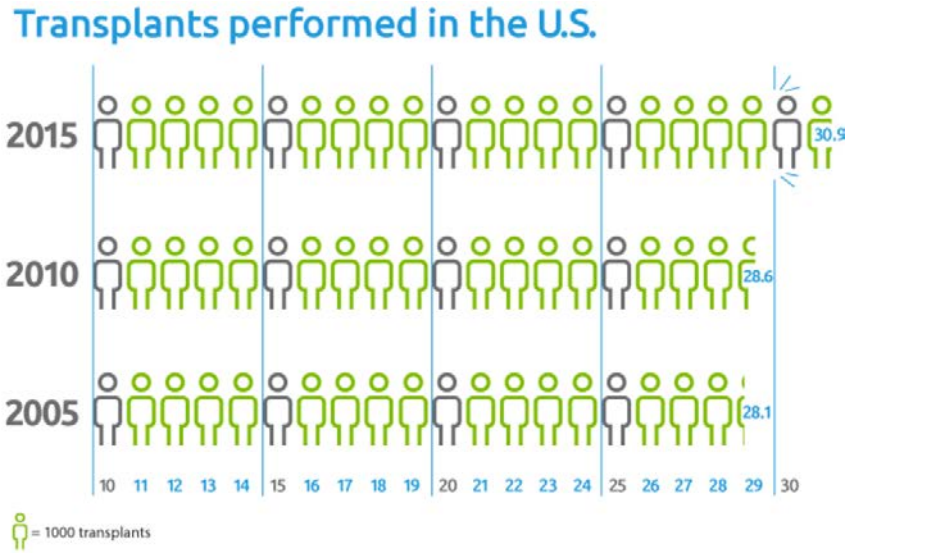
Development of a Potential At-home Assay for Tacrolimus Monitoring Using a Microsampling Device

Leeds Pathology

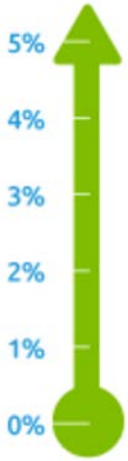
neoteryx™
The promise of microsampling, delivered

transplants performed



30,973 organ transplants in 2015

5% increase from 2014 to 2015*

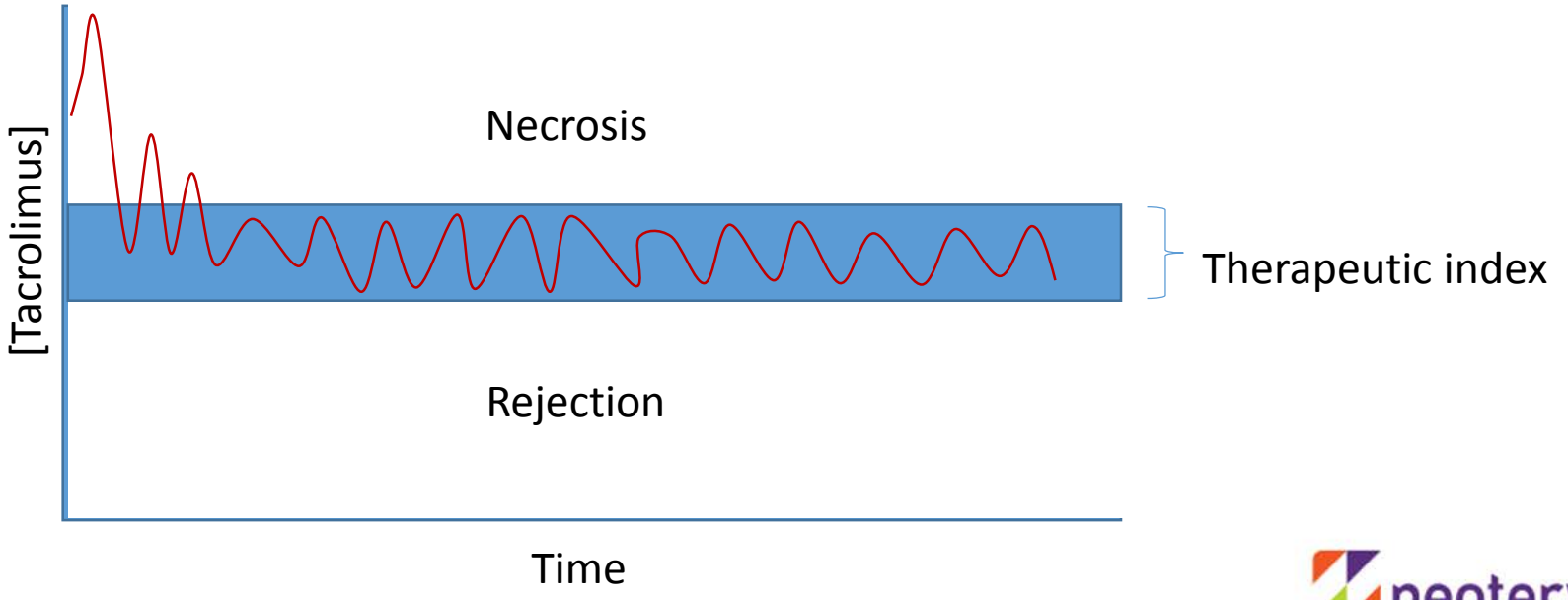


neoteryx™
The promise of microsampling, delivered

https://optn.transplant.hrsa.gov/

immunosuppressants background

- Prevent rejection of organs after transplantation
- Concentration is persistently monitored to ensure they are in therapeutic window
- Low dosing = organ rejection / High dosing = toxic side effects



neoteryx
The promise of microsampling, delivered

Methods

Current Wet Method (Zinc Sulfate Precipitation)

1. Defrost aliquots of calibrators and QC material/remove and allow them to reach room temperature.
2. Vortex mix and centrifuge samples for 5 minutes at 10,000 rpm.
3. Label sufficient 1.5mL microcentrifuge tubes
4. Place 25µL of calibration standards/QC/ patient sample into appropriate tube
5. Add 100µL 0.1M zinc sulphate solution followed by 250µL 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.

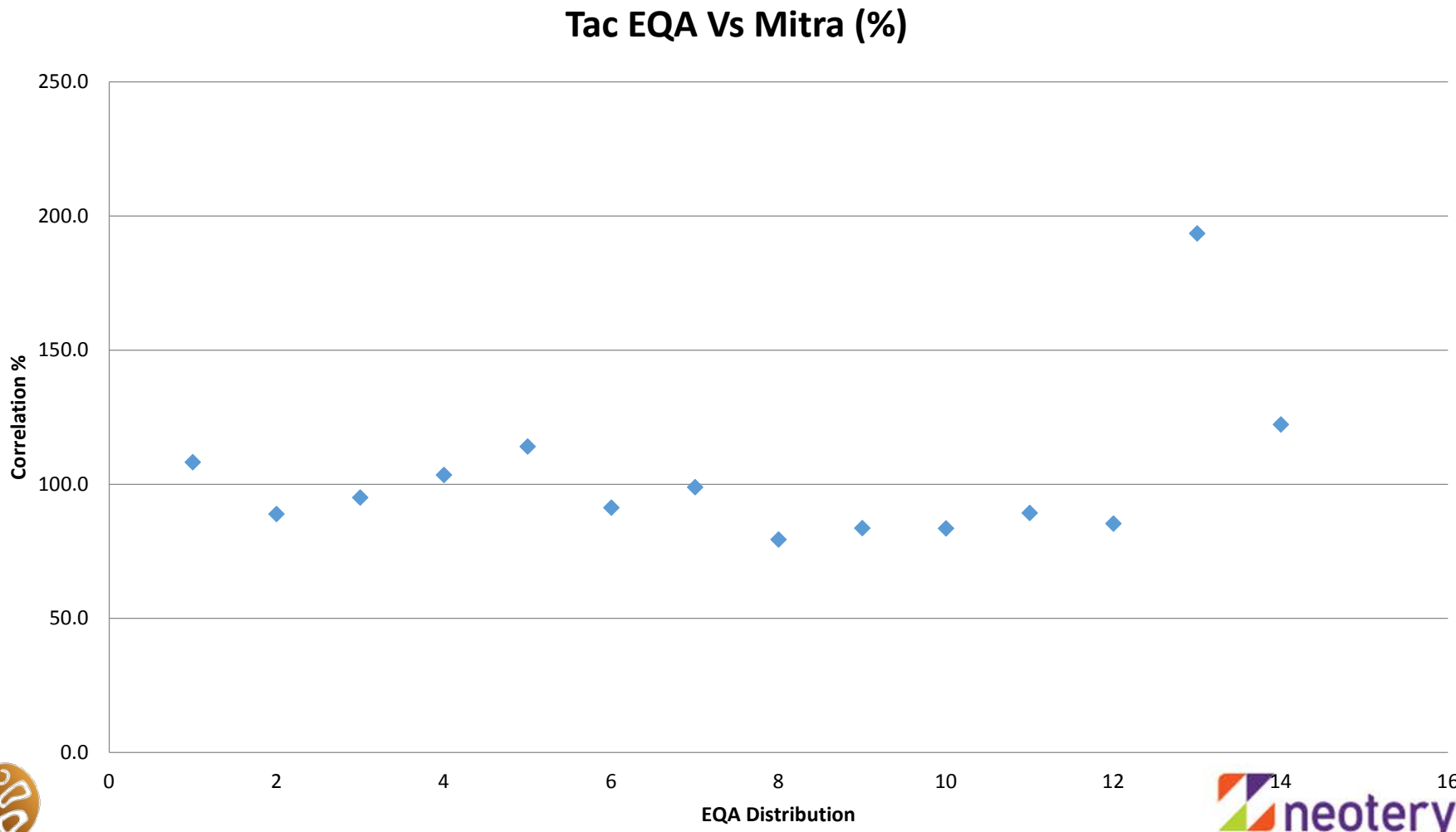
Mitra Method (MeOH Extraction)

1. Mitra tips were sampled using blood containing EDTA from microcentrifuge tubes following the instructions for use.
2. Samples were allowed to completely dry
3. Tips were removed and placed into a 96-well collection plate containing 200 µL of Methanol (containing IS)
4. Collection plate was shaken on an orbital shaker for 1 hour (1100 RPM)
5. The solvent was evaporated and reconstituted in initial mobile phase for injection.

Leeds Pathology

neoteryx™
The promise of microsampling, delivered

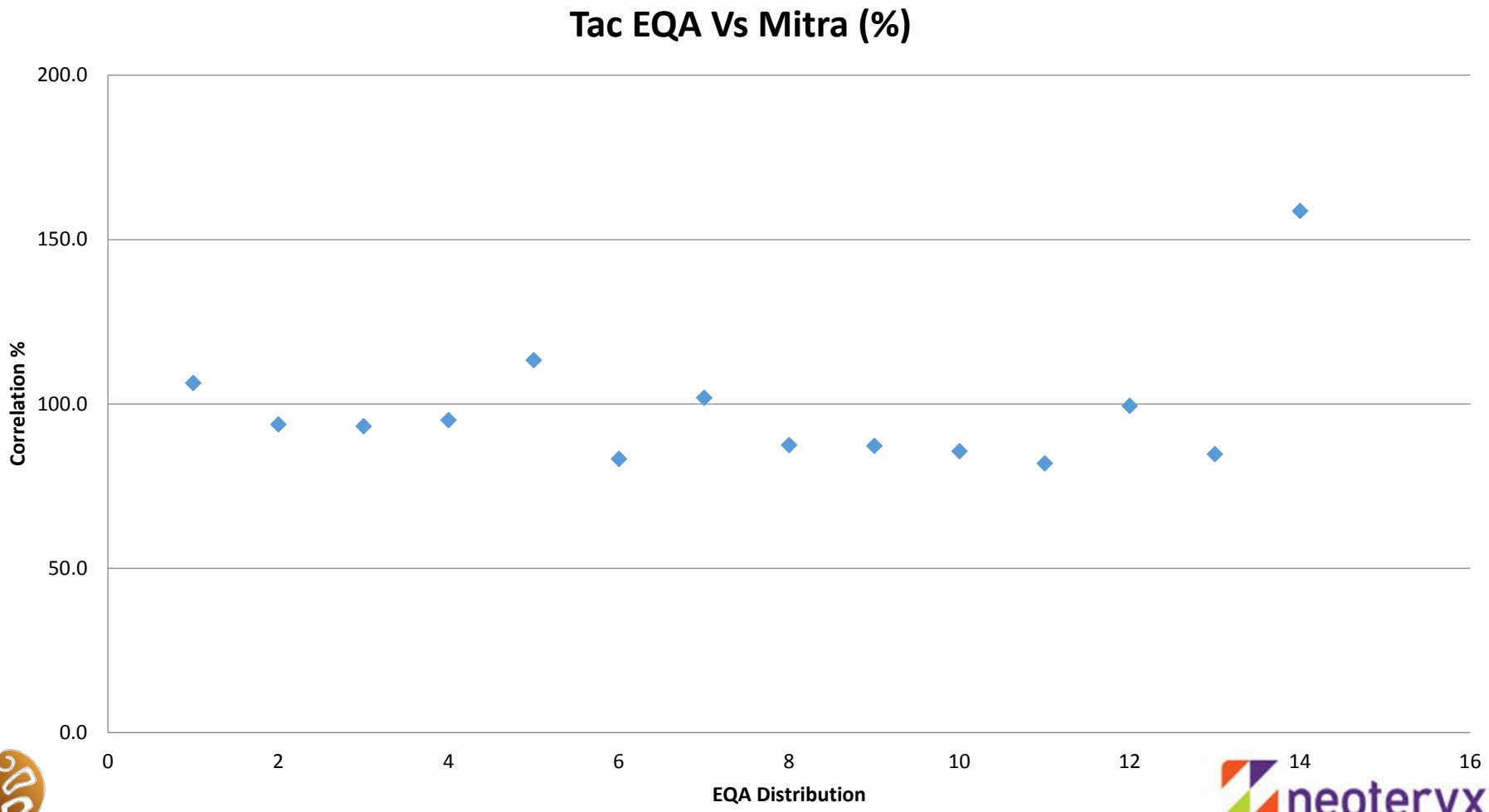
Tacrolimus EQA



Leeds Pathology

neoteryx™
The promise of microsampling, delivered

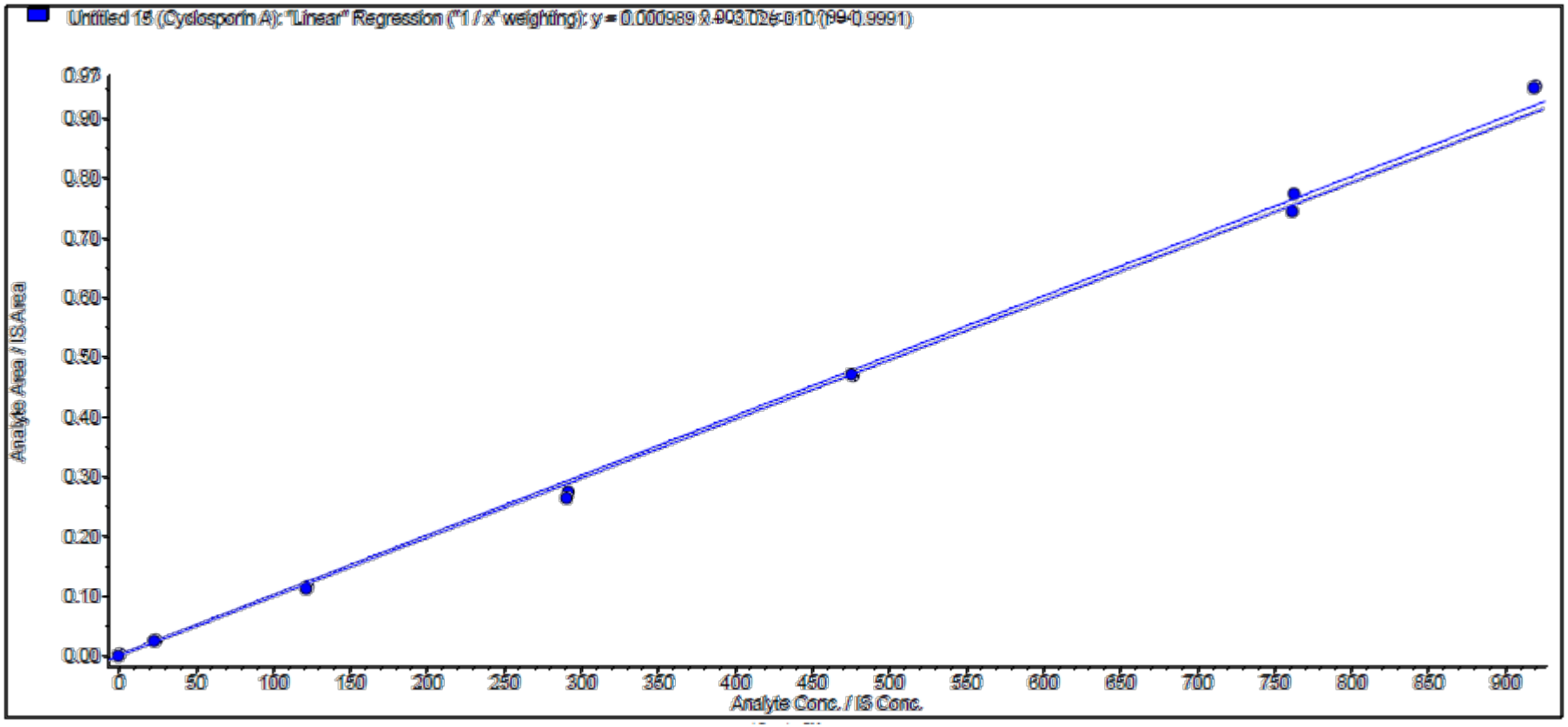
Cyclosporin EQA



Leeds Pathology

neoteryx
The promise of microsampling, delivered

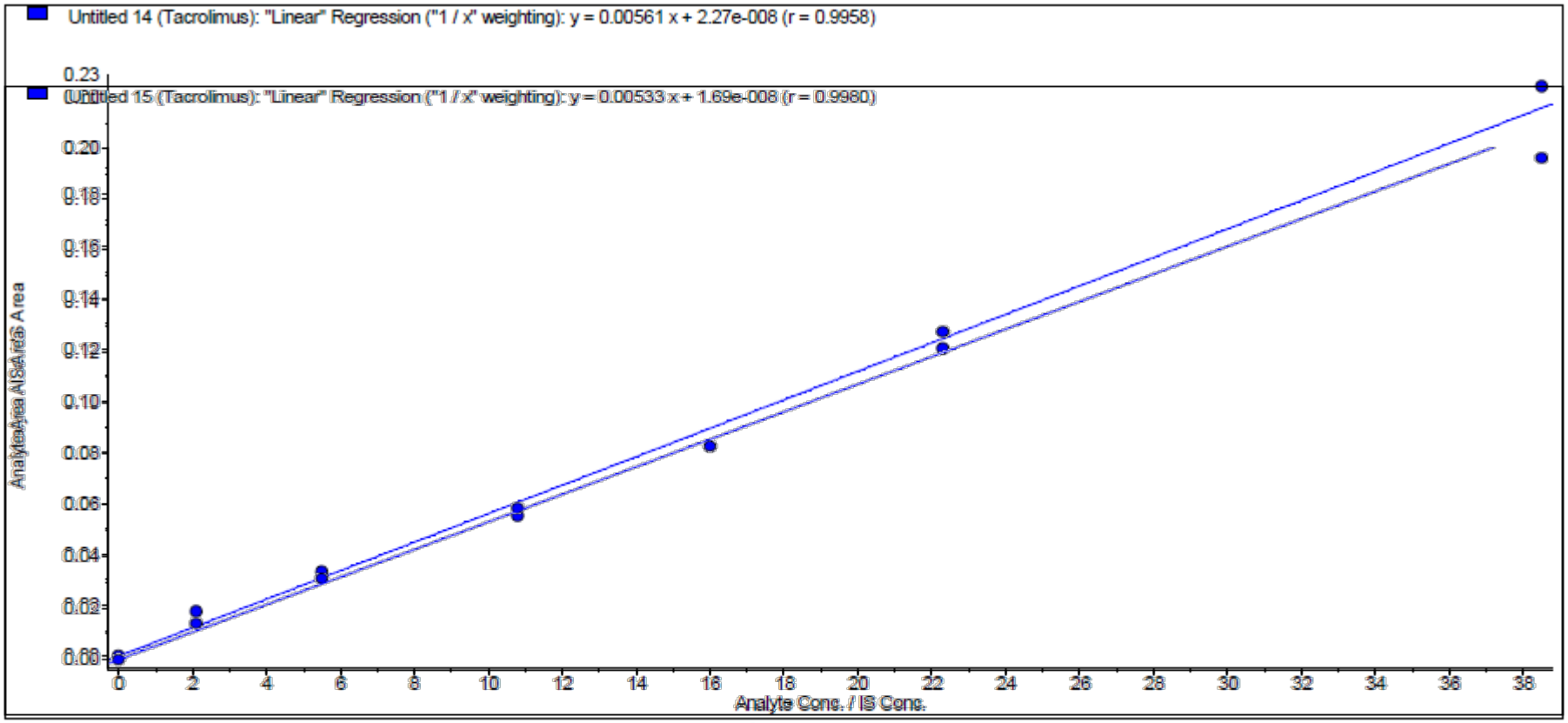
CSA Stability Over 7 Days



Leeds Pathology

neoteryx™
The promise of microsampling, delivered

Tac Stability Over 7 Days

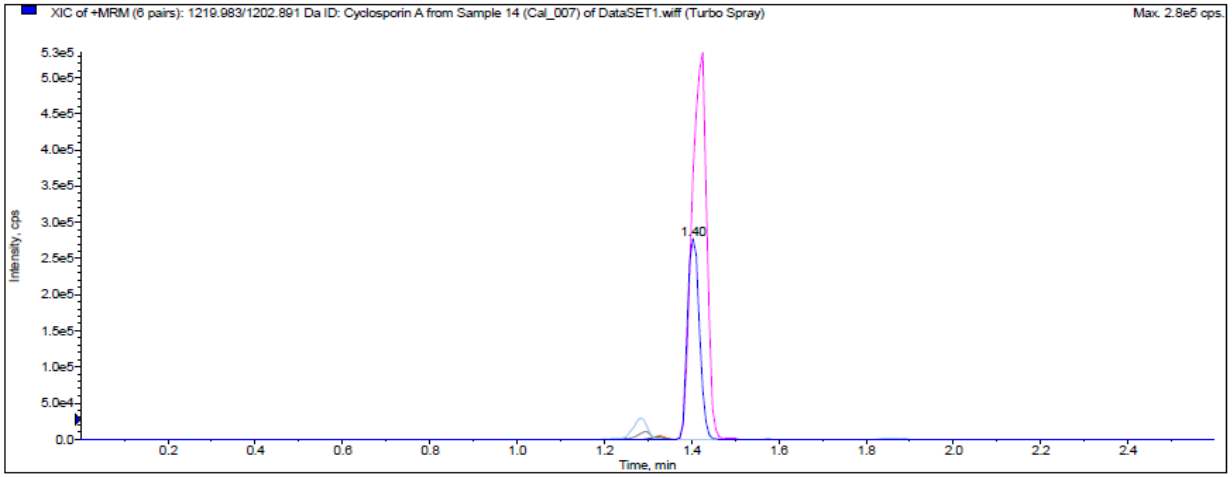


Leeds Pathology

neoteryx™
The promise of microsampling, delivered

Multiple Site Study

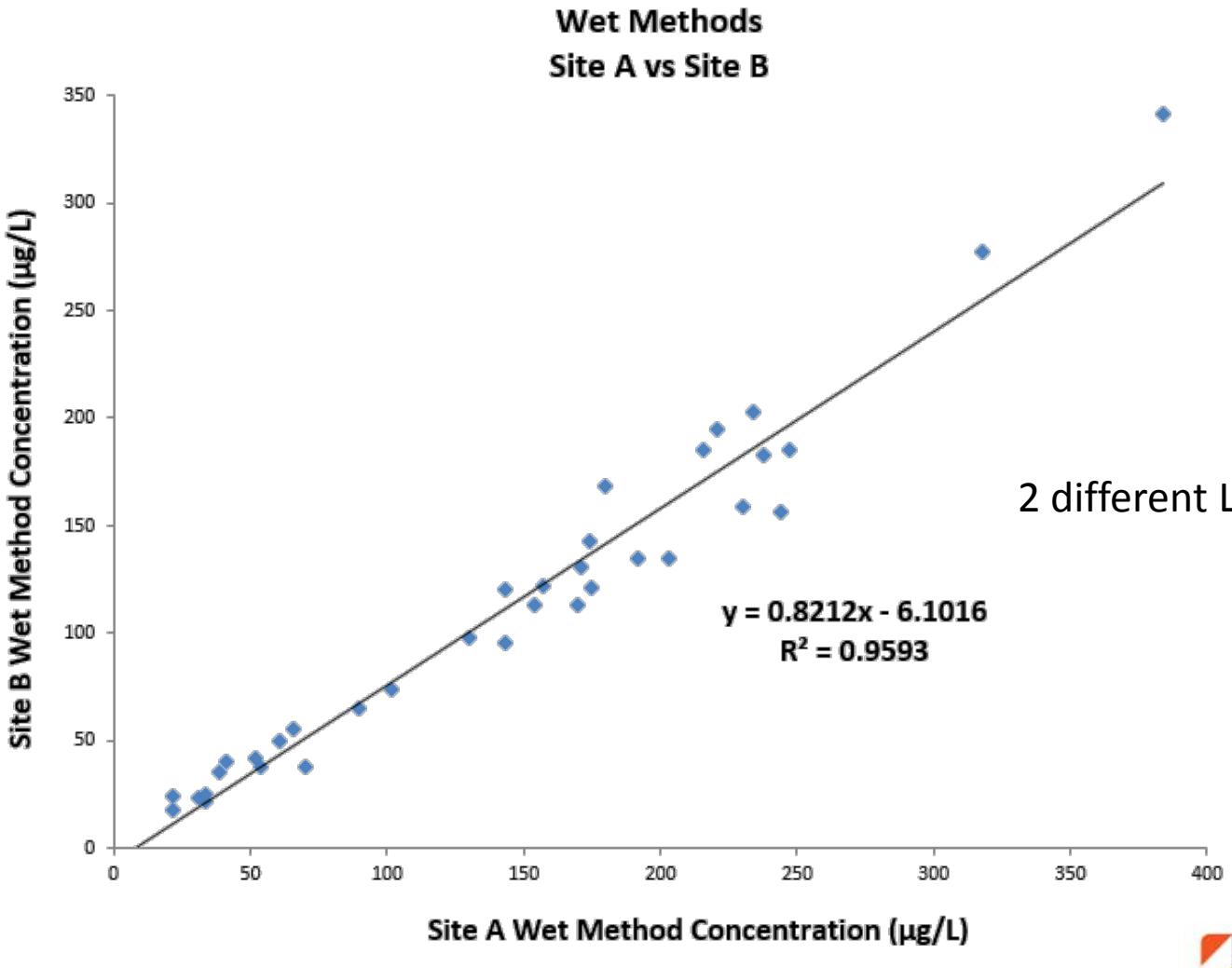
- Two Sites (A and B); Hospitals in the UK.
- 36 Samples provided by Site A
- Wet Extraction vs. Mitra
- API-4000
- Low QC = 25 ng/mL



Leeds Pathology

neoteryx™
The promise of microsampling, delivered

Correlation (Wet Methods)



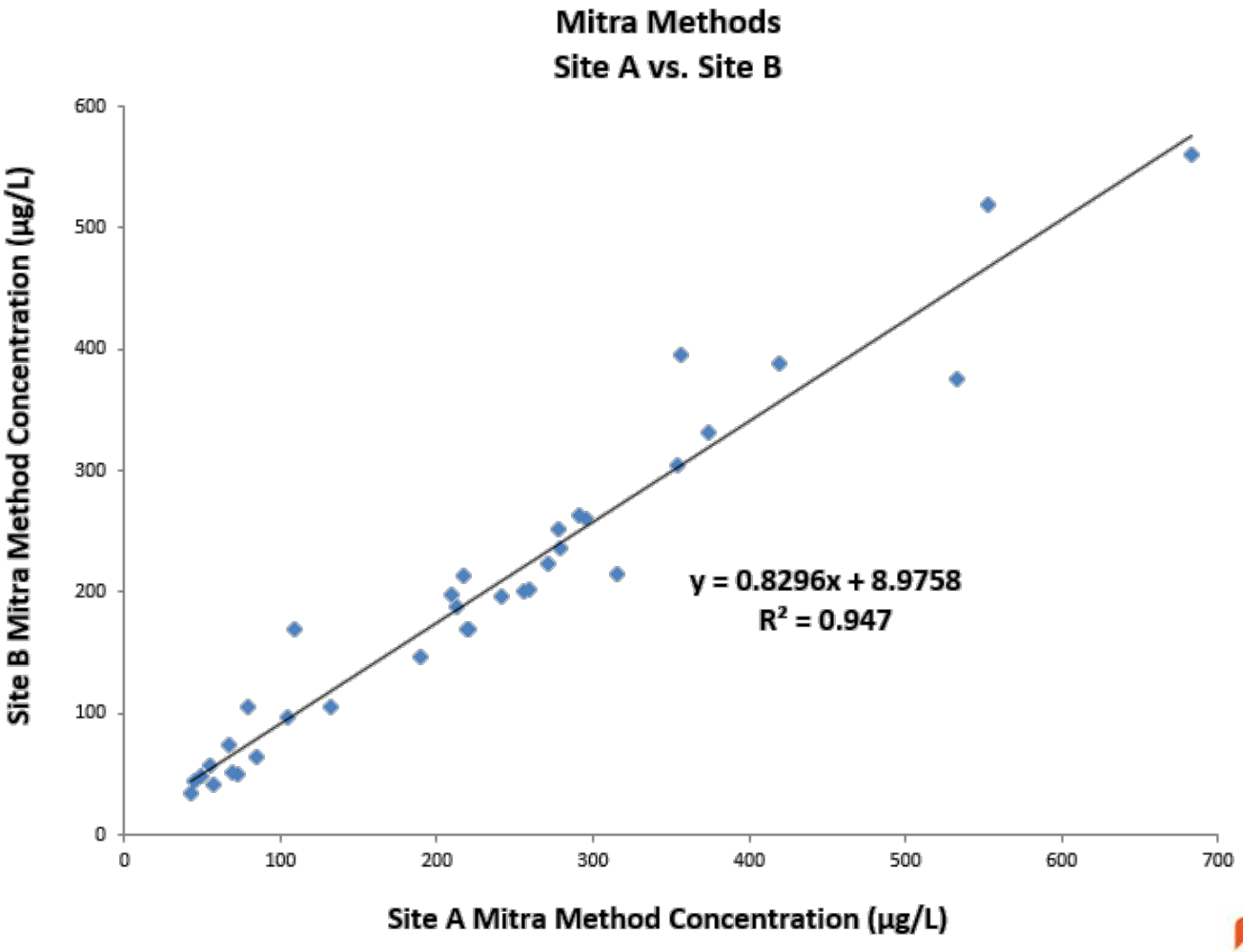
2 different LC MS method

- Strong Correlation; Negative Bias (18%; B vs. A)

Leeds Pathology

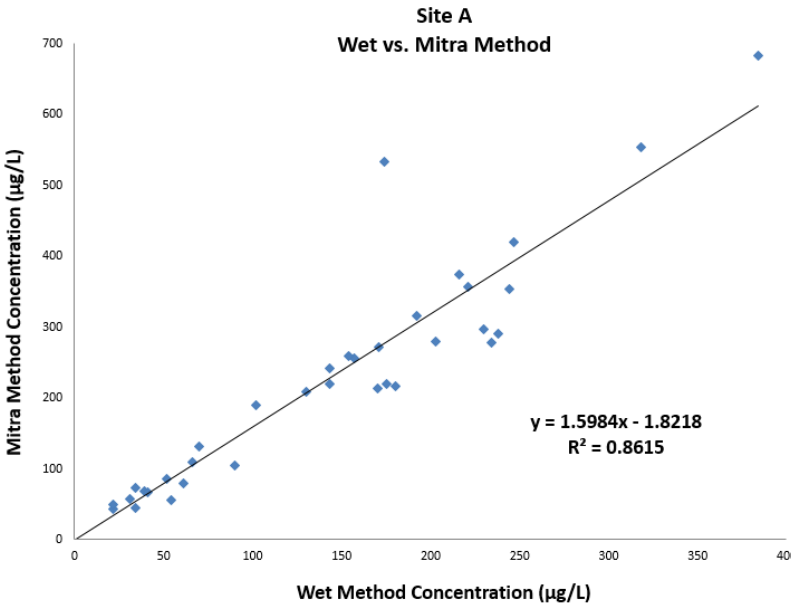
neoteryx™
The promise of microsampling, delivered

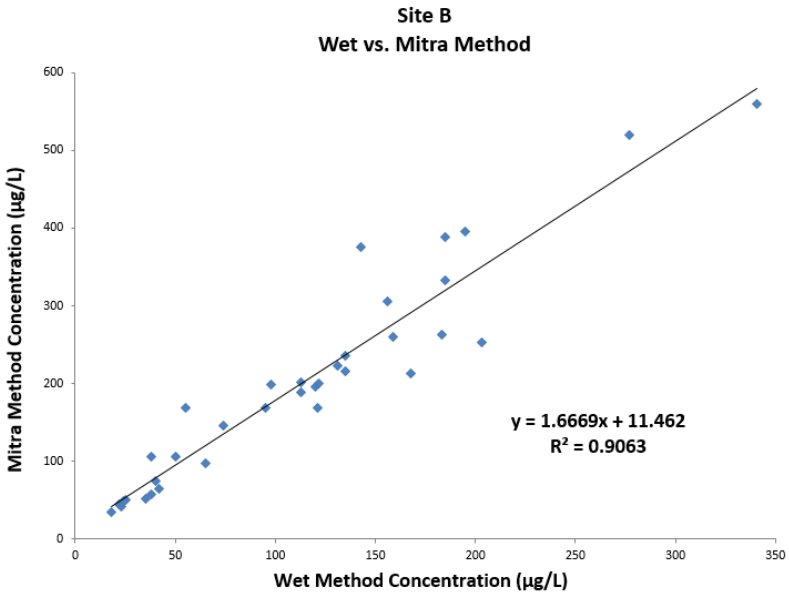
Correlation (Mitra Methods)



- Strong Correlation; Negative Bias (18%; B vs. A)

Correlation (Wet vs Mitra)





- Strong Correlation at both sites (>.86)
- Positive Bias (60%; Mitra vs. Wet)
- Extraction May have been more efficient

Leeds Pathology

neoteryx™
The promise of microsampling, delivered

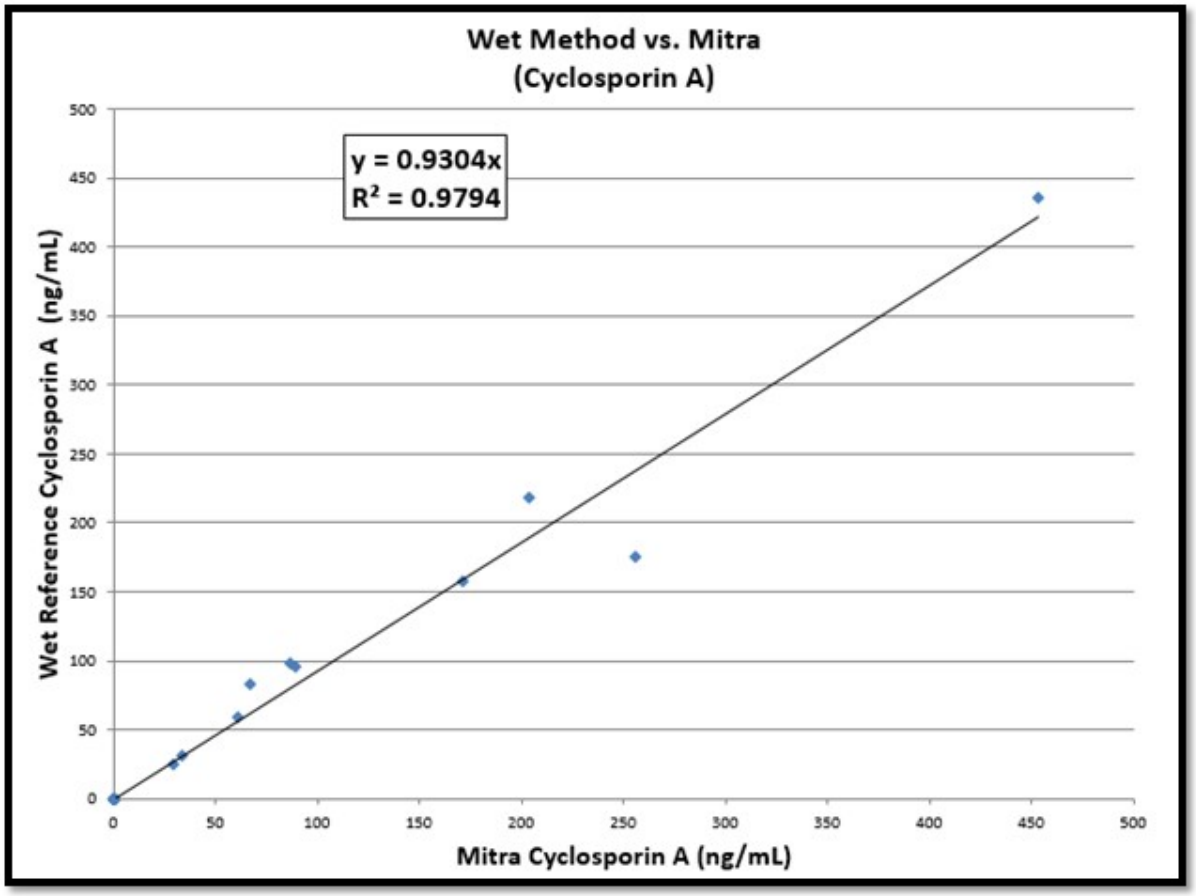
Alternate Mitra Extraction Protein Precipitation (Site B)

1. Add dried tips to 100 uL of water/5% MeOH (including IS) in square well collection plate
2. Plate shake for 20 min
3. Remove the tips which should be light pink
4. Add 100uL ZnSO4 (2.88g in 100mL)
5. Plate shake for 5 min
6. Add 100 uL of ACN
7. Plate shake for another 5 min
8. Spin (we transferred to Eppendorf tubes for this)
9. Transfer to autosampler vial and inject

Leeds Pathology

neoteryx™
The promise of microsampling, delivered

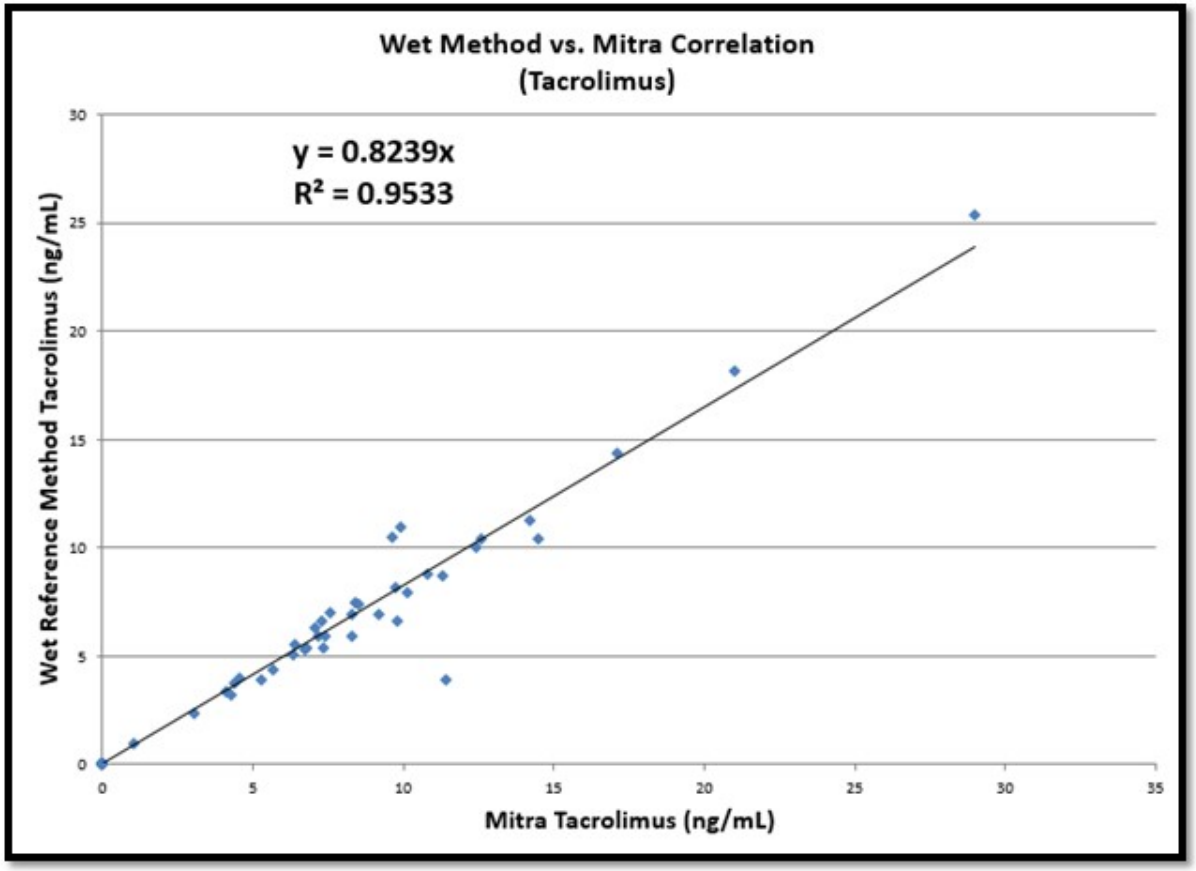
Protein Crash Method CSA



Leeds Pathology

neoteryx™
The promise of microsampling, delivered

Protein Crash Method Tac



Leeds Pathology

neoteryx™
The promise of microsampling, delivered

Conclusions and further investigations

- Strong Correlations can be built between standard methods and Mitra methods using simple extraction methods.
- Has good potential for home sampling option, in particular Paediatric patients.
- Need to explore venous draw vs finger-prick on as small patient cohort.
  - The current validation carried out has been on blood samples with anticoagulant and not intended use of product

Leeds Pathology

neoteryx™
The promise of microsampling, delivered

Leeds Pathology

Thank you

All expenses occurred in the workup and development of the sampling device were jointly funded by LTH and Neoteryx

neoteryx™
The promise of microsampling, delivered

Patient stability data Tac

Tac 2			wet method	day 1 (5uL injection)	day 7 (10 uL injection)
9044382	3	15/04/2016	3.17	2.14	3.22
9044361	6	15/04/2016	6.00	3.45	6.72
9044335	8	15/04/2016	7.71	4.09	6.66
3479879	12	15/04/2016	12.40	7.54	10.5
3479727	15	15/04/2016	14.60	6.55	14.3
9044390	20	15/04/2016	20.10	12.6	19
NEQAS					
6530991				3.85	4.63
6530992				12.7	11.4
6530993				7.01	6.18

Leeds Pathology

neoteryx™
The promise of microsampling, delivered