a review of extraction methods using Mitra® from the current literature.

immunoassays

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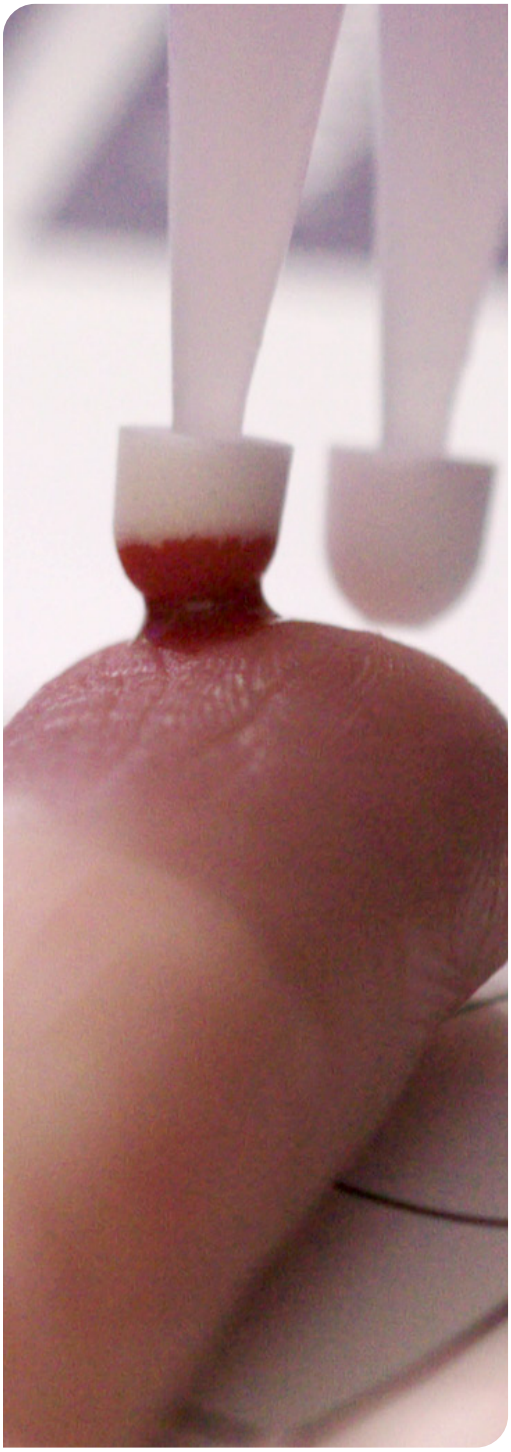
The blood microsampling innovator

Introduction

Laboratories around the world are rapidly developing immunoassays for COVID-19. There are a growing number of papers detailing successful on-boarding of Mitra® microsampling devices, with VAMS® technology, for a variety of analytes including immunoglobulins. One area of continued discussion is how to optimize extraction methods to achieve strong signals and stable extracts. This mini-review summarizes the current understanding of extraction methodology of Mitra in such ligand binding assays.



Luminex® - based Assay

Serological Assay on a Multiplex System to Measure > 33 Flu Strains

doi.org/10.1017/cts.2019.410

Summary

This study illustrates a three-way bridge between traditional phlebotomy, on-site capillary blood samples using Mitra® microsamplers, and at-home capillary blood samples collected with Mitra microsamplers. The application was to quantify anti-hemagglutinin antibodies for >30 strains of influenza. Results of all three sampling approaches were consistent and very good stability was reported (21 days ambient in the lab and samples survived being posted to the lab at the height of summer). Blood values corrected to serum levels with high concordance by measuring Hb of same extracts and converted to Hematocrit (HCT) using a well-constructed algorithm.

Extraction Method

Mitra Tips (10 µL) soaked in 200 µL of extraction buffer [PBS + 1% Bovine Serum Albumin (BSA) + 0.25 Tween] in 1 mL 96 well plates, shaken overnight. These were then further diluted 1:250 to give a final dilution of 1:5000. 200 µL of the final dilution was then added to a clear bottomed 96 well plate. For the immunoassay extraction (MAGPIX® Multiplex Reader, Luminex Co, TX), 50 µL of beads mix (containing 30 separate recombinant HAs was added to each well of the plate). Plates were then incubated with gentle shaking for 2h at room temperature (RT) then 3x washed with (PBS + 1% Bris + 0.1% BSA) using a magnet to immobilize the beads. Following this, IgG-PE secondary Ab was added, and the plates were incubated for another 2h. Three more washes were carried out and then the beads were resuspended in drive fluid.

Assays using Enzyme Linked ImmunoSorbent Assay (ELISA)

- **Successful Analytical Development of 7 Monoclonal Therapeutic Antibody Drugs.**
  doi.org/10.4155/bio-2018-0010

  Summary
  Quantitative recoveries of all drugs in typical serum concentrations, showing 1-month stability at 1 month at RT for 2 days at 37 °C. Both potassium and Hb were evaluated to correlate to serum levels.

  Extraction Method
  20 µL tips were extracted overnight in 0.5 - 1 mL of PBS containing 0.05% Tween and 0.05% NaN_3. Concentration measurements were performed using in-house ELISAs for radioimmunoassay (RIA) for one of the drugs.

- **Clinical Bridging Study on 40 IBD Patients Taking Infliximab**
  doi.org/10.1111/bcp.13939

  Summary
  Patients performed home sampling using Mitra® and then mailed the collected samples to the lab for comparison to venous serum samples collected in the clinic. Excellent correlation (≥ 0.965) comparing fingerpick (dried blood) to venous serum. HCT compensation used by measuring Hb left no bias in the data.

  Extraction Method
  All IFX concentrations were measured by an in-house ELISA. The Mitra 20 µL tips filled with dried blood were removed from the sampler bodies and eluted in 0.5 - 1 mL elution buffer (PBS / 0.05% Tween / 0.05% NaN_3) vigorously shaking overnight (≥ 17 hours) on an orbital shaker. After removal, the eluate was kept at 4°C until measurements were performed.

- **Preclinical PK Measurement of mAb Therapies Trastuzumab and Daclizumab**
  doi.org/10.4155/bio-2018-0228

  Summary
  Pharmacokinetic (PK) bridging study between serum, wet whole blood, and dried blood on Mitra®. HCT correction was performed by measuring the HCT levels of one of the male rats. Excellent extraction efficiency and PK curves generated. The paper concludes that Mitra microsampling results are equivalent to those derived from both serum and liquid whole blood sampling. Stability work was not conducted.

  Extraction Method
  Sampled Mitra® tips (10 µL) were dried overnight, the tips were removed from the sampler body and added to 2 mL microtube where 200 µL of PBS was added. Tips were shaken at 500 rpm at RT for 1h then centrifuged and the supernatant was then processed on the ELISA plate. The blocking reagent used in the bioanalytical assay was 5% (w/v) BSA (SeraCare Life Sciences, MA, USA) in PBS (Gibco Laboratories, MD, USA). The ELISA capture was goat anti-human IgG (Novus, CO, USA) and the detection was HRP-conjugated goat anti-human IgG (Southern biotech, AL, USA). No further details of the ELISA assay were given

Assays using ELISA (continued)

- **Quantification of Saxitoxin in Human Blood by ELISA**
  doi.org/10.1016/j.toxicon.2017.05.009

  Summary
  Successful quantitative detection of saxitoxin from 0.020 to 0.80 ng/mL in human whole blood and from 0.06 to 2.0 ng/mL in dried human blood. Stability was within acceptance criterion over 21 days. Quote from the paper: *"The use of the microsampling devices in this ELISA allows for standardized sampling and because dried blood is stable at RT, shipping charges are much less than shipping refrigerated liquid blood. Finally, this method requires no blood processing or sample clean-up before analysis making it extremely user-friendly and simple to perform."*

  Extraction Method
  Mitra® samplers (10µL) were removed from the sampler bodies and added to the wells of the ELISA plate. Enzyme conjugate solution (50 µL) and polyclonal rabbit antibody solution (50 µL) were dispensed into each well. The plate was covered and shaken for 1h using successive intervals of 30 seconds of shaking at 800 RPM followed by 2 minutes of no agitation. The contents were emptied and washed (x4) with wash buffer (330 µL). Substrate solution (100 µL) was added to each well of the plate, covered and, and shaken (1min, 600 rpm). The plate was covered and incubated (RT, 30 min). Stop solution (100 µL) was then added to each well of the ELISA plate, and the absorbance (450 nm) was read immediately on a PowerWave™ HT microplate spectrophotometer (Biotek, VT).

- **Measurement of IGF-1 by ELISA**
  doi.org/10.1016/j.ghir.2019.12.001

  Summary
  IGF-1 from human capillary blood extractions from Mitra® (20 µL) could be measured for serum concentrations of > 50 ng/mL. Samplers were stable for at least a month at RT.

  Extraction Method
  An aqueous extraction of Mitra tips was performed containing 150 µL of 0.9% NaCl (this provided the most signal compared to PBS and Water). Sample tips were removed into a 2 mL tube (Eppendorf LoBind) and incubated on a rotating wheel (20 rpm) or on a thermomixer for 1h to 1 night at either RT or 4°C. Very little evaporation was observed. Final "Best" protocol was 1-hour incubation on rotating wheel at 20 RPM at RT, with 0.9% NaCl and no anti-protease.

Assays using Chemiluminescence Immunoassay (CLIA) & ELISA

Measurement of C-peptide on CLIA and ELISA

doi.org/10.1177/1932296818763464

Summary
C-peptide is a measure of pancreatic B cell function to help diagnose and monitor the decline of Type-1 diabetes patients. The results showed good agreement to plasma concentrations using HCT correction algorithm. And, no decrease (0.1%) of C-peptide was observed over 48h storage. Also, the Mitra® device gave more reproducible C-peptide results than DBS, with a mean CV% of 4.3% compared to 26.4%.

Extraction Method
Mitra® tips (10 µL) were removed from the sampler bodies and placed into a mini spin column at RT. For the CLIA, 140 µL of antibody conjugate was added, and for the ELISA 110 µL of assay buffer was added. The tubes were shaken at RT (900 rpm) for 1 hour after which they were centrifuged (13,000 rpm) for 3 minutes. The supernatant was

Assays using CLIA & ELISA (continued)

transferred into 1.5 mL microtubes. For ELISA, 75 µL of the supernatant in assay buffer was added to the ELISA plate and shaken at RT for 1h and then washed. 200 µL of Tubular Basement Membrane Antibody was added to each well and incubated for 15 min at RT. Finally,10 µL of stop solution was added. The plate was read using a Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific™) at 450 nm. For CLIA, 100 µL labeled antibody (using an acridinium ester) was added to each well, then 125 µL of extracted blood in antibody conjugate was added. The plate was incubated for 37° C for 2 hours washed and read on a Centro LB 960 microplate luminometer (Berthold Technologies GmbH & Co, Germany).

Considerations for Lateral Flow Immunoassay for COVID-19

Presently there are no papers on using Mitra® in conjunction with lateral flow immunoassay, however, there have been approaches with whole blood where blood is diluted in buffer and the buffer is added to a lateral flow device. One possible method, to combine Mitra sampling with lateral flow immunoassay, is to drop a Mitra tip (10 µL) into buffer (100 µL) as described in the C-peptide paper for ELISA. Likewise, allow the blood to soak in buffer for 1h in order to produce blood water, and then centrifuge. The supernatant (50 µL) could be added to the lateral flow device. Data on such an approach will be added to this document in due course.