	108133
778	37560	35622	35701	34433
259	13103	12571	12236	11944
86.4	4403	4365	4114	4194
28.8	1530	1467	1529	1345
14.4	768	815	777	701
0	80	73	70	64

*Post Extraction Spike (Extraction control): mAb A spiked into dried blood tip extract

Materials and Methods

Standard Curve comparison

- Post extraction spike (Extraction Control): calibrators spiked into blank blood VAMS tip extract
- VAMS Tip curve (dried blood matrix, stored at room temperature)
- Wet blood curve (fresh blood)
- Surrogate matrix curve (stored at -70ºC)



Sample preparations

- Pooled blood: 10 individual blood lots were pooled
- QCs: 10µL VAMS Mitra tip QCs prepared using mAb A spiked in pooled blood, dried overnight under biological hood and store at ambient temperature.
- Calibration Standards: prepared in surrogate matrix solution and stored at -70ºC.

Evaluated LBA PK validation parameters using MSD ECL(Electrochemiluminescence) assay

Is there matrix interference from dried blood extract?

To understand dried blood extract matrix, mAb A was spiked in wet blood and in dried blood extract and tested along with VAMS tip samples (Table 2).

Table 2. Comparison of calibrator ECL response from wet blood and dried blood extract

mAb A (ng/mL)	Mean ECL Response		
	Wet Blood	Post Extraction Spike*	VAMS Sample
7000	457704	396397	352917
2333	137761	120164	127363
778	44492	37972	41847
259	15006	13365	12172
86.4	4796	4331	4411
28.8	1690	1519	1613
14.4	838	767	704
0	73	66	82

*Post Extraction Spike (Extraction control): mAb A spiked into dried blood tip extract

- Some matrix interference (proportional, mean 11±2.2% ECL reduction) observed from dried blood when compared with wet blood.

Table 3. Test of assay interference from naked VAMS tips

mAb A (ng/mL)	Mean ECL Response		% Difference
	Buffer	VAMS Tip Extract*	
7000	502693	493756	1.8
2333	154871	150914	2.6
778	48732	47924	1.7
259	16504	16623	0.7
86.4	5527	5637	2.0
28.8	1919	1884	1.8
14.4	966	962	0.4
0	86	81	5.8
Mean			2.1

*mAb A was spiked into naked VAMS tip extract

- There was no interference observed from the Mitra tip components alone. The interference seems to be coming from extracted dried blood components (Table 3).

Development of Surrogate Matrix

Reliability/convenience of calibration curve

Surrogate matrix curve > Post extraction curve > VAMS tip curve

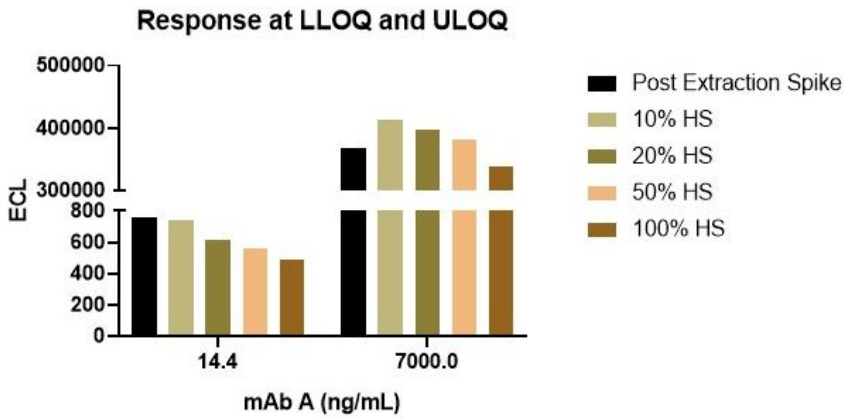


Figure 1. Test of human serum as surrogate curves. Calibration standards were spiked into 10-100% human serum. ECL counts were compared with those from post extraction spiked solution curve.

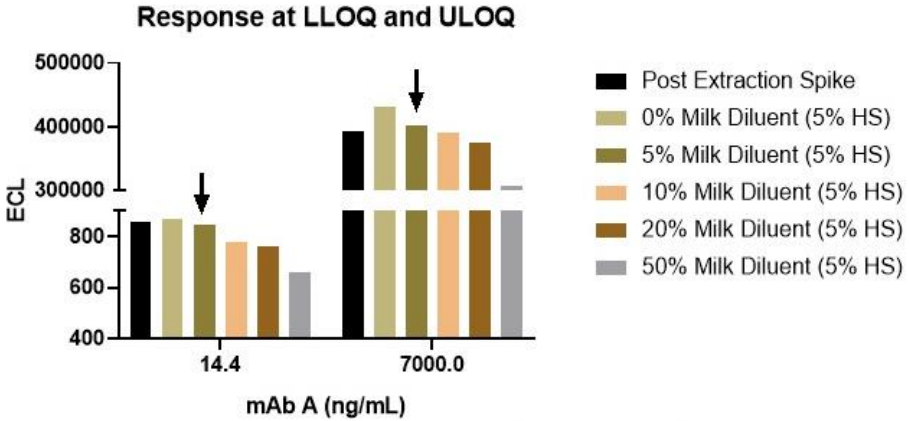


Figure 2. Surrogate curves. Calibration standards were spiked into 5 % human serum (5% HS) in combination with 0-50% of Milk Diluent. ECL counts were compared with those from post extraction spiked solution curve

Curve % match
(Post spike vs 5%MD)

LLOQ: 98.7 %
ULOQ: 102%
7 points Mean: 98.4%

After evaluating numerous combinations of animal/human sera, 5% human serum in combination with 5% KPL Milk Diluent (SeraCare) in 1% BSA/PBST showed near perfect match (Figure 2).

Accuracy and Precision and Matrix Selectivity

Using calibration standards in surrogate matrix stored at -70ºC, VAMS dried blood QC samples stored at room temperature were evaluated.

Table 4. Accuracy and Precision Profile of VAMS QCs (n= 6 runs)

QC Sample	Nominal Concentration (ng/mL)	Intra-Assay	Inter-Assay		
			Precision (%CV)	Accuracy (%RE)	Total Error[1] |%RE| + %CV
LLOQ	14.4	7.6	7.6	0.9	8.5
LQC	28.0	6.4	7.3	8.2	15.5
MQC	300	5.1	5.3	-1.7	7.0
HQC	5600	5.4	5.8	-7.1	12.9
ULOQ	7000	3.4	4.9	-7.8	12.7

[1] Total error may not equal the sum of its components due to rounding in the final displayed numbers.

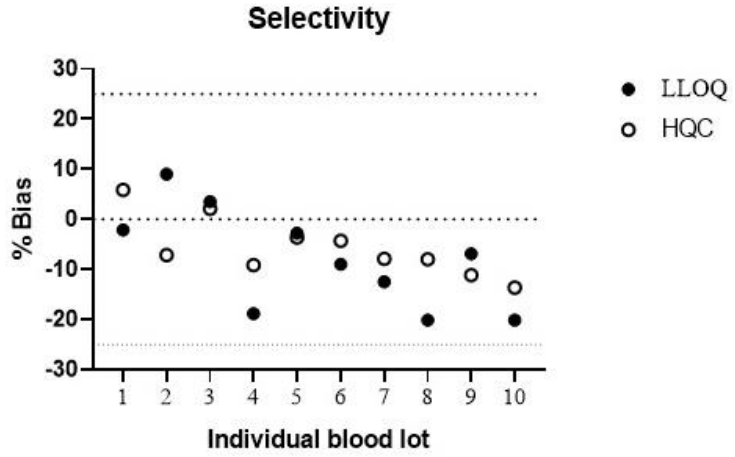


Figure 3. Matrix Selectivity from 10 individual blood lots

- Five levels of QCs were evaluated in 6 independent runs. A profile comparable to serum PK assay was obtained (Table 4).
- VAMS tips prepared using 10 healthy individual blood lots spiked at HQC and LLOQ passed all acceptance criteria within 20% bias (Figure 3)

Stability and Dilutional Linearity

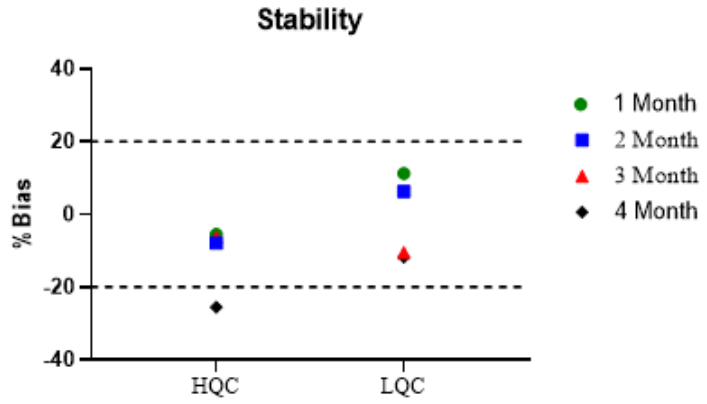


Figure 4. Stability of VAMS Mitra QCs at room temperature.

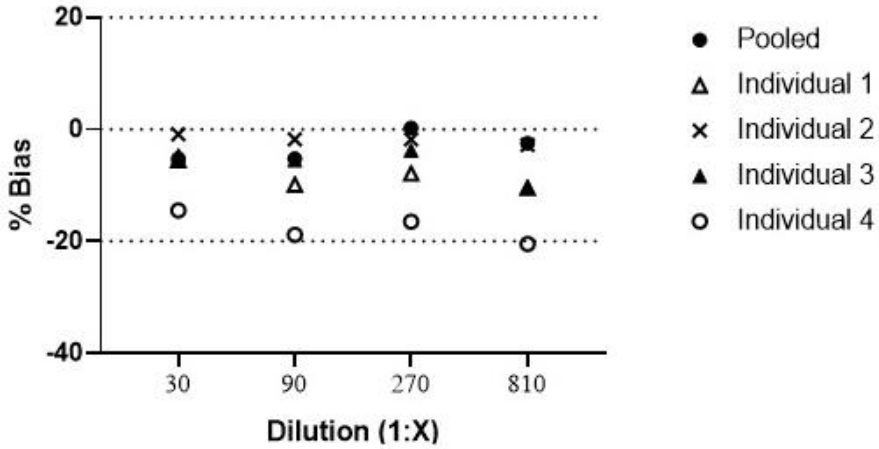


Figure 5. Dilutional Linearity evaluation using surrogate matrix.

- VAMS dried blood QC samples at HQC and LQC were stable up to 3 months at ambient temperature. A recovery of VAMS HQC tip was somewhat impacted at 4 month of storage at room temperature (Figure 4).
- DQC (dilutional QC) VAMS tip sample prepared in a pooled blood and 4 individual blood lots were extracted and the surrogate matrix was used as sample diluent to ensure that there was no variation in individual sample dilution (Figure 5)

Conclusions

- Extraction method for “mAb A” from VAMS dried blood tip was established.
- Surrogate matrix (5% human serum in combination with 5% Milk Diluent in 1% BSA/PBST) was successfully utilized to construct calibration curve that can be stored at -70^{o}C as single use aliquots and used as sample diluent.
- Stability for VAMS dried blood sample at ambient temperature is established for 3 months which provides sufficient time from blood collection on VAMS Mitra tips to PK analysis.
- The result from this feasibility study demonstrates the potential of using a patient-centric sampling procedure for mAb biotherapeutic clinical studies. However, it should be cautioned that study sample variability has not tested.

Future evaluation plans: test other mAb drugs, Tasso-M20 (convenient for self blood collection at home), parallel study with serum PK assay in clinical settings.

Acknowledgments

Regulated Bioanalysis, Merck & Co., Inc., West Point, PA, USA

- Huizhi (Iris) Xie
- Ming Wang
- Linlin Luo
- Eric Woolf

- Huaping Tang (currently at GSK)

Questions

Contact information

dong.hun.lee@merck.com

Dong Hun (Don) Lee, Ph.D.
Assoc. Principal Scientist
Regulated Bioanalysis, PCD
Merck & Co., Inc.
West Point, PA 19486 USA