



# Immunosuppressant Monitoring by LC-MS/MS Using Mitra™ Microsampling Devices

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Mass Spectrometry: Applications to the Clinical Lab 2016  
February 23, 2016

# Disclosure

- Relevant Financial Relationships:
  - Neoteryx provided:
    - Microsampling devices
    - One night hotel/dinner for MSACL meeting

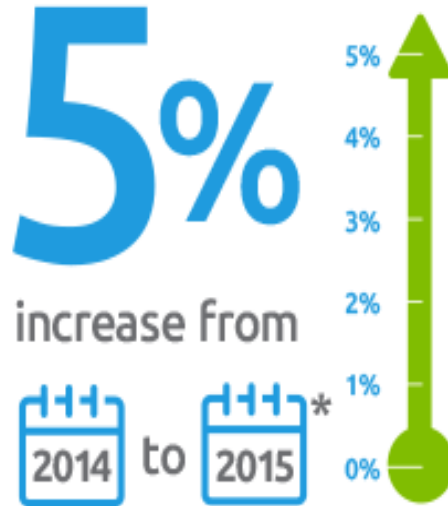
# Objectives

- Determine the feasibility of measuring Tacrolimus and Cyclosporine A by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) using a dried whole blood sample (20  $\mu$ L) on a microsampling device compared to 200  $\mu$ L EDTA whole blood.
- Demonstrate the importance of therapeutic drug monitoring of immunosuppressant's in transplant patients and how microsampling devices might benefit patient care and satisfaction.

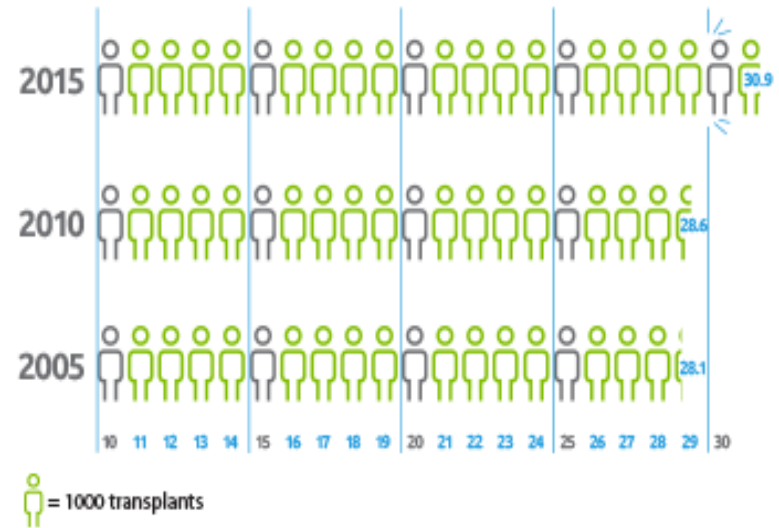
# Organ Transplants in the United States



**30,973** organ transplants in 2015

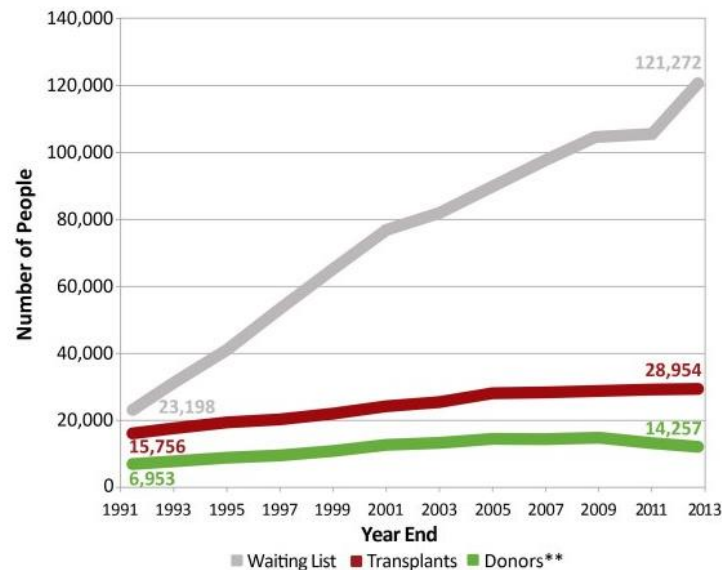


## Transplants performed in the U.S.



# Organ Donor Statistics in the United States

- ~85 people receive an organ transplant each day
- ~22 people die each day waiting for an organ transplant
- 121,439 people on the waiting list (78,031 active) as of 2-22-16



# Organ Donor Statistics in the United States

- ~85 people receive an organ transplant each day
- ~22 people die each day waiting for an organ
- 121,439 people on the waiting list (78,031 active)
- % of recipients still living 5-years post-transplant (Dec 4, 2012):
  - Kidney (deceased donor): 83.4%
  - Kidney (living donor): 92%
  - Heart: 76.8%
  - Liver (deceased donor): 74.3%
  - Liver (living donor): 81.3%
  - Lung: 55.2%

# Immunosuppressive Agents

- Definition:
  - Anti-rejection medications to inhibit or prevent activity of the immune system.
- Use:
  - Reduce and prevent the rejection of transplanted organs and tissues.
- Small Molecule Preparations:
  - Calcinerin inhibitors
    - Cyclosporine A (Neoral ® and Sandimmune ®)
    - Tacrolimus (Prograf ®)
  - Antiproliferative agents
    - Azothioprine
    - Mycophenolate mofetil (CellCept ® and Myfortic ®).
  - mTOR inhibitors
    - Sirolimus (Rapamune ®)
  - Corticosteroids

# Calcineurin Inhibitors

- How do they work?
  - Inhibit T Cell activation and interleukin-2.
    - Interfere with the IL-2 Gene transcription essential for activation and proliferation of cytotoxic t-cells in response to alloantigens.
  - Tacrolimus is good at preventing rejection and has better long term graft survival outcomes.



# Tacrolimus (Prograf®)

- Therapeutic Drug Monitoring (TDM) is important
  - Narrow Therapeutic Window:
    - Concentration too low:
      - Increased risk of rejection
    - Concentration too high:
      - Hypertension
      - Photosensitivity
      - Hyperglycemia
      - Insomnia
      - Hyperkalemia
      - Nephrotoxicity
      - Neurotoxicity

# Example Trough Tacrolimus TDM Goals

- Kidney Transplant:
  - 1<sup>st</sup> month: Trough level at 8-10 ng/mL
  - >1<sup>st</sup> month: Trough level at 6-8 ng/mL
- Pancreas Transplant:
  - 1<sup>st</sup> month: Trough level at 10-12 ng/mL
  - 1-4 months: Trough level at 8-10 ng/mL
  - >4 months: Trough level at 6-8 ng/mL
- Liver Transplant:
  - First 60 days: Trough level at 6-12 ng/mL
  - 60-120 days: Trough level at 4-10 ng/mL
  - >120 days: Trough level at 4-8 ng/mL
- **Goal Levels are always individualized to each patient's situation.**

# Experimental Design

- Compare 200  $\mu\text{L}$  EDTA whole blood to 20  $\mu\text{L}$  dried blood collected on the Mitra™ microsampling device



VS.



- Measure Tacrolimus and Cyclosporine A by LC-MS/MS
  - 20  $\mu\text{L}$  tip + 150  $\mu\text{L}$  CLWR w/ IS; Vortex 20 min @ 2500 rpm
  - 150  $\mu\text{L}$  0.1 M  $\text{ZnSO}_4$ ; Vortex 5 min @ 2500 rpm
  - 150  $\mu\text{L}$  MeOH; Vortex 5 min @ 2500 rpm;
  - Centrifuge; Transfer supernatant to new plate for analysis

# Microsampling Precision: Tacrolimus

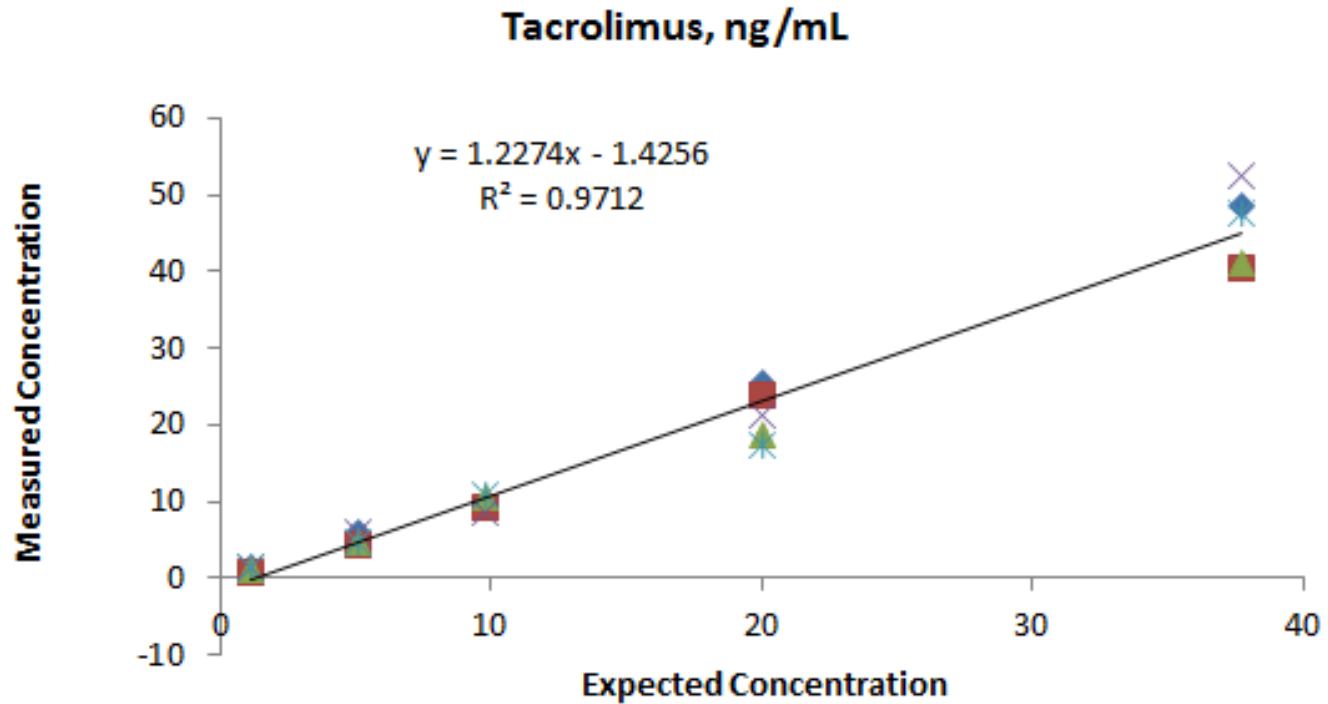
- Intra-assay ( $n=10$ )

Tacrolimus	Level I	Level II	Level III
Mean (ng/mL)	6.1	16.8	35.1
SD	0.44	1.50	4.02
% CV	7.3%	8.9%	11.5%

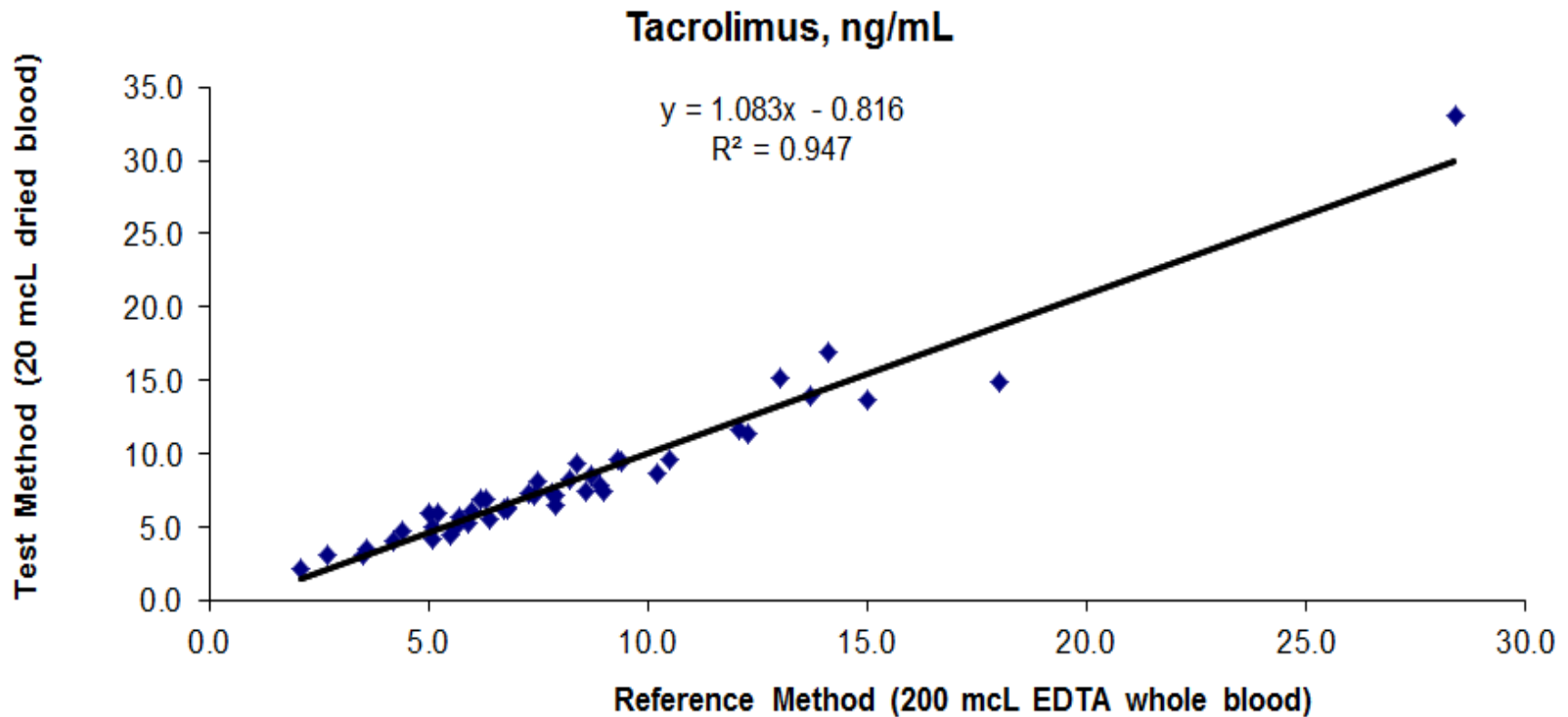
- Inter-assay ( $n=3$  days)

Tacrolimus	Level I	Level II	Level III
Mean (ng/mL)	5.4	16.0	32.1
SD	0.49	1.59	2.66
% CV	9.1%	9.9%	8.3%

# Microsampling Linearity: Tacrolimus



# Microsampling Accuracy: Tacrolimus *n*=45 patients



# Microsampling Precision: Cyclosporine

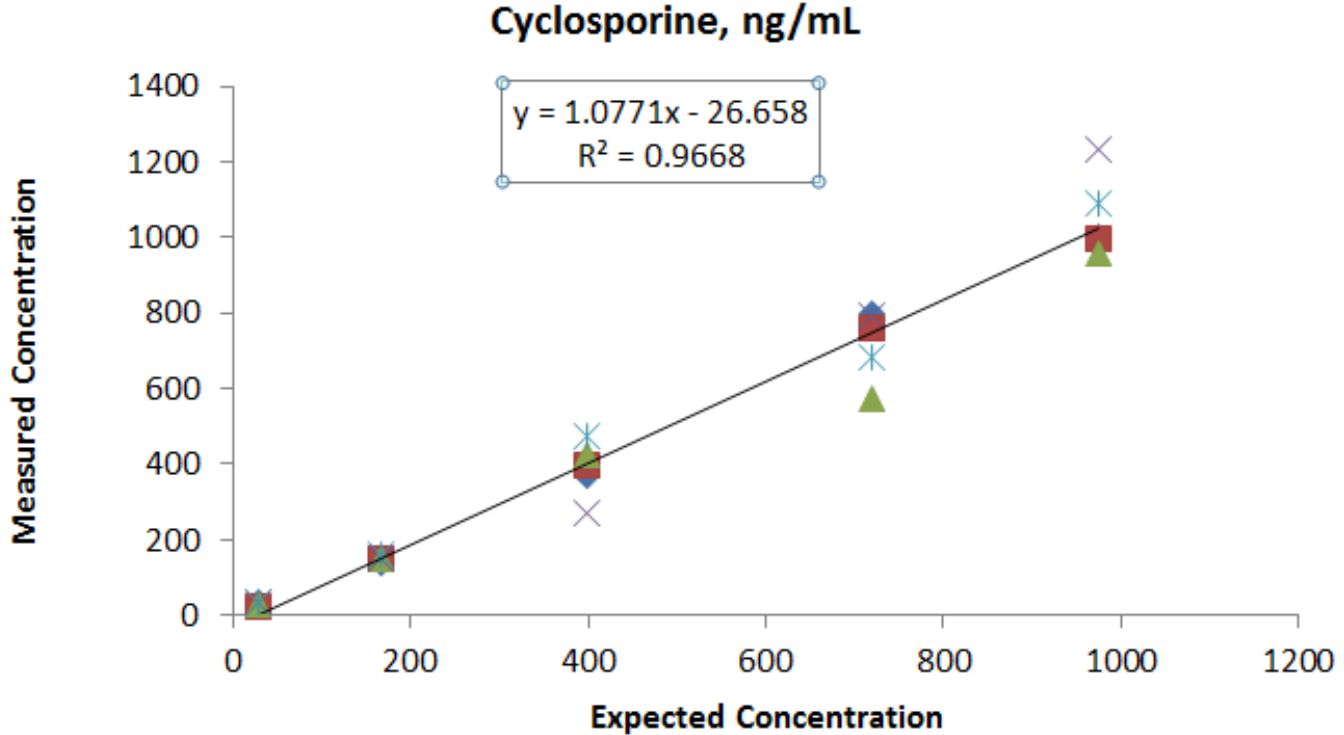
- Intra-assay ( $n=10$ )

Cyclosporine	Level I	Level II	Level III
Mean (ng/mL)	138	375	646
SD	7.9	35.1	65.3
% CV	5.7%	9.4%	10.1%

- Inter-assay ( $n=3$  days)

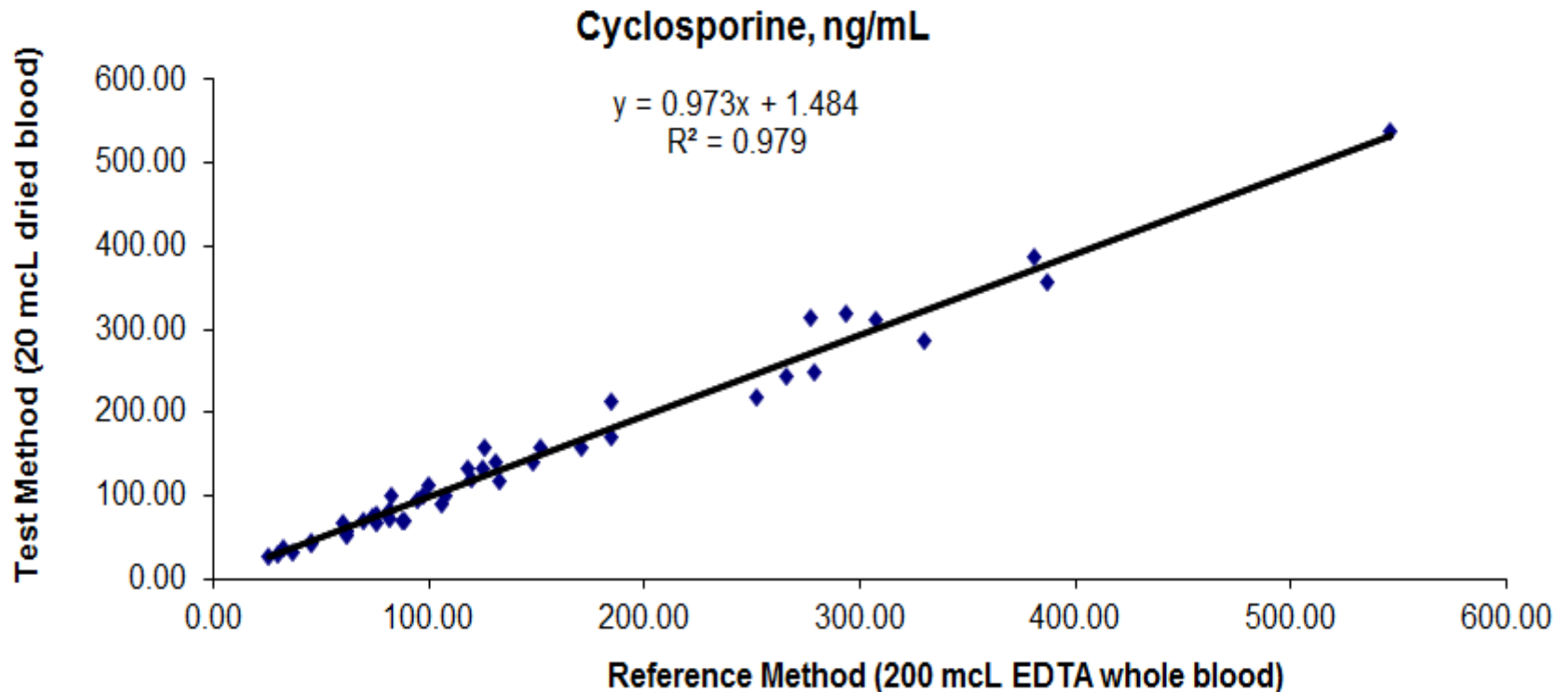
Cyclosporine	Level I	Level II	Level III
Mean (ng/mL)	120	342	564
SD	9.8	33.3	53.8
% CV	8.1%	9.7%	9.5%

# Microsampling Linearity: Cyclosporine





# Microsampling Accuracy: Cyclosporine *n*=45 patients



# Microsampling Carryover

Analyte	Average Blank % LLOQ Area following High sample	Criteria: Must be <50% LLOQ
Tacrolimus	43%	Pass
Cyclosporine	10%	Pass

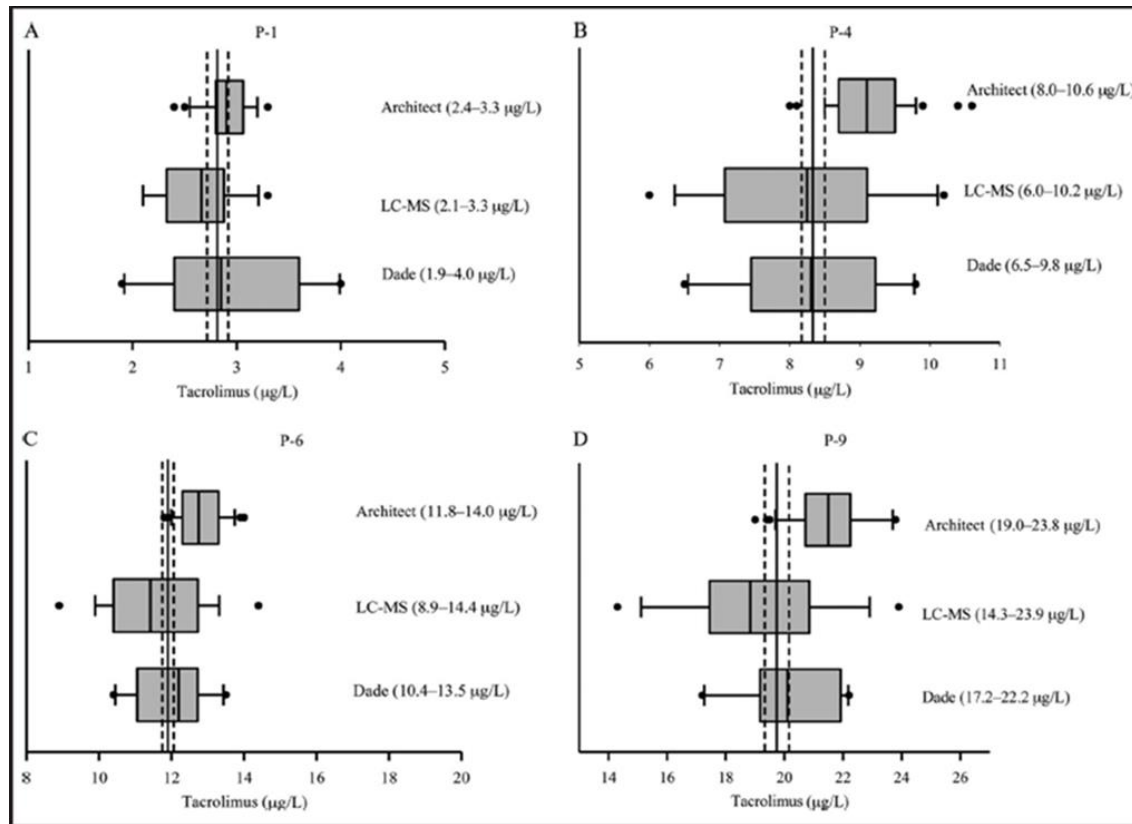
# Conclusions

- Preliminary validation data of Mitra™ microsampling device used to measure both Tacrolimus and Cyclosporine showed acceptable:
  - Precision (Intra- and Inter-day)
  - Linearity
  - Accuracy
  - Carryover
- Future studies with patient self-collected samples will be performed to fully characterize variability in TDM results

# Potential Benefits

1. Reduced blood draw (20  $\mu$ L vs. 4 mL)
  - A. Especially important for pediatric transplant patients
2. Ability to collect sample at home
  - A. Improved patient satisfaction
3. Centralized TDM of immunosuppressant's
  - A. Decreased shipping costs (DBS vs. tube)
  - B. Immunosuppressant testing is NOT standardized/harmonized

# We Still Need to Standardize Immunosuppressant Testing



**Fig. 1.** Box-and-whisker plots of Architect, LC-MS, and Dade Dimension test values for samples P-1 (A), P-4 (B), P-6 (C), and P-9 (D).

Vertical solid line across the plots, reference value determined by EM-IDMS; dashed lines, associated expanded uncertainty; values in parentheses, range of tacrolimus concentration values obtained by each tacrolimus test method.



## Questions & Discussion