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Quantitation of the COVID-19 Neutralizing Antibody Bamlanivimab* Using Mitra® Microsampling and Mass Spectrometry

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OVERVIEW

PURPOSE

To demonstrate the applicability of Mitra® microsampling for the quantitation of a therapeutic neutralizing antibody using a hybrid LC-MS/MS approach.

METHODS

A bioanalytical assay initially developed and validated for bamlanivimab in human serum was transferred to the Mitra® microsampling format.

Human whole blood fortified with bamlanivimab was sampled onto 10µL Mitra®. Samples were desorbed by impact-assisted extraction (IAE) prior to enrichment with Protein G coated paramagnetic particles and trypsin digestion. Data was acquired by LC-MRM on a SCIEX Triple Quad 5500.

RESULTS

Addition of mild detergents and chelators to the desorption buffer significantly improved extraction of bamlanivimab from the Mitra® sorbent. The assay demonstrated comparable performance to the already developed serum-based assay, in terms of linearity, specificity, precision, and accuracy. Bamlanivimab quantitation from Mitra® was not affected by blood hematocrit.

INTRODUCTION

The COVID-19 pandemic was caused by the outbreak of a β-coronavirus, leading to a global health emergency. The speed at which the virus has spread emphasized the urgency to identify and develop effective new therapies. Bamlanivimab is a neutralizing antibody directed against the SARS-CoV-2 spike glycoprotein that plays an essential role in viral infection (**Figure 1**).

The ability to accurately measure therapeutic agents is pivotal during clinical safety/efficacy testing. The development of microsampling techniques in recent years presents an attractive alternative to overcome challenges associated with traditional sampling procedures. This work will demonstrate the applicability of dried blood Mitra® microsampling coupled with a hybrid LC-MS/MS approach for the quantitation of bamlanivimab.

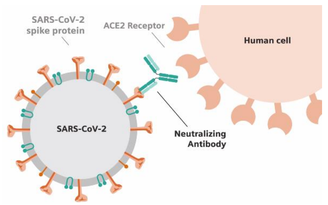

Figure 1. Bamlanivimab SARS-CoV-2 neutralizing antibody mechanism of action (Source: https://www.sinobiological.com/research/virus/sars-cov-2-neutralizing-antibody)

METHODS

REFERENCE STANDARD

Bamlanivimab reference material (LY3819253, 70.4 mg/mL; lot #EL19677-008-API) used in this research was provided by Eli Lilly.

BIOANALYTICAL PROCEDURES

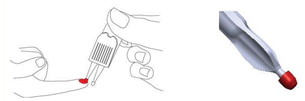

Figure 2. Human whole blood samples fortified with bamlanivimab were absorbed onto 10 µL Mitra® (Neoteryx) and dried at room temperature.

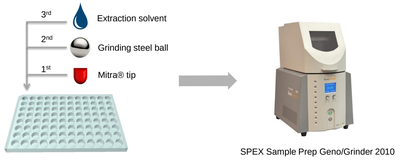

Figure 3. Impact-Assisted Extraction (IAE). The Mitra® tips are transferred to a plate, followed by a 5/32" stainless steel ball and extraction buffer (400 µL). Tips are left soaking for 10 minutes followed by two rounds of five-minute mixing at 1750 strokes/minute on a SPEX Geno/Grinder. Extracted samples (25 µL) are transferred to a KingFisher plate for processing.

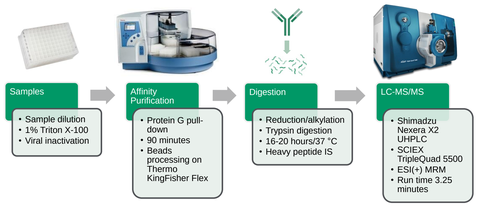

Figure 4. Hybrid LC-MS/MS approach. Samples are purified using Protein G pull-down, followed by protein reduction, alkylation, and trypsin digestion. A bamlanivimab-specific peptide derived from the CDR-H2 region and its corresponding heavy-labeled peptide are monitored by LC-MRM.

RESULTS

METHOD ADAPTATION TO MITRA® MICROSAMPLING

The first step of the bamlanivimab serum assay involves a 40-fold dilution of a 25 µL serum sample in 1 mL of 1% Triton X-100 solution. To keep the sample dilution comparable, bamlanivimab from a 10 µL Mitra® microsample was desorbed using 400 µL of extraction buffer.

Bamlanivimab desorption efficiency from Mitra® is presented in **Figure 5**. The addition of detergents significantly improves desorption from Mitra® tips, probably due to enhanced protein solubility and permeation into the porous Mitra® tip. Interestingly, the addition of EDTA to the buffer significantly improved recovery compared to PBS alone. Extraction buffer containing 1% Triton X-100 and 50 mm EDTA was used to characterize the assay performance.

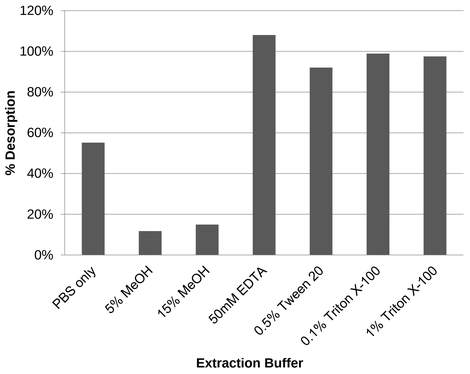

Figure 5. Bamlanivimab desorption from Mitra®. Desorption efficiency was evaluated by extracting bamlanivimab Mitra® human whole-blood microsamples prepared at ULOQ concentration. For comparison, non-extracted samples were prepared by extracting blank Mitra® human whole blood and adding a pure solution containing bamlanivimab (representing 100% recovery) at the end of the impact-assisted extraction (IAE) procedure. Once desorbed, the samples were prepared by affinity purification, reduction, alkylation, and trypsin digestion, followed by LC-MRM analysis.

SENSITIVITY, PRECISION, AND ACCURACY

For the qualification of this hybrid assay, criteria of accuracy of +/- 20% (+/- 25% at the LLOQ) and precision ≤ 20% CV (≤ 25% CV at the LLOQ) are used.

The assay is linear (weighted ($1/x^2$) linear regression), precise, and accurate within an analytical range of 5.00 to 250.00 µg/mL using 10µL Mitra® microsamplers. Representative chromatograms of extracted blank and LLOQ are shown in **Figure 6**.

The precision and accuracy of bamlanivimab were determined at LLOQ, low, medium, and high QC sample concentrations). Overall, the human bamlanivimab whole-blood Mitra® assay demonstrated comparable sensitivity, precision, and accuracy to our validated bamlanivimab serum assay (**Table 1**).

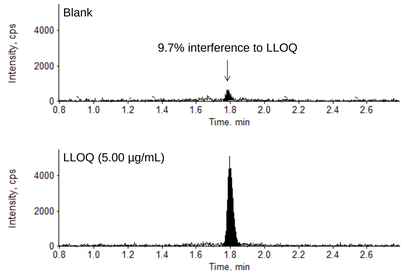

Figure 6. Chromatograms of an extracted blank and LLOQ in human blood Mitra®. Bamlanivimab surrogate peptide IIPILGIANYAQK is monitored.

Table 1. Between-run (interday) precision and accuracy comparison for bamlanivimab in serum and human blood Mitra®

	Serum Assay Validation Interday n = 5	Human Blood Mitra® Adaptation Interday n = 4
LLOQ QC - 5.00 µg/mL		
Between-run accuracy (%Bias)	-3.2%	-1.5%
Between-run precision (%CV)	7.2%	11.6%
Non LLOQ QC		
Between-run accuracy (%Bias)	0.4% to 1.2%	-3.9% to 3.4%
Between-run precision (%CV)	5.3% to 6.0%	4.5% to 13.1%

METHOD QUALIFICATION

Evaluations performed and results obtained as part of method qualification are summarized in **Table 2**. Determination of bamlanivimab from Mitra® was not affected by blood hematocrit. The stabilities of bamlanivimab were demonstrated in Mitra® tips at different temperatures, primary extraction plate, and processed samples.

Table 2. Bamlanivimab method qualification summary

Evaluation	Results	
Specificity	Significant interference (>20% LLOQ) observed in one out of seven regular blank matrix lots screened	
Carryover	No significant carryover observed	
Hematocrit Effect	Low Hematocrit (25%)	High Hematocrit (55%)
Accuracy (%Bias)	-7.4% to 3.7%	-7.0% to -1.7%
Precision (%CV)	2.5% to 4.1%	8.4% to 10.7%
Matrix Effect (as Matrix Factor (MF))	Low QC Mean Analyte MF: 0.9460 Mean IS MF: 1.0508 Mean IS-Normalized: 0.9008 %CV: 3.6	High QC Mean Analyte MF: 0.9533 Mean IS MF: 1.0139 Mean IS-Normalized: 0.9403 %CV: 1.9
Dilution Integrity	187.50 µg/mL diluted five-fold Accuracy (%Bias): -4.3% Precision (%CV): 4.8%	
Recovery of analyte	Recovery from impact-assisted extraction 78.7% to 81.2% Recovery from Protein G pull-down 92.2% to 82.3%	
Stability in whole blood	Confirmed up to 2.0 hours at 22°C nominal % deviation: -7.7% for low QCs and -1.8% for high QCs	
Stability in the primary extraction plate	Confirmed up to 5 days at 4°C nominal Accuracy (%Bias): -8.8% for low stability QC and -2.1% for high stability QC	
Processed samples stability	Confirmed up to 48.0 hours at 4°C nominal Accuracy (%Bias): -4.7% for low stability QC and -10.2% for high stability QC	
Stability on Mitra®	Confirmed up to 12 days at 22°C nominal with desiccant Accuracy (%Bias): -7.0% for low stability QC and -4.5% for high stability QC Confirmed up to 3 days at 37°C nominal with desiccant Accuracy (%Bias): -5.0% for low stability QC and -2.9% for high stability QC Confirmed up to 3 days at 50°C nominal with desiccant Accuracy (%Bias): -0.4% for low stability QC and 3.4% for high stability QC	

CONCLUSION

This research project supports the applicability of Mitra® microsampling along with hybrid LC-MS/MS analysis for the determination of monoclonal antibody therapeutics. It will be interesting to demonstrate whether the approach is applicable to other therapeutic mAbs, using more selective extraction procedures such as immunocapture.

* Bamlanivimab was authorized for emergency use in Nov 2020 but was revoked by the FDA in April 2021 at the request of Eli Lilly and Company as part of the strategy to transition to the use of bamlanivimab in combination with etesevimab, which achieved EUA in the US in February, 2021.