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clinical study

Evaluation of the Mitra® Microsampling Device for Immunosuppressants Monitoring

Successful adherence monitoring by giving the patient a more convenient experience

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Abstract

Immunosuppressants are drugs routinely prescribed to transplant patients in order to suppress the body's ability to reject the transplanted organs. Doctors must individualize a patient's immunosuppressant drug therapy to obtain the optimum balance between therapeutic efficacy and the occurrence of adverse effects, a practice commonly referred to as Therapeutic Drug Monitoring (TDM). Unfortunately, many solid-organ-transplant recipients do not adhere to their immunosuppressant therapy as prescribed[1], which can lead to devastating consequences[2]. A novel microsampling device (Mitra™) has been evaluated in the clinical setting to support the monitoring of immunosuppressant therapies in transplant patients. This device has been designed to easily and reproducibly collect a small volume (e.g. 10 µL) of whole blood in a hematocrit-independent manner, which facilitates blood draws in the clinic or at home by the patients themselves.

For these current evaluations, a two-site study was conducted with the goal to measure the correlation between the Mitra microsampling technique and standard wet blood sampling. Four immunosuppressants (Cyclosporin A, Tacrolimus, Sirolimus, Everolimus) in 36 patient samples were studied, however this study focuses on the results for Cyclosporin A. The correlation of the wet methods commonly used at each site and a Mitra methanol extraction method (additional extraction methods were also evaluated) is very strong with R^2 values at 0.86 (site A) and 0.91 (site B), respectively. Since both sites achieved a strong correlation for this bridge study, a corrective fit can be applied to account for the assay bias as is the common practice in the industry. This strong correlation opens up the possibility for using Mitra microsampling for immunosuppressant monitoring with the added benefits of an automated, economical workflow, a reduced blood collection requirement, and a better patient experience with at-home sampling. The ultimate hope is that adherence can be more closely monitored by providing patients a more convenient option for delivering their blood samples to the lab.

Introduction

Immunosuppressants or immunosuppressant therapy (IST) are drugs routinely prescribed to transplant patients in order to suppress the body's ability to reject the transplanted organs. Doctors must individualize a patient's immunosuppressant drug therapy to obtain the optimum balance between therapeutic efficacy and the occurrence of adverse effects, a practice commonly referred to as Therapeutic Drug Monitoring (TDM). This analysis, commonly performed on a LC/MS/MS platform, is fairly complex since efficacy, pharmacokinetics, and toxicity must be closely evaluated by frequent blood tests, especially just after the transplant operation. Unfortunately, many solid-organ-transplant recipients do not take their IST as prescribed[1]. Nonadherence to IST after transplantation is one of the leading causes of allograft rejection, graft loss, and death. Despite the devastating consequences[2] of IST nonadherence, which include decreased quality of life, increased health care costs, need for dialysis, morbidity, and mortality, reported nonadherence rates range from 5 % to 68 %[1]. Therefore, adherence, defined as the extent to which a person's behavior conforms to medical or health advice, is a critical issue in transplant medicine and institutions are seeking solutions that allow them to more closely monitor their transplant patients.

Mitra dried matrix microsampling, based on volumetric absorptive microsampling (VAMS™) technology, is gaining attention as an alternative to the conventional wet whole blood sample collection and analysis workflow used for IST monitoring. There are several compelling reasons for this. First, with improvements in LC-MS/MS technology, the blood volume requirements for bioanalysis make it unnecessary to collect such large volumes of blood prior to analysis. Second, Mitra microsampling, relative to wet whole blood sampling, offers a simplified sample collection, storage, shipping, and sample preparation process. Third, there is the capability to automate the Mitra microsampler sample preparation. Fourth, and perhaps most importantly, Mitra microsampling offers the opportunity to send at-home sample collection kits to IST patients. The at-home kits are a significant convenience to patients in that they simply collect their own sample at home and mail it back to the lab for analysis. They no longer have to sit in a waiting room for a phlebotomist to draw their blood. Because they are no longer required to visit the hospital and or clinical reference lab for weekly (or even daily/hourly) blood draws the hope is that they will be more diligent about providing a sample regularly, which can be monitored for adherence.

In this two-site study (site A: Leeds Hospital; site B: Royal Liverpool University Hospital), the goal was to measure the correlation between the Mitra microsampling technique and standard wet blood sampling. Four immunosuppressants (Cyclosporin A, Tacrolimus, Sirolimus, Everolimus) in 36 patient samples (provided by site A) were studied, however this study focuses on the results for Cyclosporin A. For more detailed study information on the other compounds, contact Neoteryx.

Materials and Methods

Calibration Curve Generation

Calibration curves were generated and triplicate QCs at 4 concentrations matched the calibration curves within expected limits (± 20 %). The clinical samples from the two sites were then analyzed for their concentration of Cyclosporin A by both the Mitra method and the wet chemistry method and were then correlated to each other

Wet Sample Preparation Methods

Zinc Sulfate Precipitation (Site A Method)

1. Defrost aliquots of calibrators and QC material/remove and allow them to reach room temperature
2. Vortex mix samples
3. Label sufficient 1.5 mL microcentrifuge tubes
4. Place 20 µL of calibration standards/QC/ patient sample into appropriate tube
5. Add 80 µL 0.1 M zinc sulphate solution and vortex mix for 10 seconds each
6. Add 250 µL working Cyclosporin D internal standard
7. Cap and vortex samples for 10 seconds each
8. Centrifuge tubes for 5 minutes at 10,000 rpm
9. Decant 200 µL of supernatant into a microtitre plate
10. Place the plate into the LC auto-sampler in the appropriate position. It is now ready for injection

Zinc Sulfate Precipitation (Site B Method)

1. Defrost aliquots of calibrators and QC material/remove and allow them to reach room temperature
2. Vortex mix samples
3. Label sufficient 1.5 mL microcentrifuge tubes
4. Place 25 µL of calibration standards/QC/ patient sample into appropriate tube
5. Add 100 µL 0.1 M zinc sulphate solution followed by 250 µL working Cyclosporin D internal standard using a repeater pipette
6. Cap and vortex samples for 20 seconds each
7. Centrifuge tubes for 5 minutes at 10,000 rpm
8. Place tubes in worklist order in auto-sampler plate and remove tube lids
9. Cover with re-sealable film and heat seal
10. Place the plate into the LC auto-sampler in the appropriate position. It is now ready for injection

The Mitra Microsampler class I medical device is for direct specimen collection of blood and other biological fluids. It is not specific to any clinical test, and is not for use in diagnostic procedures. Use of the Mitra Microsampler in Laboratory Developed Tests (LDTs) requires further processing including the establishment of performance characteristics and successful validation by the laboratory in a manner consistent with CLIA requirements.

VAMS Collection & Sample Preparation Methods

MeOH extraction (Site A & B)

1. Mitra sampling tips collected 10 µL of patient blood, containing EDTA, from microcentrifuge tubes following the steps in the instructions for use
2. Samples were allowed to completely dry (at least 40 minutes)
3. Add dried sampling tips were removed and placed into a 96-well collection plate containing 200 µL of methanol (containing internal standard)
4. Collection plate was shaken on an orbital shaker for 1 hour @ 1100 rpm
5. Remove the sampling tips which should be a pale reddish color.
6. The solvent was evaporated and samples were reconstituted in initial mobile phase for injection
7. Transfer solvent to autosampler vials and inject into LC-MS/MS

Zinc Sulfate Precipitation *(Site B only)*

1. Mitra sampling tips collected 10 µL of patient blood, containing EDTA, from microcentrifuge tubes following the steps in the instructions for use
2. Samples were allowed to completely dry (at least 40 minutes)
3. Add dried sampling tips to 100 µL of water/5 % MeOH (including IS) in square well collection plate
4. Plate shake for 20 min
5. Remove the sampling tips which should be light pink
6. Add 100 µL $ZnSO_4$ (2.88 g in 100 mL)
7. Plate shake for 5 min
8. Add 100 µL of ACN
9. Plate shake for another 5 min
10. Spin (we transferred to Eppendorf tubes for this)
11. Transfer to autosampler vials and inject into LC-MS/MS

LC-MS/MS Method

System:	Shimadzu UFLC / ABI 4000
Column:	Kinetex 2.6 µm C18 50 x 2 mm ID (Phenomenex)
Temperature:	60 °C
Flow Rate:	0.6 mL/min
Injection Volume:	5 µL
Mobile Phase:	A: 1 L 100 % methanol / 0.143 g acetic acid / 1 mL formic acid B: 1 L 100 % water / 0.143 g acetic acid / 1 mL formic acid

Gradient:

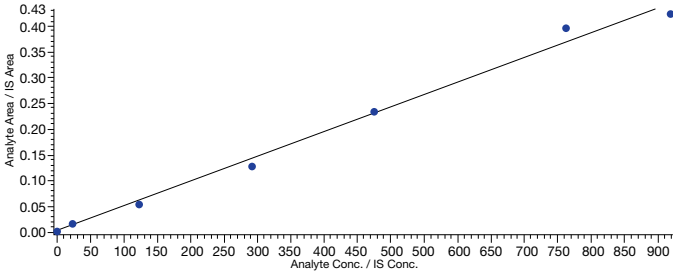
Time (min)	% B
0	60
1	0
1.6	0
1.61	60
2.6	60

Results and Discussion

Calibration and QCs

Due to the importance of immunosuppressant assays every data point is checked by the analyst, thus a robust assay is vital[3]. To ensure that the assay is accurate and precise, and there is no drift in the data, each batch is run with whole-blood calibration and QC samples. The number of these vary from hospital to hospital. For this study we mimicked the standard hospital practice[4] and generated calibration curves with a low QC equal to 25 ng/mL up to a high QC of 1,070 ng/mL (**Figure 1**). Triplicate QCs at four concentrations matches the calibration curves within expected limits (± 20 %). The clinical samples from the two sites were then analyzed for their concentration of Cyclosporin A by both the Mitra method and their wet method and were then correlated to each other. Therapeutic ranges for Cyclosporin A are generally based on trough concentrations (i.e. blood testing occurs immediately before a scheduled dose), and optimal response for most individuals occurs with trough levels at 100-400 µg/mL[5]. If testing is performed at any time prior to the next scheduled does the reported concentrations will be higher. Dosing levels typically decrease in the months after transplant, and a reasonable therapeutic default reference range for testing at a trough is 50-400 µg/mL.

Figure 1.
Calibration curve for Cyclosporin A



Correlation

Figures 2 and **3** show the correlation of the wet method commonly used at each site and the Mitra methanol extraction method. The correlation between the data sets is very strong with R^2 values at 0.86 (site A) and 0.91 (site B), respectively. There also appears to be a uniform assay bias determined at both sites where the Mitra assay yields a concentration that is around 60 % higher than the result obtained via the wet methods. This was due to the difference between using QCs which are freeze dried and "fresh" patient samples. The immunosuppressants were trapped in the dried whole red blood cells and impossible to extract without lysis of the extraction which is the reason why the zinc sulfate precipitation method works better than the methanol extraction. Since both sites achieved a strong correlation for this bridge study, a corrective fit can be applied to account for the assay bias as is the common practice in the industry.

Figure 2.
Leeds Hospital (site A) correlation between the standard wet method they use for extraction of immunosuppressants vs. the Mitra microsampling methanol extraction.

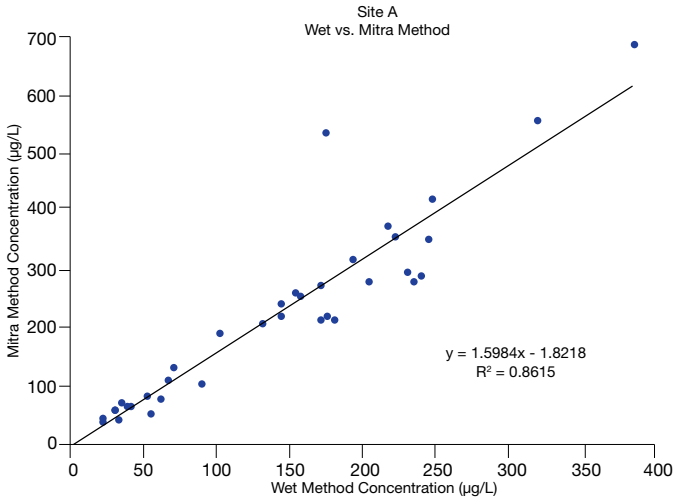
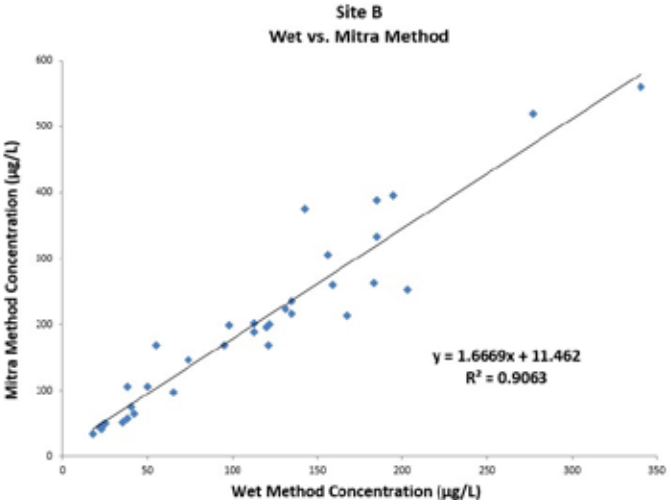


Figure 3.
Royal Liverpool University Hospital (site B) correlation between the standard wet method they use for extraction of immunosuppressants vs. the Mitra microsampling methanol extraction.



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Results and Discussion (continued)

Correlation (continued)

Figure 4 shows the correlation between the two wet methods run at the two different sites whereas **Figure 5** shows the correlation between the two Mitra methods run at each site. Both figures show essentially the same result, there is an assay bias between the two sites in each method where the result at site B is around 20% lower than the concentrations determined by site A. The assays bias are similar and the correlation coefficients are ≥ 0.94 in both sets of data indicating that the data from both sites correlates in the same way regardless of whether the method is performed via traditional wet methods or the Mitra method.

Figure 4.
Leeds Hospital (site A) standard wet method vs. Royal Liverpool University Hospital (site B) standard wet method.

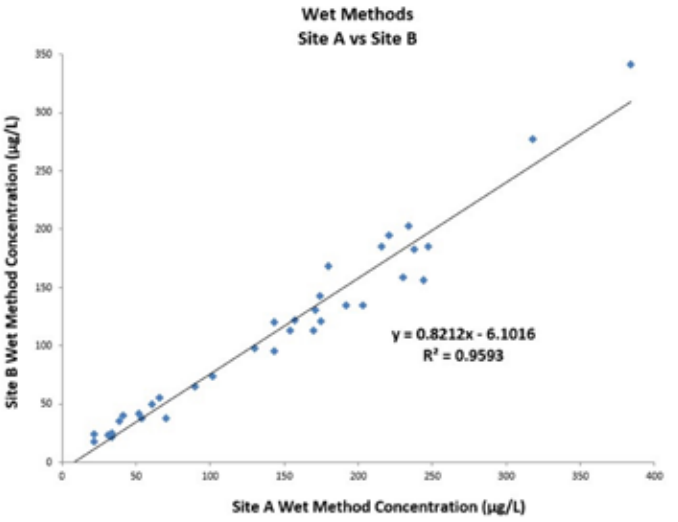
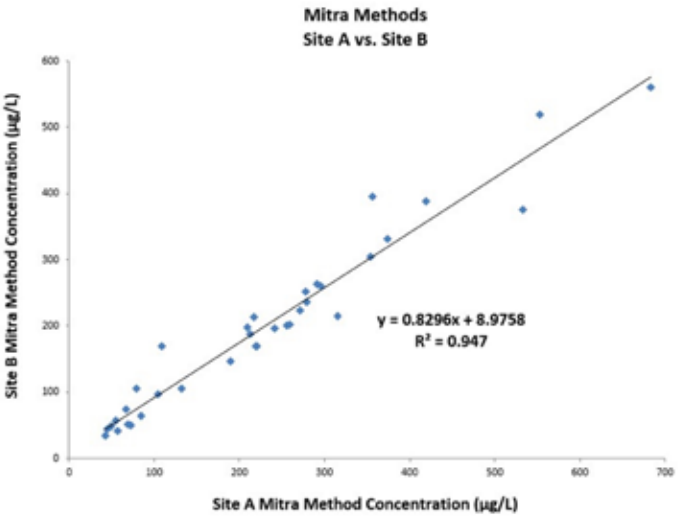


Figure 5.
Leeds Hospital (site A) vs. Royal Liverpool University Hospital (site B) for the Mitra method



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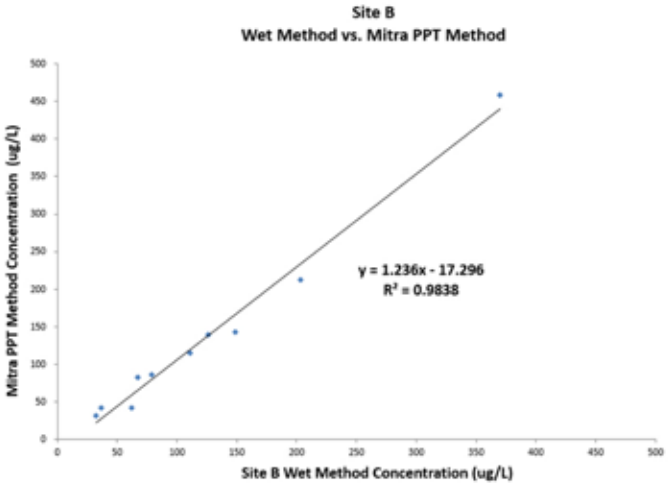
Correlation After Mitra Method Optimization

After observing the positive assay bias at both sites, an effort was made to modify the extraction methodology. It was decided to adapt a zinc sulfate blood precipitation approach to an extraction of the Mitra microsampler. This method was developed and then employed by site B against a pool of ten of their patient samples (**Figure 6**). With this new method, the assay bias reduced to ~1.2 µg/L, and the correlation between the wet and dry methods increased to R^2 0.98. Efforts are being made to improve further on this extraction, and then to expand the work to include additional sites and samples.

Figure 6.
Royal Liverpool University Hospital (site B) correlation between the standard wet method they use for extraction of immunosuppressants vs. the Mitra microsampling zinc sulfate precipitation method.



There is still more work to be done on the analytical side, but we were not expecting to get such good results on the first pass. Further work will entail looking at further improving the extraction experiment. It is hoped that improvements in extraction will help to reduce further any assay bias. Work in-house on other analytes have shown that using prolonged vortexing and also sonication have significantly improved absolute extraction efficiencies. Similar strategies will be employed with optimization of this assay.

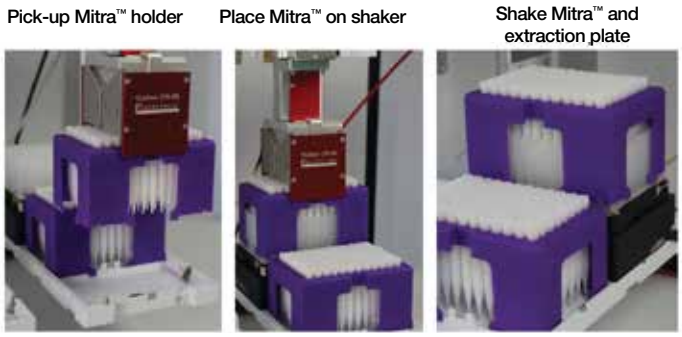
Results and Discussion (continued)

Standard Wet Sampling vs. Mitra Microsampling

This study, as evidenced by the correlation data, shows that Mitra microsampling is a potential alternative to traditional wet sampling methods and can be used to overcome the drawbacks of wet sampling that surround sample preparation and patient sampling. One drawback of the standard wet method is that sample preparation is rather laborious and difficult to automate. The simpler Mitra method, on the other hand, can be automated on a liquid handler leveraging the 96-rack format as seen in **Figure 7**.

Figure 7.

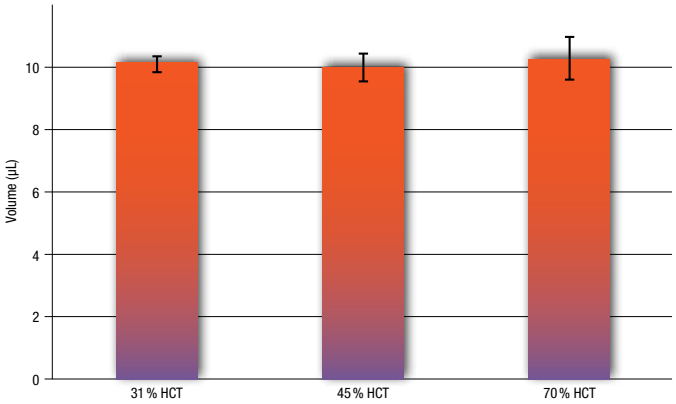
Automation example on the Mitra PAL® RTC instrument (Brechbühler AG)



Another drawback of traditional wet sampling is the amount of blood taken for the immunosuppressant assay. Only 20-100 µL is required for the test depending on the sample preparation method and LC-MS/MS instrument sensitivity, but several milliliters of blood is sent to the lab. This becomes an issue when working with pediatric patients where it is difficult to draw large volumes of blood or with very ill patients where blood flow is low. The Mitra microsampling device reliably collects 10 µL (**Figure 8**) of blood, regardless of hematocrit percentage, from a fingerstick. Many times additional blood is taken at the same time for a creatinine measurement, which is even more burden on the patient. To address this, we are currently investigating a Mitra microsampling method for it.

Figure 8.

Average volume collected by the Mitra Microsampler is 10.1 µL with a 3.5 % RSD



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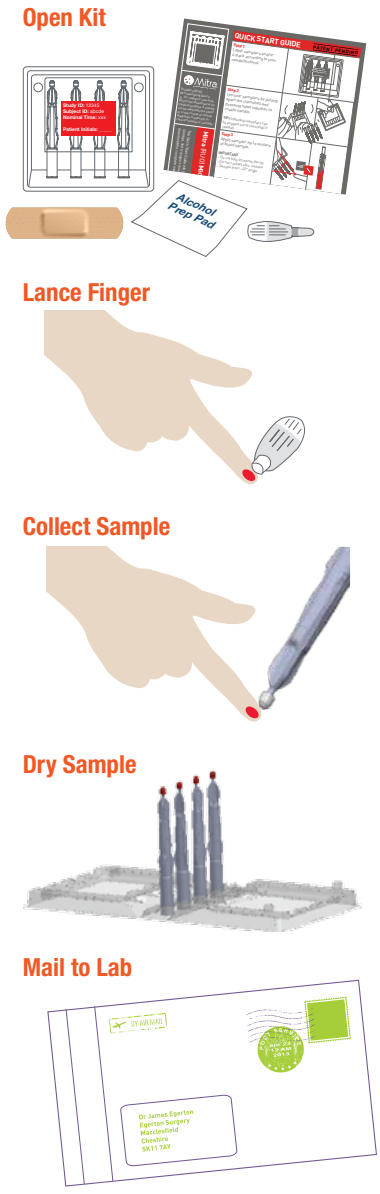
The inconvenience patients experience driving to and from the clinic time specific post-dose blood draws and the irritation of sitting in a lab waiting room is a third drawback of the traditional wet sampling method. At-home sampling kits containing a Mitra microsampler give the patients a more convenient, peaceful experience (**Figure 9**) because they can draw their own quantitative sample in the comfort of their own home and simply mail it back to the lab for analysis. This convenience factor can potentially lead to patients being more diligent about providing their sample to be monitored for adherence. Time required to go to the hospital will be for IST discussions with the physician not for blood draws with the phlebotomist. Other advantages to at-home sampling include less burden on clinic resources and less chance of the patient being exposed to sick patients in the hospital which is an issued when their immune system is compromised.

Figure 9.

Patient experience using a Mitra home sampling kit.



Conclusions

This study has shown that with little method development and simple extraction protocols, strong correlations can be built between standard wet sampling methods and the Mitra microsampling one. This strong correlation opens up the possibility for using Mitra microsampling for IST monitoring with the added benefits of an automated, economical workflow, a reduced blood collection requirement, and a better patient experience with at-home sampling. The ultimate hope is that adherence can be more closely monitored by providing IST patients a more convenient option for delivering their blood samples to the lab.

Literature Cited

1. Chisolm M, Lance CE, Mulloy LL. Patient Factors Associated With Adherence to Immunosuppressant Therapy in Renal Transplant Recipients. Am J Health Syst Pharm. 2005; 62 (17):1775-1781.

2. Prendergast MB, Gaston RS. Optimizing Medication Adherence: An Ongoing Opportunity To Improve Outcomes After Kidney Transplantation. Clinical Journal of the American Society of Nephrology. Clin J Am Soc Nephrol 2005 May; 1305–1311.

3. Peterson AM, Takiya L, Finely R. Metaanalysis of trials and interventions to improve medication adherence. Am J Health-Syst Pharm. 2003; 60:657-65.

4. Kahan BD, Keown P, Levy GA, et al: Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. Clin Ther 2002

5. http://www.childrensmn.org/manuals/lab/Chemistry/028766.pdf

Ordering Information

Part No.	Description	Unit
10005	10 µL Mitra Microsampler 96-rack	1 ea
20005	20 µL Mitra Microsampler 96-rack	1 ea
10006	10 µL Mitra Microsampler 96-rack	6/pk
20006	20 µL Mitra Microsampler 96-rack	6/pk
10004	10 µL Mitra Microsampler 4-sampler Clamshell	52/pk
20004	20 µL Mitra Microsampler 4-sampler Clamshell	52/pk
10002	10 µL Mitra Microsampler 3-sampler Clamshell	52/pk
20002	20 µL Mitra Microsampler 3-sampler Clamshell	52/pk
10001	10 µL Mitra Microsampler 2-sampler Clamshell	52/pk
20001	20 µL Mitra Microsampler 2-sampler Clamshell	52/pk
20101	20 µL Mitra Microsampler 96-autorack	1 ea
20100	20 µL Mitra Microsampler 2-sampler Cartridge / Desiccant Pack	8/pk
100	Mitra Drying Rack	1 ea
108	Mitra Drying AutoRack	1 ea
102	Mitra Sampling Tool	1 ea
103	96-Well Collection Plate, 2 mL Round Well, Round Bottom, 8 mm	50/pk
104	Protein Precipitation Plates	2/pk
105	Pierceable Sealing Mat, 96-Round Well, 8 mm, Silicone	50/pk
106	Pre-Slit Sealing Mat, 96-Round Well, 8 mm, Silicone	50/pk
107	Sealing Tape Pad	10/pk

CE IVD

The Mitra Microsampling Device is a FDA listed Class 1 device (D254956). Neoteryx complies with FDA good manufacturing practices, CFR 820 regulations, and ISO 13485

TN38760616_W

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