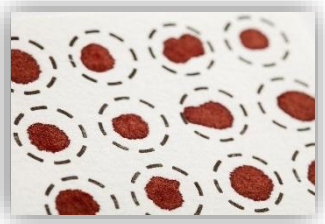
neoteryx™

The promise of microsampling, delivered

introduction to neoteryx services/products, publications/resources, and future plans

microsampling devices

	DBS card	Capillary tube
		
No hematocrit (HCT) bias		x
Automatable extractions		
Quantitative volume collection		x
User-friendly sampling		
No freeze/thaw cycle	x	
No cold chain/biohazard shipping	x	
No sub-punching required		x

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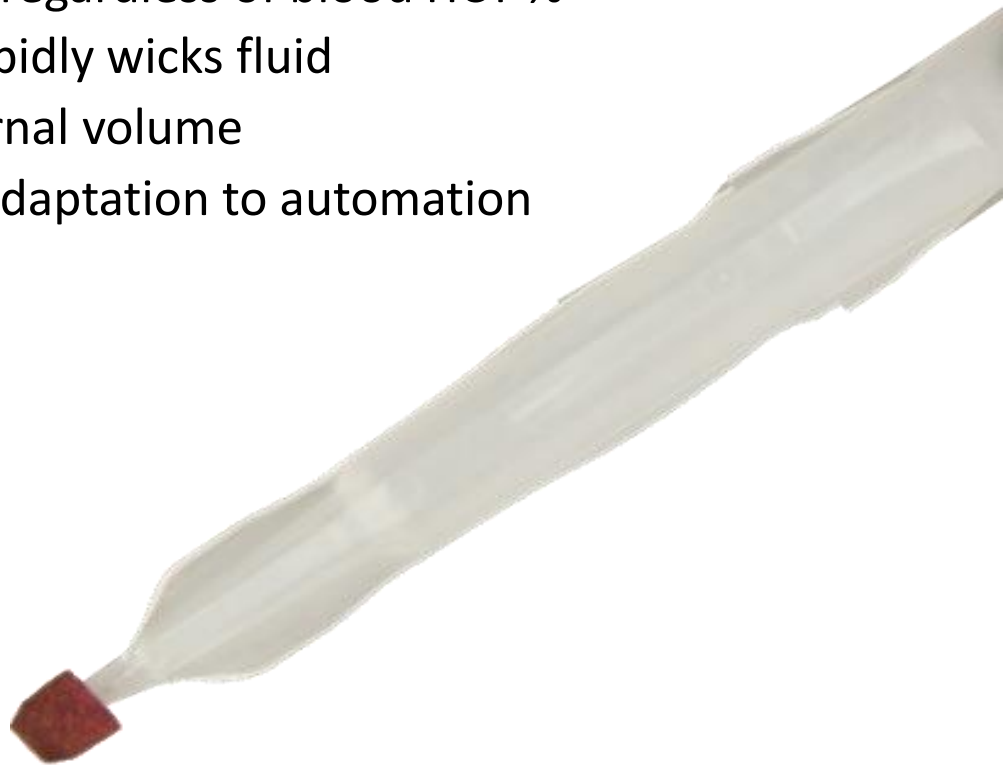
next generation Mitra® microsampling device

- Based on Volumetric Absorptive Microsampling (VAMS™) technology
  - Collect 10 or 20 µL in seconds, regardless of blood HCT %
  - Hydrophilic porous material rapidly wicks fluid
  - Fixed, highly reproducible internal volume
  - Sampler body allows for easy adaptation to automation



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.

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. The Mitra microsampler is patent pending.

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video of rapid, simple sample collection

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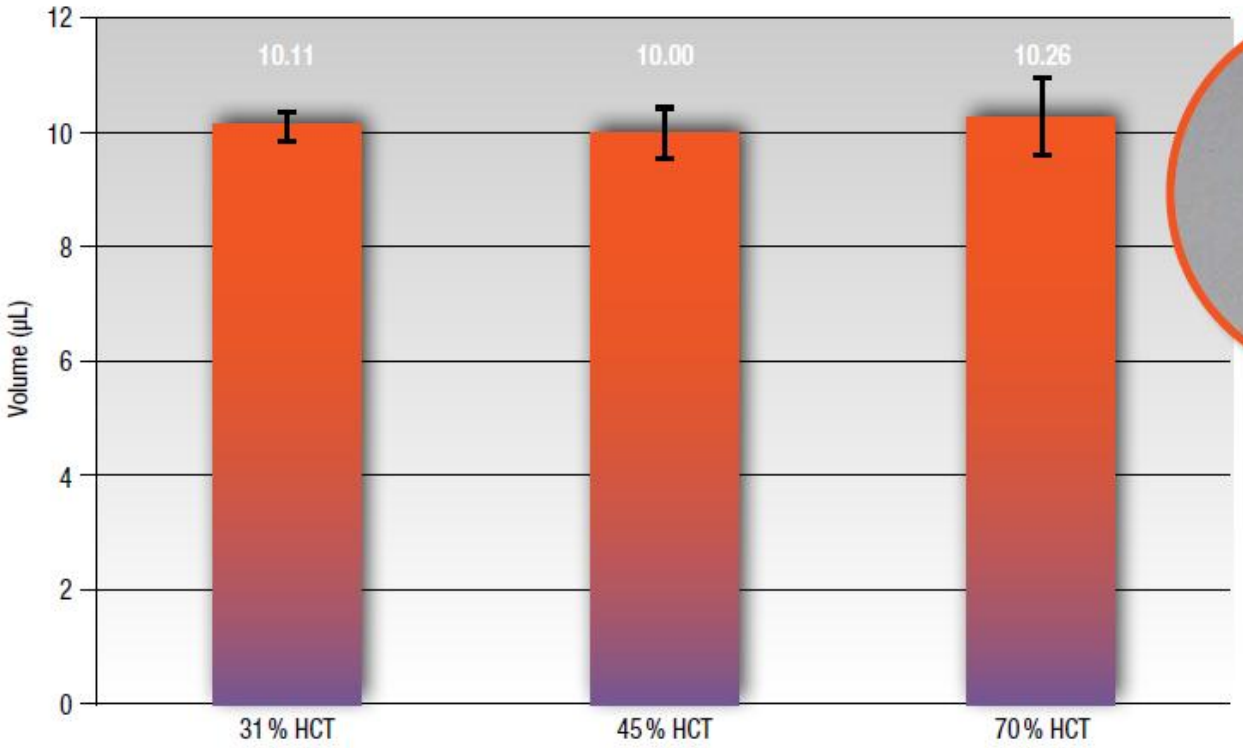
HCT independent collections (10 µL)



Overall average = 10.1 µL
Overall RSD = 3.5 %

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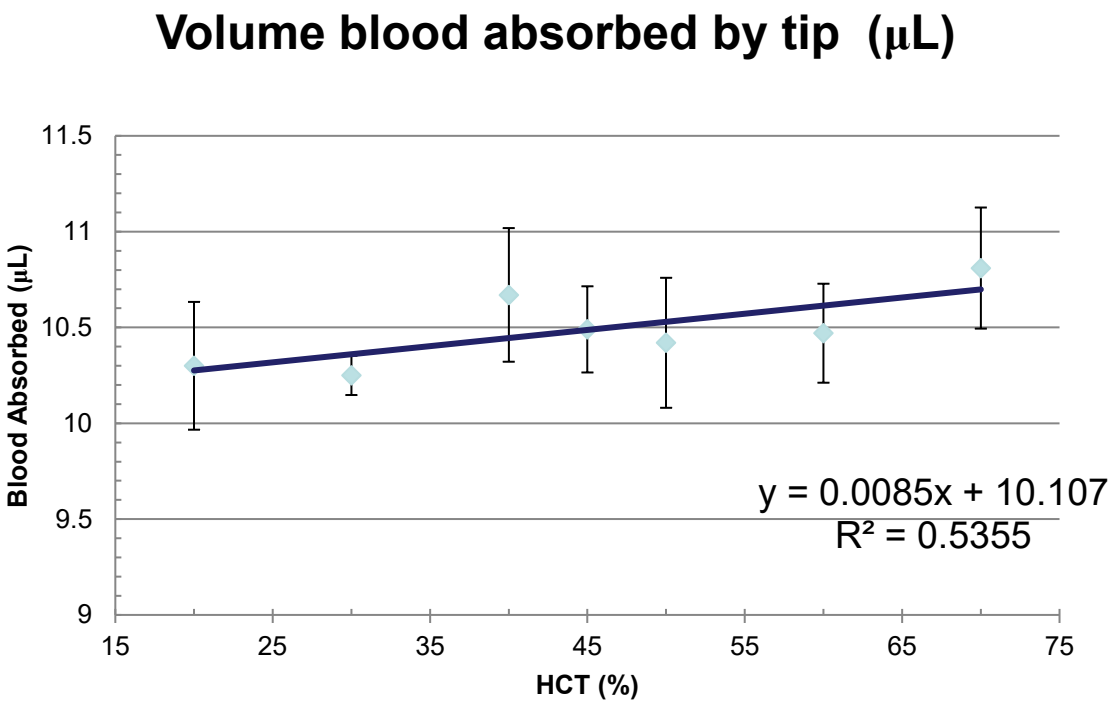
HCT effect on blood volume absorbed

- Max volume bias from 45% HCT is 3%
- 5% max volume deviation over HCT ranges 20% to 70%



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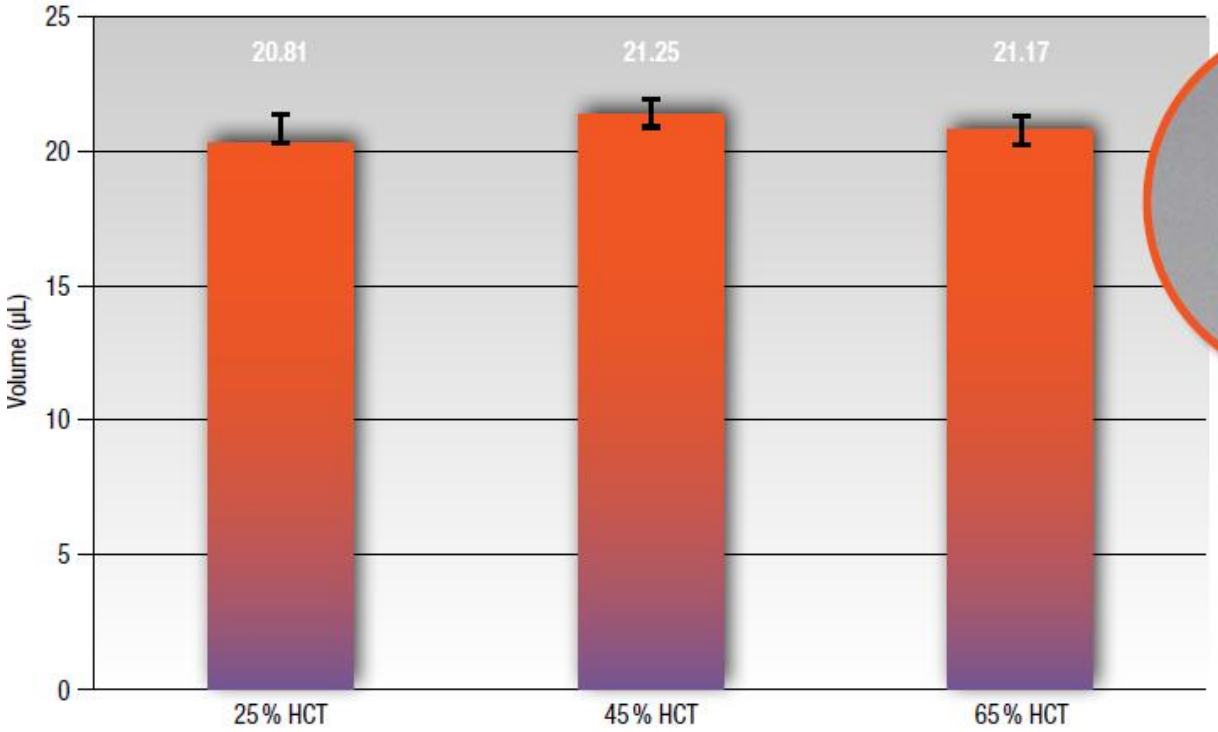
Data Courtesy of Neil Spooner and Phil Denniff, GSK (Anal. Chem., 2014, 86 (16), pp 8489–8495)

HCT independent collections (20 µL)



Overall average = 21.08 µL
Overall RSD = 2.5 %

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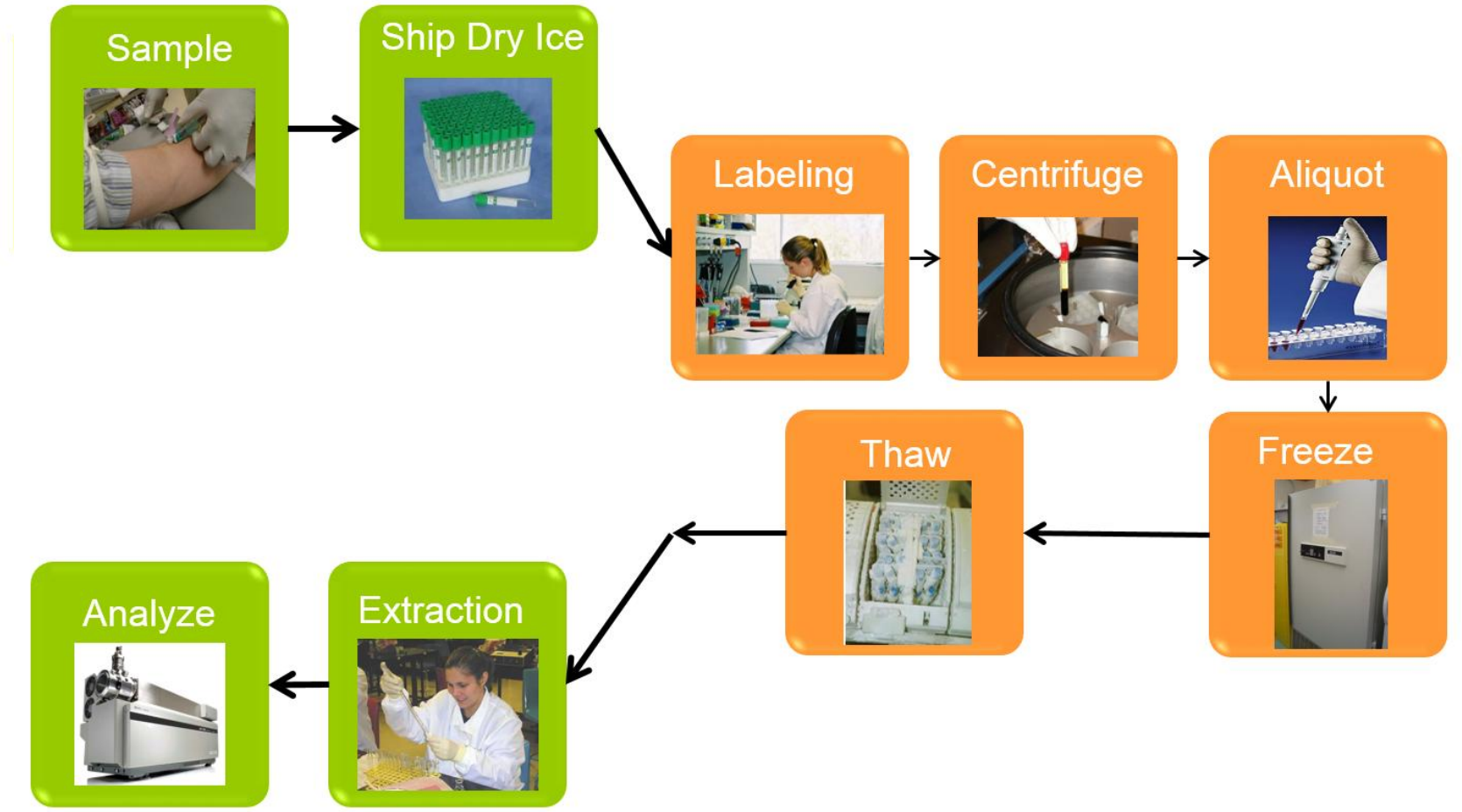
streamlined workflow



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Mitra® device formats

- Direct sampling cartridges and clamshells

- Automatable 96-racks

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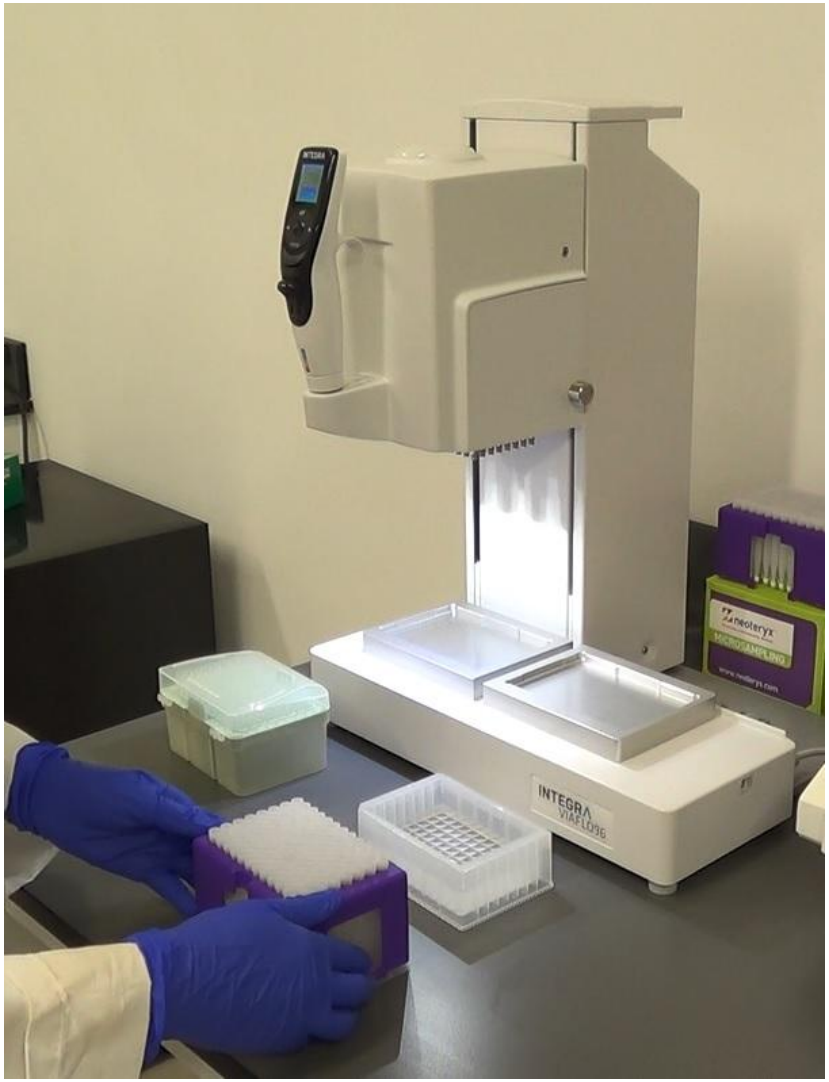
semi-automation of Mitra® device



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microsampling devices

	DBS card	Capillary tube	Mitra® device
			
No hematocrit (HCT) bias by spot migration		x	x
Automatable extractions			x
Quantitative volume collection		x	x
User-friendly sampling			x
No freeze/thaw cycle	x		x
No cold chain/biohazard shipping	x		x
No sub-punching required		x	x

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immunosuppressant monitoring

- Collaboration with Leeds Teaching Hospital, St. James Hospital (36 patients)
  - Development of an at-home assay for tacrolimus monitoring
  - Measure the correlation between in-house “wet sample” method and Mitra® dry sample method

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study design

In-House (Wet) Protein Precipitation Method

1. 25 µL of calibration standards/QC/ patient sample were placed in 1.5 mL Eppendorf® tubes
2. A solution of 100 µL 0.1 M zinc sulphate solution followed by 250 µL working IS solution (MeOH) was added to the samples
3. The tubes were capped and vortexed for 20 seconds then centrifuged for 5 minutes at 10,000 rpm
4. The supernatant was then analysed by 2D LC-MS/MS

Data courtesy of Stephen Stephenson. Leeds Teaching Hospital, St. James Hospital, England.

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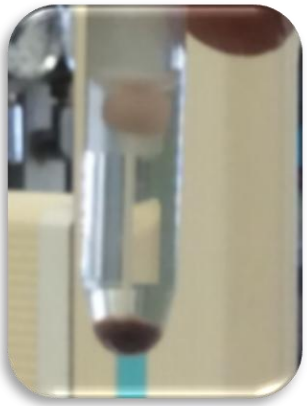
study design (continued)

Mitra Organic Extraction Method

1. Patient blood samples with calibrators (7) and QCS (4) were sampled with Mitra and dried for 1h
2. The Mitra tips were placed into a 96-well collection plate containing 200 µL of methanol (containing internal standard) and shaken for 1h (1100 rpm)
3. The tips were removed and the supernatant was dried then reconstituted in mobile phase
4. The samples were then analysed by 2D LC-MS/MS

Mitra Protein Precipitation Method

1. Patient blood samples with calibrators (7) and QCS (4) were sampled with Mitra and dried for 2h
2. The samplers were added to wells of a 96-well plate containing water (100 µL) and vortexed for ½ h after which the samplers were discarded
3. A premix of 100 µL of 8 % zinc sulfate and 100 µL of methanol with IS was added to each well and the plate was vortexed for 5 minutes
4. The samples were transferred to centrifuge tube (1.5 mL Eppendorf) and spun for 5 min
5. The samples were then analysed by 2D LC-MS/MS



Data courtesy of Stephen Stephenson. Leeds Teaching Hospital, St. James Hospital, England.

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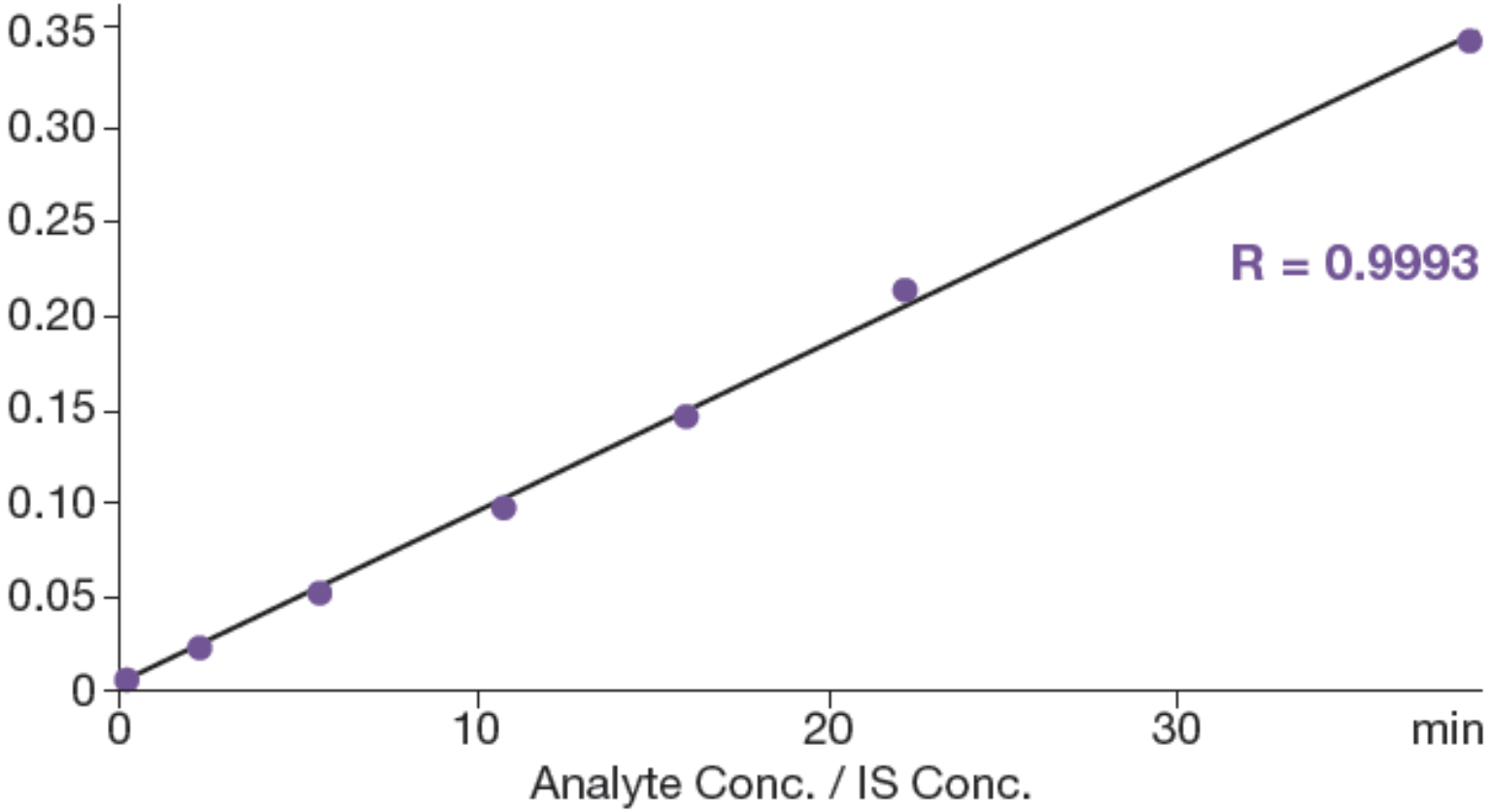
study results

- Calibration curve: tacrolimus from a Mitra® Microsampler extract



Data courtesy of Stephen Stephenson. Leeds Teaching Hospital, St. James Hospital, England.

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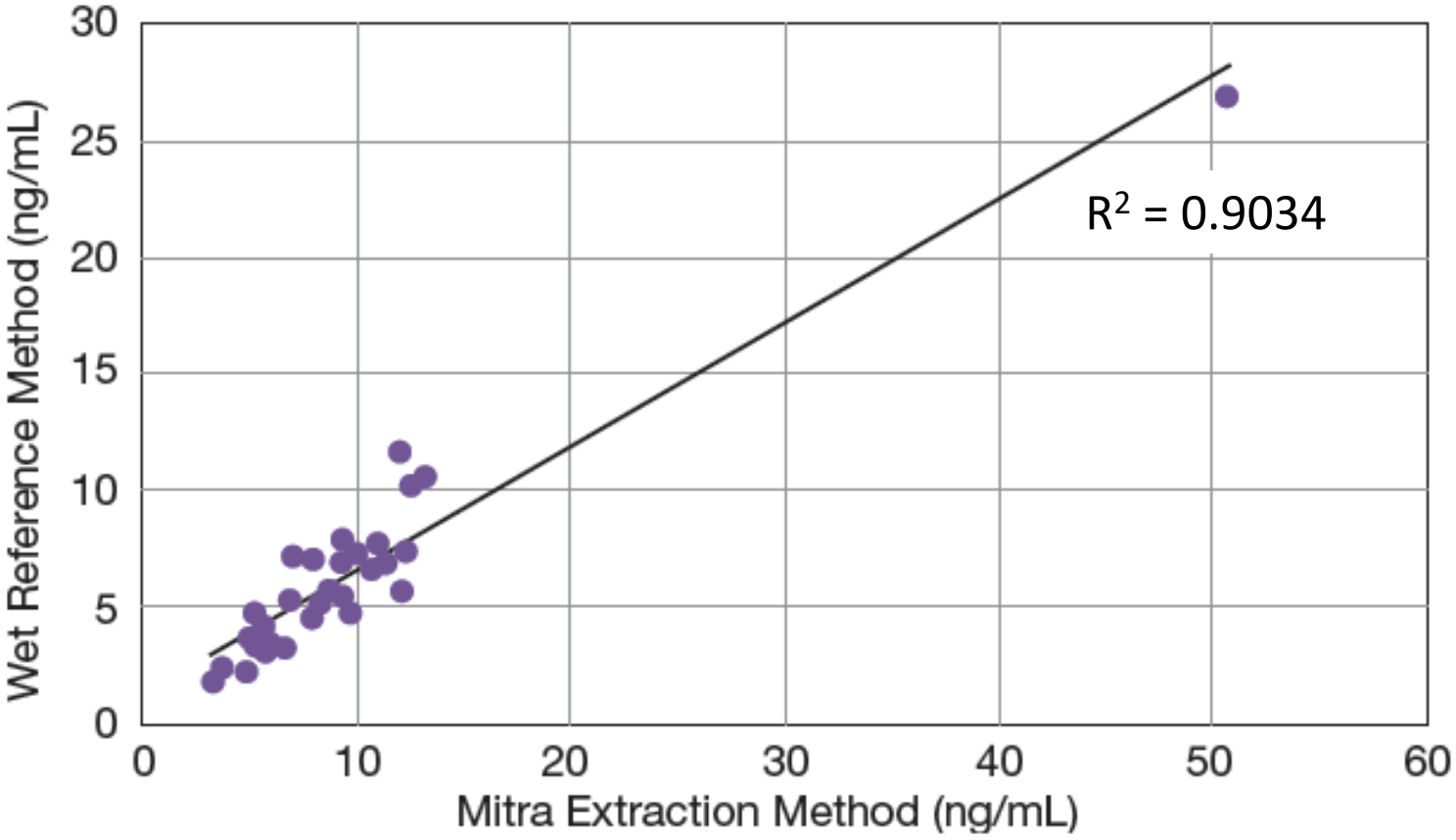
study results (continued)

- Correlation: in-house wet vs. Mitra® organic extraction method



Data courtesy of Stephen Stephenson. Leeds Teaching Hospital, St. James Hospital, England.

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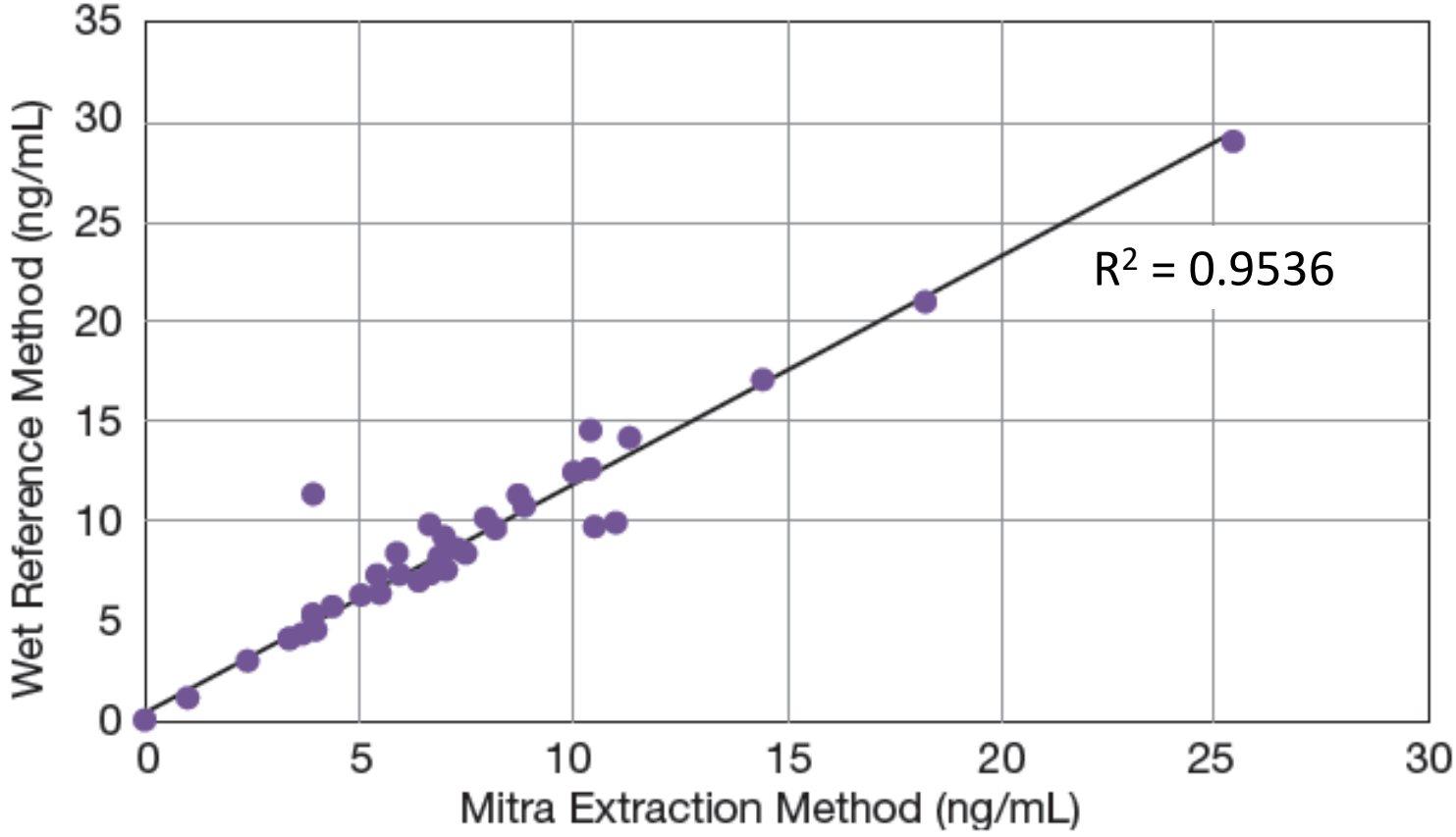
study results (continued)

- Correlation: in-house wet vs. Mitra® protein precipitation method



Data courtesy of Stephen Stephenson. Leeds Teaching Hospital, St. James Hospital, England.

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immunosuppressant monitoring summary

- Strong correlations can be built between standard methods and Mitra® microsampling methods using simple extraction methods
- Immunosuppressant assays may benefit from a protein precipitation method for extraction
- Data indicates that microsampling approaches can be applied to therapeutic drug monitoring of immunosuppressants in the clinic or potentially at-home

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molecular diagnostics

- Study by Norgen Biotek Corp for total RNA/DNA purification and detection from blood preserved on Mitra® device
  - Aims to validate if dry blood microsampling can be used as a collection device for various downstream applications
  - Is microsampling a viable solution for genotyping and biomarker identification

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study design

- DNA purification, quantitation and detection
- RNA purification, quantitation, and detection
- SNP detection
- RNA expression analysis by Next Generation Sequencing

Data courtesy Dr. Bernard Lam, et. al. Norgen Biotek Corp., Canada.

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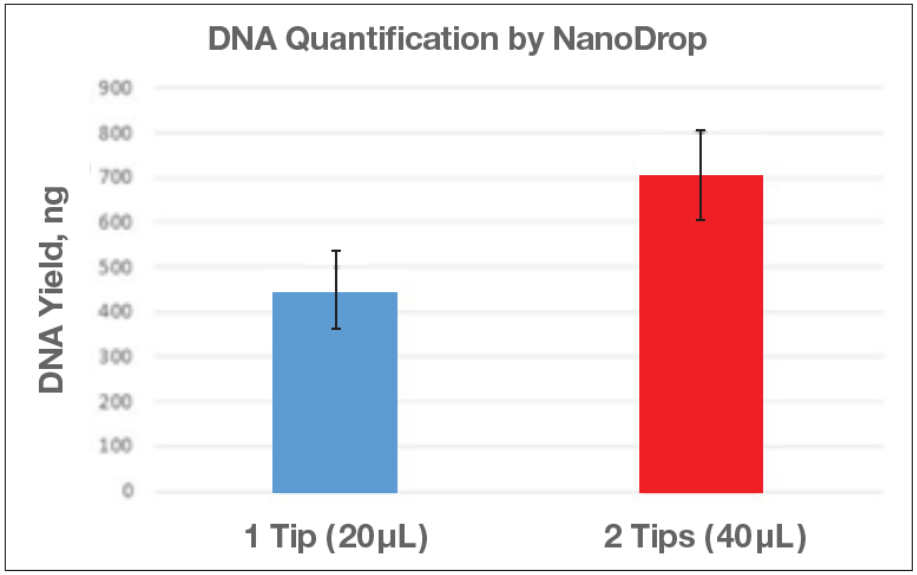
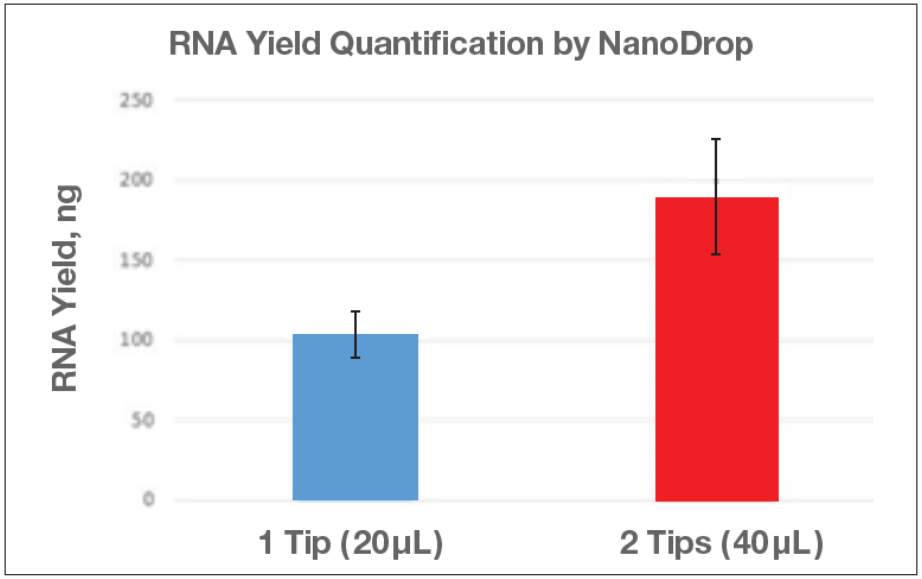
DNA and RNA quantification by NanoDrop™





Data courtesy Dr. Bernard Lam, et. al. Norgen Biotek Corp., Canada.

NanoDrop is a trademark of Thermo Fisher Scientific.

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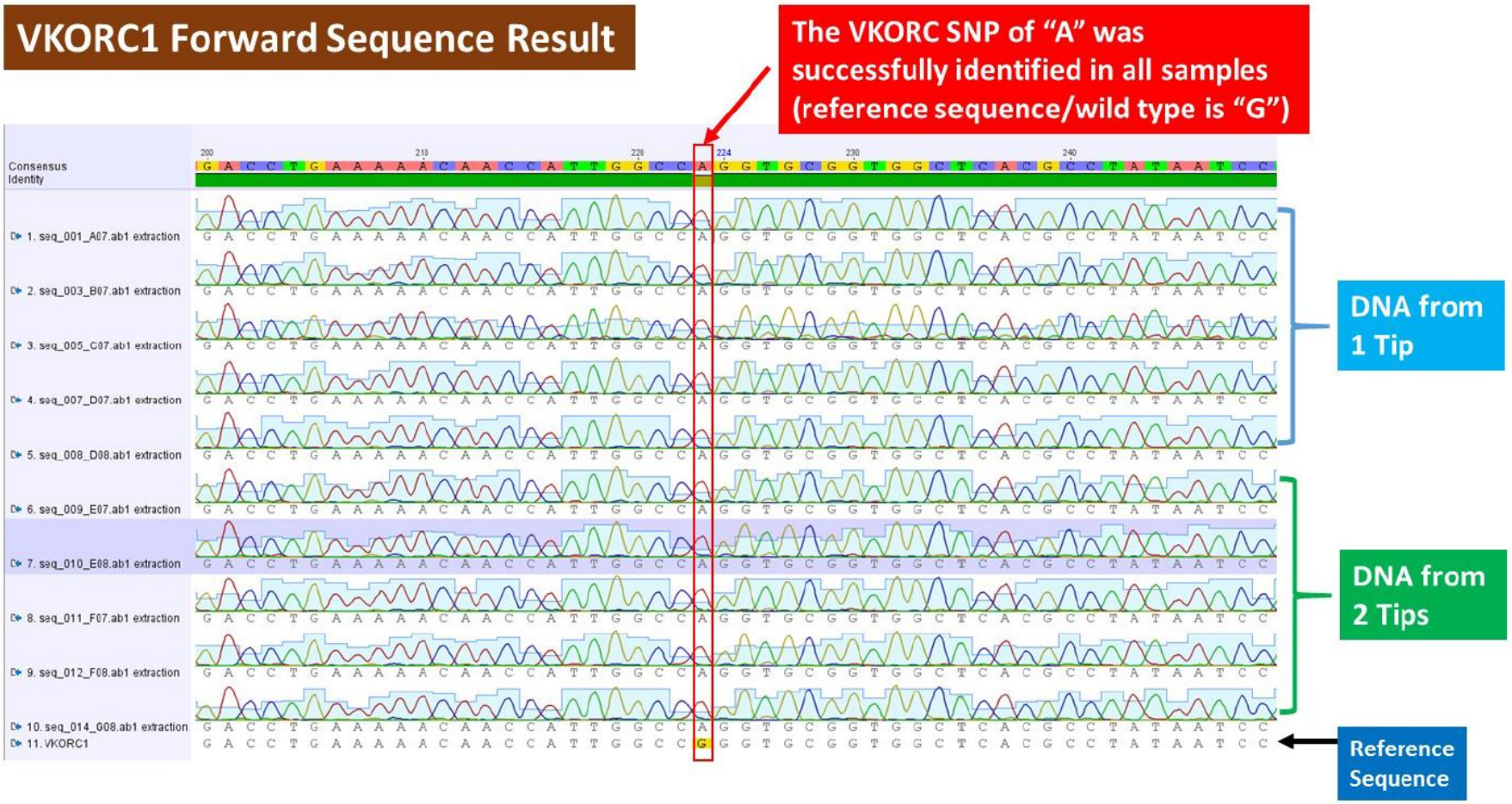
SNP identification



Data courtesy Dr. Bernard Lam, et. al. Norgen Biotek Corp., Canada.

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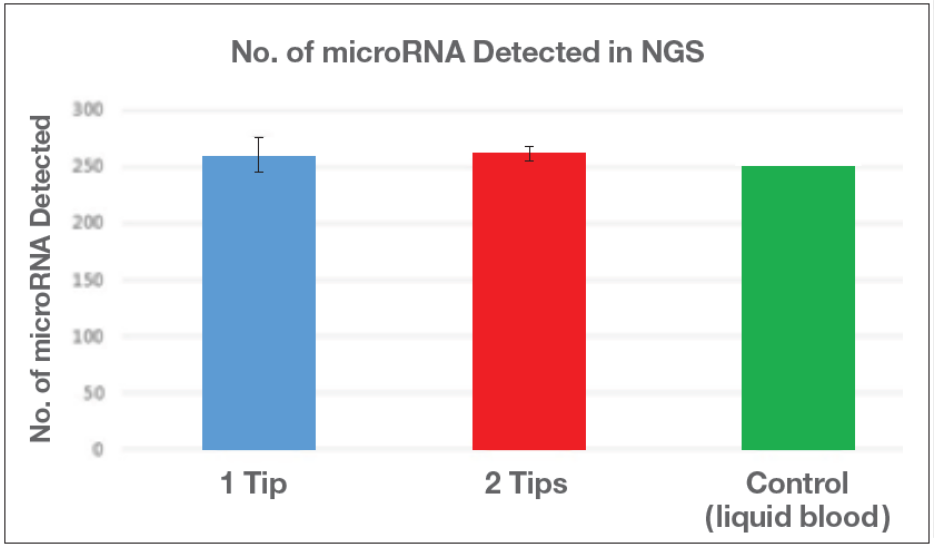
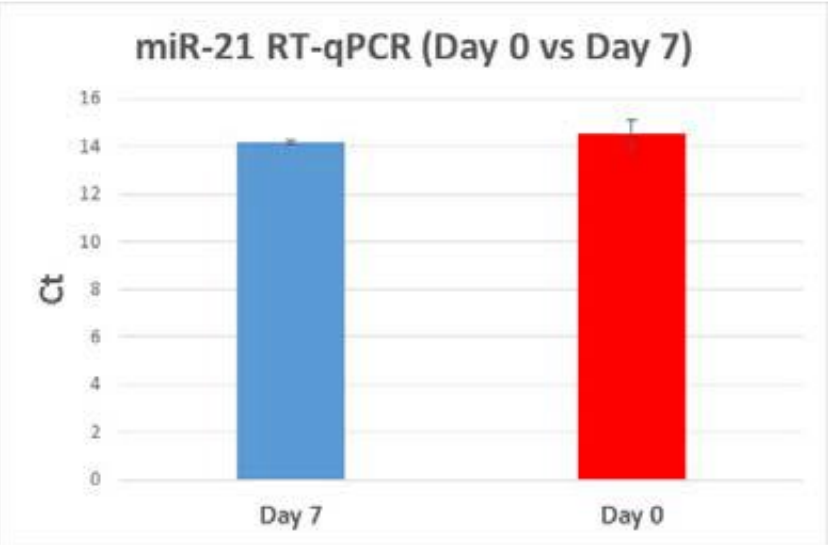
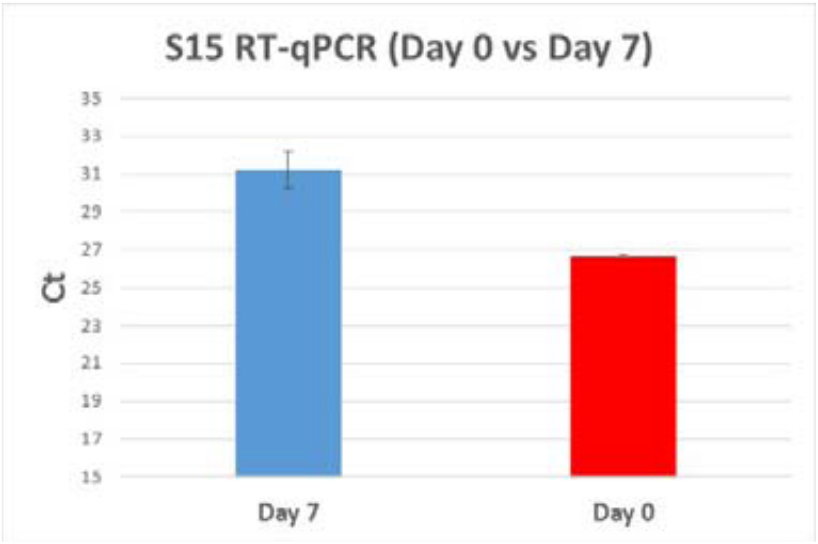
microRNA detection by NGS







Data courtesy Dr. Bernard Lam, et. al. Norgen Biotek Corp., Canada.

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molecular diagnostics summary

- Total RNA (large mRNA and microRNA) & DNA were preserved in the Mitra device

- Proportional amount of RNA or DNA was successfully purified from 1 or 2 Mitra Device

- Isolated RNA or DNA were high quality.
  - Nucleic acids purified were used successfully in downstream applications including qPCR and Sanger sequencing (DNA) & RT-qPCR and Next Gen Sequencing (Small RNA-Seq) (RNA)

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resources

VAMS™ publications

general

De Kesel, P.M.M., Lambert, W. E., Stove, C. P. (2015). **Does volumetric absorptive microsampling eliminate the hematocrit bias for caffeine and paraxanthine in dried blood samples? A comparative study.** *Anal. Chim. Acta., 881,* 65–75.

Mano, Y.; Kita, K.; Kusano, K. (2015). **Hematocrit-independent recovery is a key for bioanalysis using volumetric absorptive microsampling devices, Mitra.** *Bioanalysis, 7(15)* , 1821–1829.

Kushon, S., Bischofberger, A., Carpenter, A., Denniff, P., & Guo, Y. (2014). **A Novel Dried Matrix Microsampling Device that Eliminates the Volume Based Hematocrit Bias Associated with DBS Sub-Punch Workflows,** 62nd ASMS Conference on Mass Spectrometry and Allied Topics.

Houbart, V., Cobraiville, G., Servais, A.-C., Napp, A., Merville, M.-P., & Fillet, M. (2015). **Hepcidin determination in dried blood by microfluidic LC–MS/MS: comparison of DBS and volumetric absorptive microsampling for matrix effect and recovery.** *Bioanalysis,* 7(21), 2789–2799.

pharmaceutical

Spooner, N., Denniff, P., Michielsen, L., De Vries, R., Ji, Q. C., Arnold, M. E., … Rudge, J. B. (2015). **A device for dried blood microsampling in quantitative bioanalysis: overcoming the issues associated blood hematocrit.** *Bioanalysis*, 7(6), 653–659.

Denniff, P., Parry, S., Dopson, W., & Spooner, N. (2015). **Quantitative bioanalysis of paracetamol in rats using volumetric absorptive microsampling (VAMS).** *Journal of Pharmaceutical and Biomedical Analysis, 108,* 61–69.

Miao, Z., Farnham, J. G., Hanson, G., Podoll, T., Reid, M. J. (2015). **Bioanalysis of emixustat (ACU-4429) in whole blood collected with volumetric absorptive microsampling by LC – MS / MS.** *Bioanalysis,* 7(16), 2071–2083.

Wachtel, D., Tobin, J., Virasingh, B., Sheldon, A., Dearborn, S., Guo, Y., … Ribadeneira, M. (2016). **A 10-Point Time Course Pharmacokinetic Study Collected from a Single Mouse by use of the Mitra Microsampling Devices.** *64th ASMS Conference Poster.*

Parker, S., Roberts, J., Lipman, J., & Wallis, S. C. (2015). **Quantitative bioanalytical validation of fosfomycin in human whole blood with volumetric absorptive microsampling.** *Bioanalysis,* 7(19), 2585–2595.

Luo, Y., Korfmacher, W., Ho, S., Shen, L., Wang, J., Wu, Z., Guo, Y., Snow, G., O'Shea, T. (2015). **Evaluation of two blood microsampling approaches for drug discovery PK studies in rats.** *Bioanalysis, 7(18), 2345-2359.*

continued on other side

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VAMS™ publications

clinical research & diagnostics

Mercolini, L., Protti, M., Catapano, M. C., Rudge, J., & Sberna, A. E. (2016). **LC–MS/MS and volumetric absorptive microsampling for quantitative bioanalysis of cathinone analogues in dried urine, plasma and oral fluid samples.** *Journal of Pharmaceutical and Biomedical Analysis, 123*(10 May), 186–194.

Kipper, K., Barker, C., Lonsdale, D., Sharland, M., & Johnston, A. (2015). **Evaluation of the Mitra microsampling device for dry sample processing in a pharmacokinetic/pharmacodynamic study of beta-lactams.** *42nd Symposium of HPLC.*

Merconlini, L., Protti, M., Albers, L. J., & Reist, C. (2016). **Therapeutic Drug Monitoring (TDM) By Means Of Novel Sampling AND Extraction Procedures: A Comparative Study.** *European Bioanalysis Forum (EBF) Symposium.*

Protti, M., Capatano, M., Somaini, L., Gerra, G., & Mercolini, L. (2016). **Enhanced Stability of Dried Haematic Samples for New Psychotropic Synthetic Compound Monitoring.** *European Bioanalysis Forum (EBF) Symposium.*

Neupane, B., Mulla, H., Spooner, N., Abu-Rabie, P., Rudge, J., & Pandya, H. (2016). **Midazolam Measurement and Modelling using Matrix Samplers (The 4M's Study).** *American Pediatrics Association (APA) Conference.*

Stephenson, S., Dutton, J., Davison, A., Kushon, K., Rudge, J. (2016). **Evaluation of the Mitra Microsampling Device for Immunosuppressants Monitoring.** *Neoteryx Application Note.*

Gordon, C., Sadilkova, K., Jack, R. M., & Dickerson, J. A. (2016). **Improved Sensitivity for Immunosuppressant Monitoring in Two Dried Matrices: A Proof of Concept.** *MSACL* Conference - *Mass Spectrometry: Applications to the Clinical Lab.*

Mbughuni, M. (2016). **Immunosuppressant (Tacrolimus/Cyclosporin A) Monitoring by LC-MS/MS Using Mitra Microsampling Devices.** *MSACL Conference - Mass Spectrometry: Applications to the Clinical Lab.*

molecular diagnostics & proteomics

van den Broek, I., Fu, Q., Millis, K., Eckersley, T., Wood, W. W., Kowalski, M. P., … Eyk, J. E. Van. (2016). **Can volumetric absorptive micro sampling in the presence of a stable-isotope labeled protein standard control pre-analytical variability in proteomics?** *64th ASMS Conference on* Mass Spectrometry and Allied Topics.

El-Mogy, M., Misic, V., McGowan, S., Lam, B., & Haj-Ahmad, Y. (2016). **Total RNA/DNA Purification and Detection from Blood Preserved on a Mitra Microsampling Device.** *Neoteryx Application Note.*

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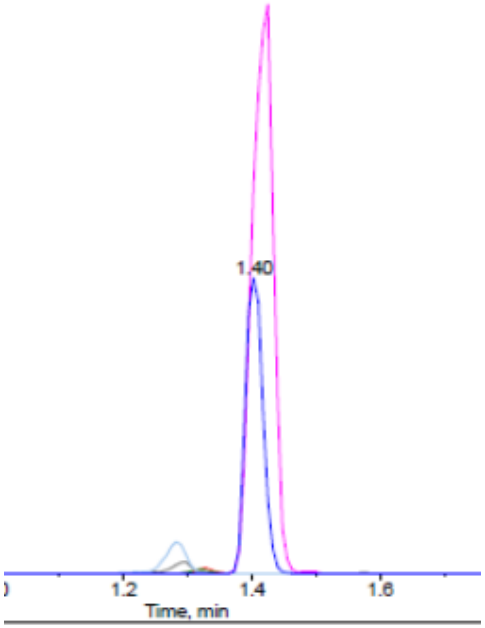
from barcode to data



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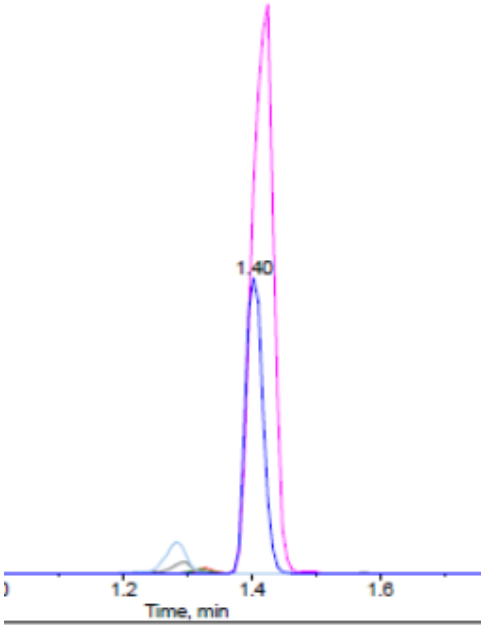
from barcode to data



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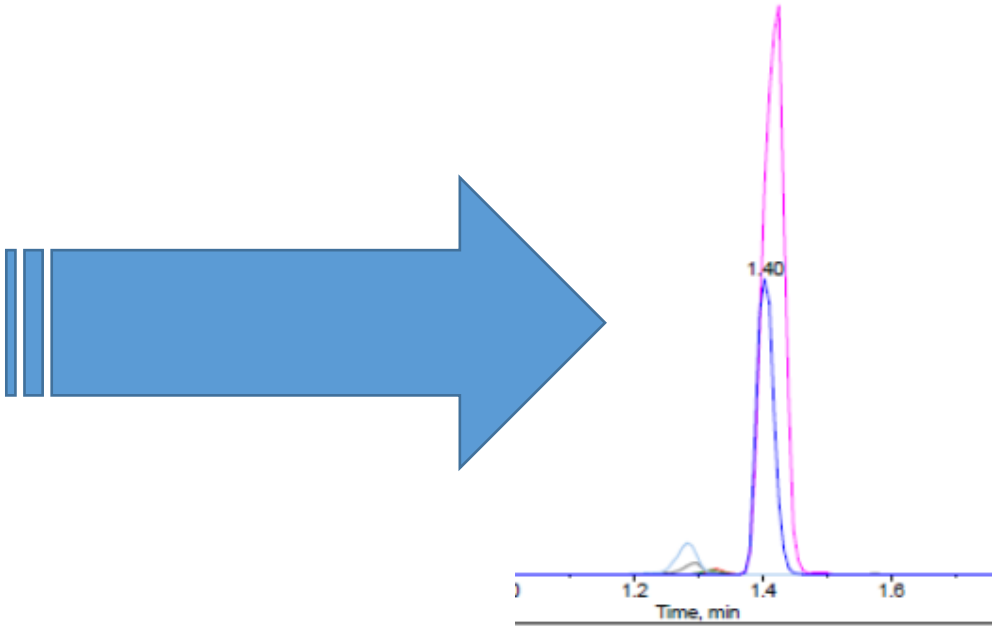
from barcode to data

Hamilton STARlet™



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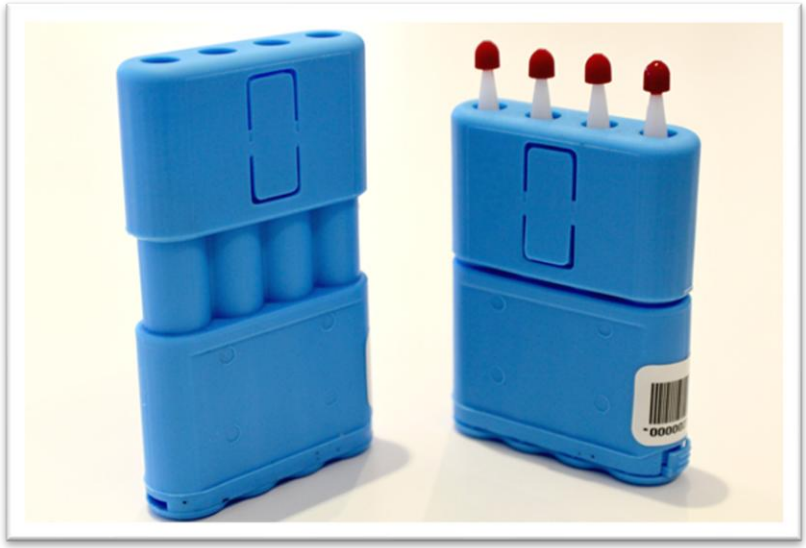
- STARlet is a trademark of Hamilton Company

Patent Pending

mitra autocartridge for walk-away automation

- Designed for use with Hamilton STARlet™ systems
- Same easy sampling and shipment as clamshell and cartridge
- Walk-away automation for reformatting and extraction



- Anticipated availability of 4-sampler Autocartridge in early 2017
- Other configurations (2 and 3-sampler) in development

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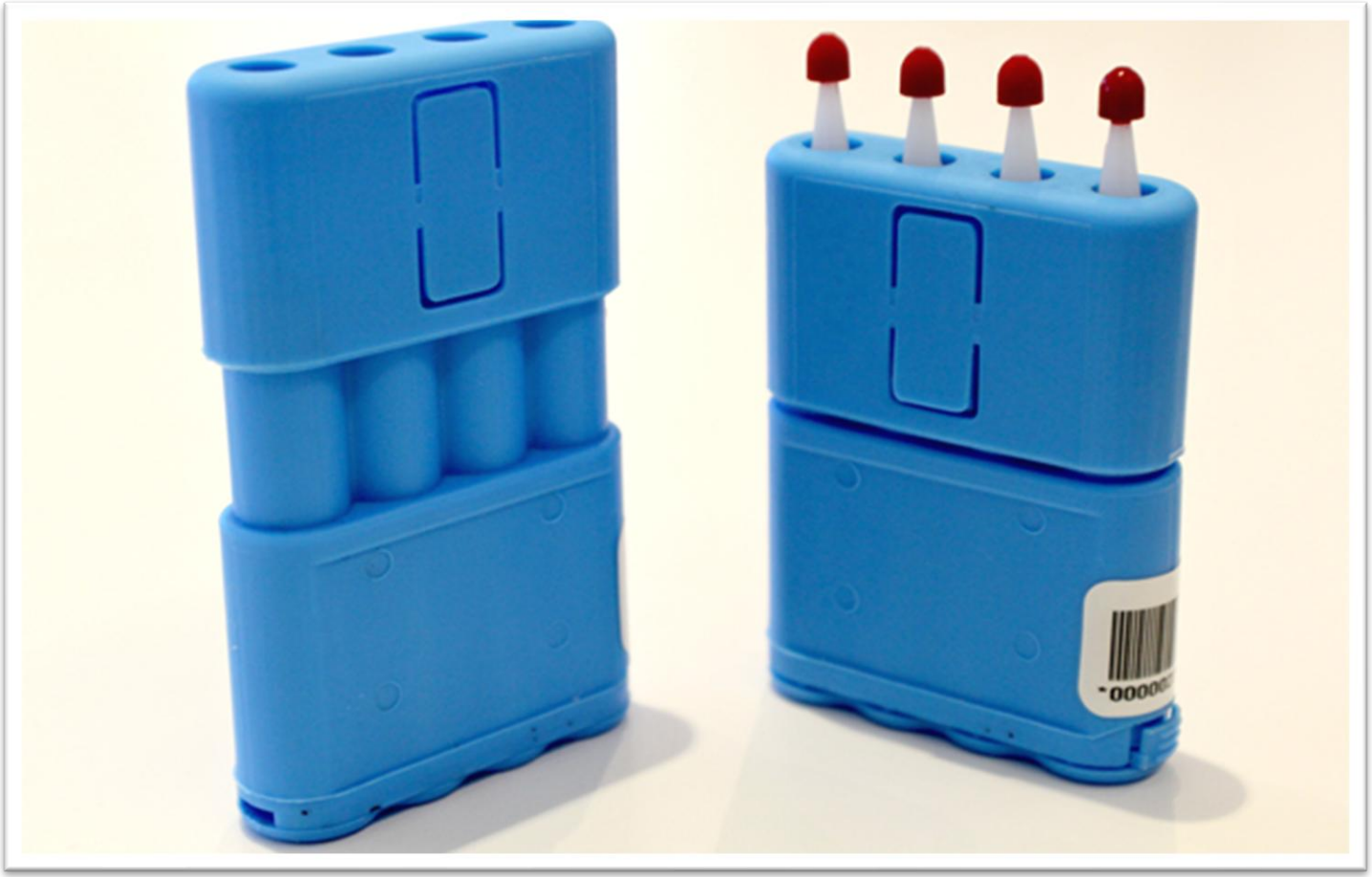
Patent Pending

autocartridge: hamilton solution for reformatting and extraction



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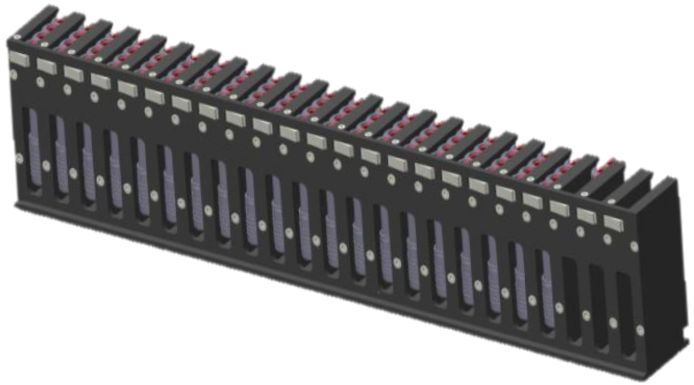
Patent Pending

autocartridge: hamilton solution for reformatting and extraction

Easy loading

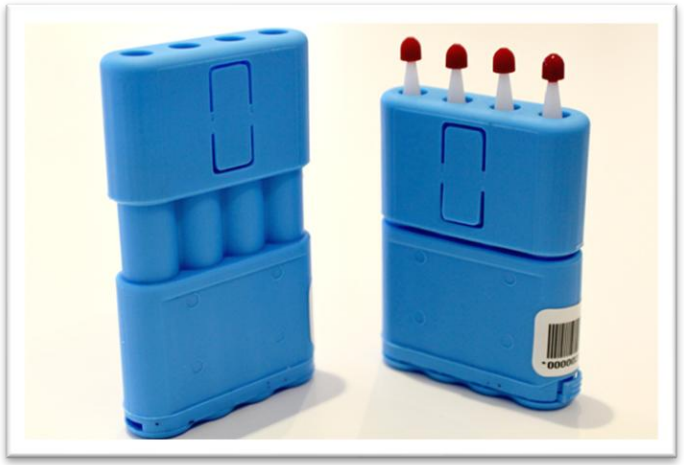
Automated Reformatting and Extraction



- STARlet is a trademark of Hamilton Company

summary

- we have come a long way in two years



- mitra is being used in a huge number of ways
  - new products
  - applications
  - understanding

- This is just the tip of the iceberg!

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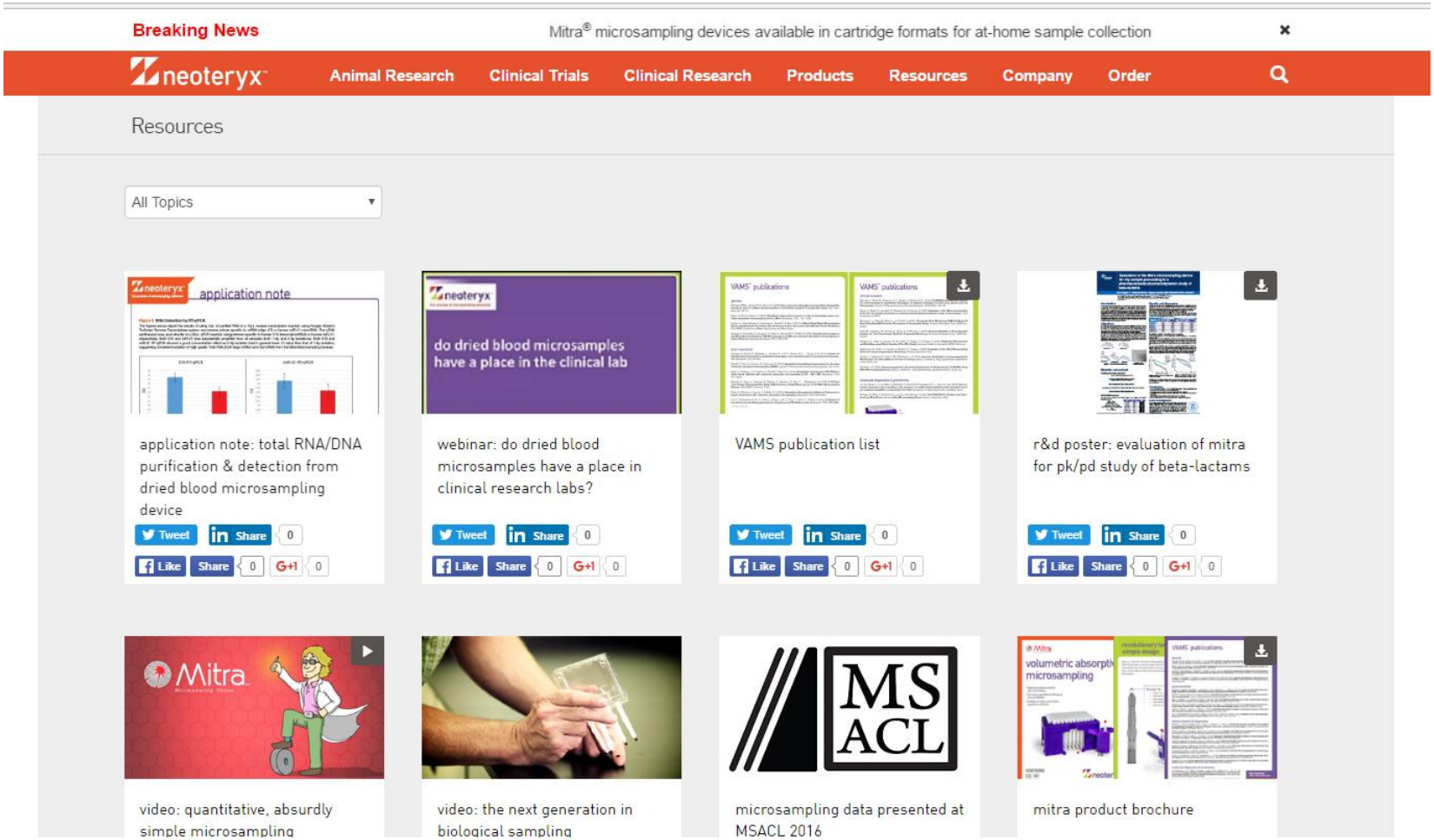
resources



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need advice on mitra method optimization and validation?
please contact the neoteryx team on info@Neoteryx.com