M1051

Application of Microsampling Techniques for Detection of Anti-drug Antibody (ADA) in Rat

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ABSTRACT

Purpose:
The purpose of this work was two-fold: (1) To test micro sampling methods of DBS and Mitra on a challenging Immunogenicity assay that had originally been developed for serum; (2) To build upon our previous in vitro study using ex-vivo spiked ADA and assess the two collection methods against the standard serum assay for measurement of ADA generated in vivo.
Methods:
Ten Sprague-Dawley rats were dosed with drug at 4mg/kg on days 1 and 7. Samples were collected pre-dose and day 16. Serum was prepared from 200 µL of blood, DBS cards were spotted with 20 µL of blood, and Mitra tips absorbed 10 µL of blood only. Custom Design of Experiments was used to evaluate elution buffer concentration, volumes, time, neutralization buffer concentration and pH to overcome challenges with test article binding to DBS paper and Mitra absorptive material.
Results:
The final extraction conditions for both DBS and Mitra were: 200 µL of 300 mM Acetic Acid and Tris buffer pH: 8.0. Elution times were 55 and 20 minutes, and Tris buffer volumes were 80 and 150 µL, respectively. Assay sensitivities were 62.5 ng/mL and 15.6 ng/mL, and the drug tolerance ratios were 100:1 and 50:1, respectively. The *p* value of correlation between serum and DBS, serum and Mitra, and DBS and Mitra were 0.0268, < 0.0001 and <0.0001, respectively. Raw signal comparison of three collection methods showed that samples with high levels of ADA in serum also measured high using DBS and Mitra. The % positive rate at 16 days in serum, DBS and Mitra were 90%, 40% and 70%, respectively.
Conclusions:
Original assay conditions could not be directly applied to DBS and Mitra due to reduction in sensitivity caused by drug binding to collection materials. After optimization DBS and Mitra still had reduced sensitivity compared to the serum method but assay sensitivity achieved for each of the methods meet and exceeded regulatory recommendations for preclinical ADA assays. These methods are all deemed suitable for detection of ADA responses in vivo.

INTRODUCTION

Previously we have shown that using samples spiked with ADA, DBS collection can yield comparable results to normal serum collection (J Immunol Methods. 2013;393(1-2):53-60).

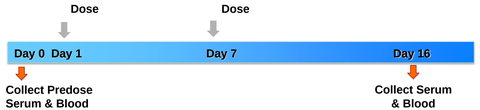
Purpose:

- To confirm previous *ex-vivo* results using ADA generated *in vivo* using a rat model
- To assess performance of a new dried sample technology: Mitra, for ADA sample collection
- To compare results generated from DBS and Mitra collection against the established serum collection

METHODS

Sprague-Dawley Rat Study Design

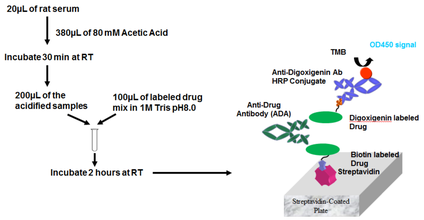
- Ten rats were dosed at 4 mg/kg of drug and samples collected at time points indicated below



Sample Collection:

- Serum: 200 µL of blood were incubated at room temperature for 30 minutes then centrifuged at 13k rpm for 5 minutes
- DBS: 20 µL of blood were placed on designated DBS cards (DMPK-C) then left to dry at room temperature for 2 hours
- Mitra: 10 µL of blood were applied to Mitra tips then dried at room temperature for 2 hours

ADA Assay Procedure:



Different collection methods required modifications of the assay to optimize analyte recovery and achieve acceptable sensitivities. Conditions tested in Design of Experiments were:

- Acetic Acid Concentration: 100 mM - 300 mM
- Acetic Acid Volume: 100-400 µL
- 1 M Tris Buffer: pH 8.0 vs pH 9.5
- 1M Tris Volume: 40-200 µL
- Extraction time: 20-60 minutes

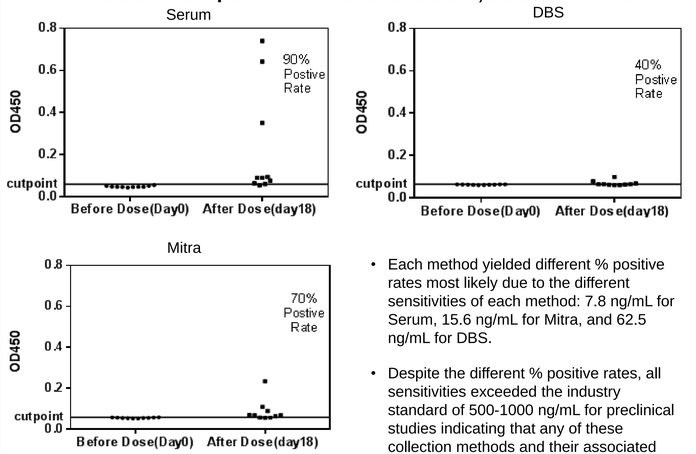
RESULTS

Optimized Assay Conditions for Serum, DBS and Mitra from DOE

Parameters	Original Serum	DBS	Mitra
Sample Volume	20 µL	2x3mm (5 µL)	10 µL
Acetic Acid conc	80 mM	300 mM	300 mM
Acetic Acid volume added	380 µL	200 µL	200 µL
Acidification time	30 min	55 min	20 min
1M Tris pH	8.0	8.0	8.0
1M Tris volume added	100 µL	80 µL	150 µL
Assay Sensitivity	7.8 ng/mL	62.5 ng/mL	15.6 ng/mL
Drug Tolerance Ratio	12.5:1	100:1	50:1

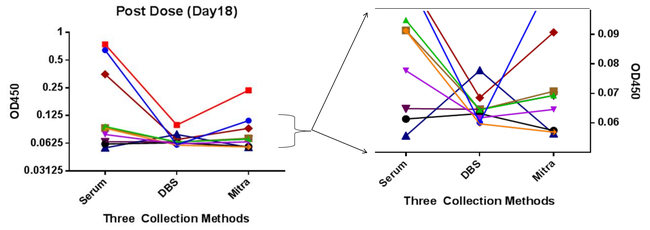
Assay sensitivities correlate directly with sample volume: 20 µL for serum; 10 µL for Mitra; and 5 µL for DBS. Note: Serum equivalents for Mitra and DBS are approximately 5 µL and 2.5 µL as they represent whole blood volumes.

Percent ADA positive animals for Serum, DBS, and Mitra



- Each method yielded different % positive rates most likely due to the different sensitivities of each method: 7.8 ng/mL for Serum, 15.6 ng/mL for Mitra, and 62.5 ng/mL for DBS.
- Despite the different % positive rates, all sensitivities exceeded the industry standard of 500-1000 ng/mL for preclinical studies indicating that any of these collection methods and their associated assays would detect relevant ADA positive animals.

Comparison of sample rank order for Serum, DBS and Mitra for OD450



- The highest three ADA samples ranked the same for all collection methods with one exception for DBS.
- For lower samples rank order is not consistent as the magnitude of differences is within the assay variability.
- Samples collected by Mitra showed the highest degree of similarly in rank order to serum suggesting under these conditions Mitra collection is more suitable than DBS collection.

CONCLUSIONS/DISCUSSION

Given the increasing global nature of clinical trials, collection methods like Mitra that do not require specialist equipment, personnel, or dry ice for shipping are need more than ever.

- **In this study we have described that micro sampling methods such as Mitra can be suitable for preclinical sample collection for ADA measurements. Furthermore these data suggest that these methods should be translatable to clinical ADA sample collection where sensitivities of between 250-500 ng/mL are required.**
- **Given the results of both the sensitivity experiment and the ability to detect positive samples in rank order similar to serum, Mitra performed better than DBS under the conditions tested.**
- **Given the data presented in this poster and the reported advantage that data generated from Mitra are not impacted by hematocrit (Bioanalysis Vol. 7, No. 6, p. 653-659) we conclude that Mitra is suitable for not only ADA collection but potentially for quantitative assays such as PK assays.**

NEXT STEPS

- **Data on stability of Mitra in real world conditions still needs to be generated to assess the ability to ship at ambient temperature, but this type of technology is clearly already useful for sites where centrifuges and -80 freezers are not necessarily available.**
- **For regulated bioanalysis, side by side analysis of serum and Mitra collections may be expected. Discussions amongst industry and regulatory agencies will be necessary to determine the best path forward for deploying Mitra collection method in the regulated bioanalysis arena.**
- **However, for non quantitative measurements such as ADA, and particularly for exploratory biomarkers where data are not used for decision making, there is greater potential for immediate deployment of these technologies.**

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