

Immunosuppressant therapeutic drug monitoring in whole blood on volumetric absorptive microsamples: a development on i601 mass spec by Roche®

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Background and objective

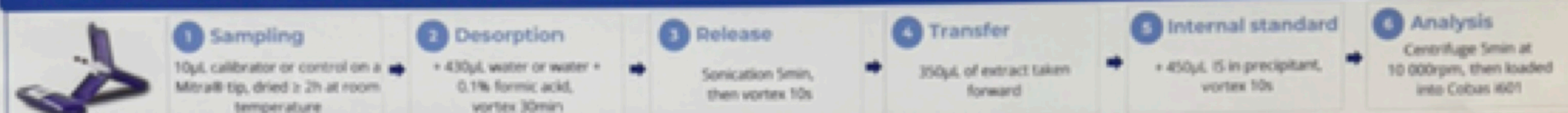
LC-MS/MS is the reference technique for immunosuppressant therapeutic drug monitoring. The Cobas i601 (Roche®) makes it a routine tool: a fully automated, random-access triple-quadrupole system integrated into the clinical chemistry line, with a 6.8-fold shorter turnaround than conventional LC-MS/MS.

The sample itself, however, has not changed: transplant recipients still come in for a venipuncture at every scheduled measurement. Dried microsampling has been routine practice in newborn screening for decades, and volumetric absorptive microsampling now brings it a fixed 10 µL of blood from a fingerprick¹. Adapting the assay to these samplers would open a route towards home sampling, and a real gain in quality of life for patients.

Objective

Adapt the i601 assay to 10 µL Mitra® tips for ciclosporin A, tacrolimus, sirolimus and everolimus^{1,2}, and test whether 0.1 % formic acid in the extraction solvent improves performance.

Methods: VAMS workflow on the Cobas i601



Design. Recipe® calibrators G1–G7 and Chromsystems® controls, 4 levels × 6 replicates, 3 independent runs per solvent, 768 measurements, no missing data.

Statistics. Three calibration models were compared on each curve, all excluding the blank³. Quantification for the performance analysis used 1/x² weighting⁴; intermediate precision by one-way ANOVA; *t* tests on pooled within-run variances.

Acceptance criteria were ± 15 % for both bias and CV, ± 20 % at the lowest level⁵.

Calibration model and accuracy at the lowest calibrator

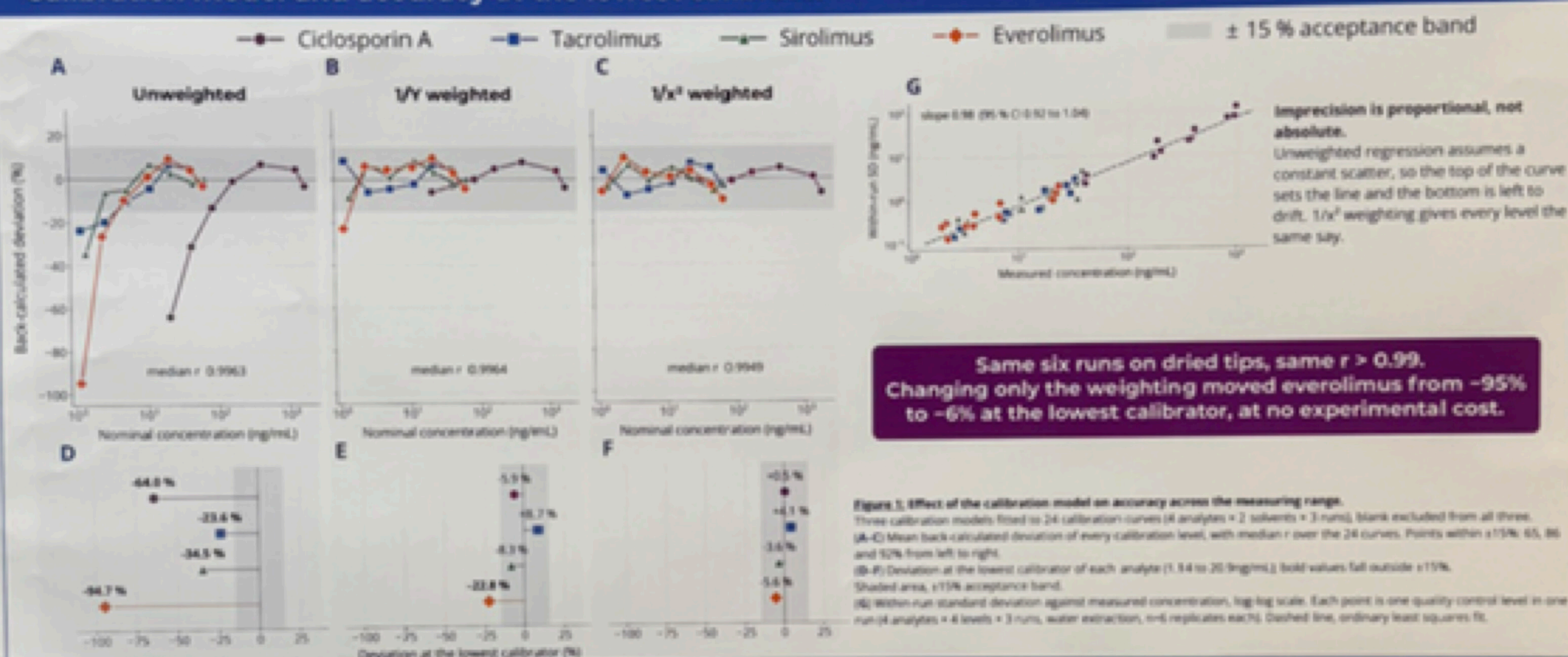
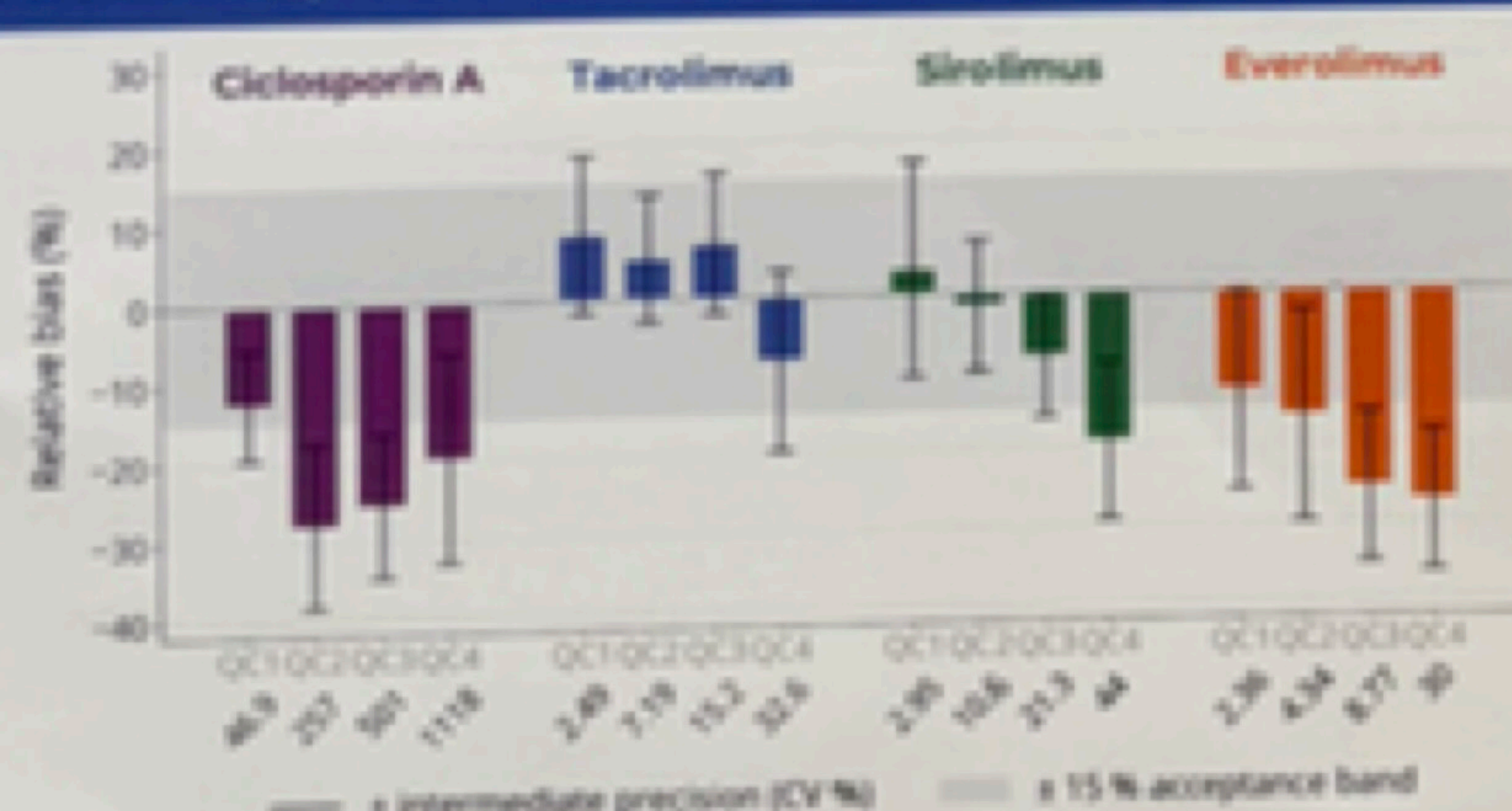


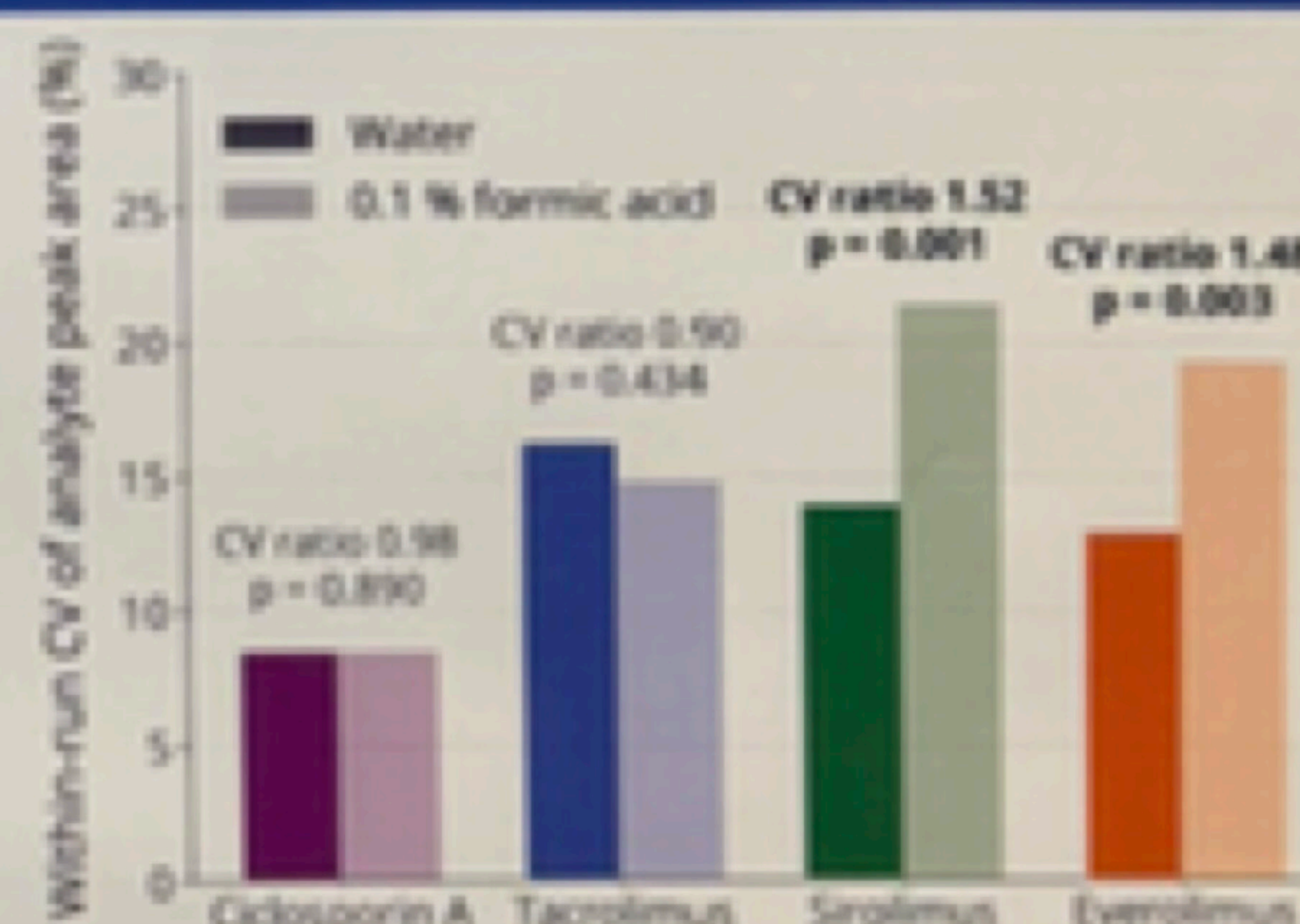
Figure 1. Effect of the calibration model on accuracy across the measuring range. Three calibration models fitted to 24 calibration curves (4 analytes × 2 solvents × 3 runs), blank excluded from all three. (A–C) Mean back-calculated deviation of every calibration level, with median *r* over the 24 curves. Points within ±15%, 65, 86 and 92% from left to right. (D–F) Deviation at the lowest calibrator of each analyte (1.14 to 20 ng/mL); bold values fell outside ±15%. Shaded area, ±15% acceptance band. (G) Within-run standard deviation against measured concentration, log-log scale. Each point is one quality control level in one run (4 analytes × 4 levels × 3 runs, water extraction, n=6 replicates each). Dashed line, ordinary least squares fit.

Trueness, precision and effect of formic acid



Intermediate precision falls between 9.7 and 11.3% for all four analytes on a 10 µL dried tip. Mean bias per analyte ranges from +3.1 to -21.4% against controls from another manufacturer.

Figure 2. Trueness and precision on quality control material. Relative bias against target value, water extraction, 1/x² weighted calibration. Error bars, one intermediate precision CV (one-way ANOVA, 3 runs × 6 replicates, n = 18 per level). Second tick line, target concentration in ng/mL. Calibrators and controls come from different manufacturers. Shaded area, ±15% acceptance band.



0.1 % formic acid changed no response and raised the scatter of the two analytes it was meant to rescue by half. Water remained the reference condition for the performance analysis.

Figure 3. Effect of 0.1 % formic acid on repeatability. Within-run CV of the analyte peak area, before internal standard normalisation. Variances pooled over 4 control levels × 3 runs on log-transformed areas (60 df per solvent), compared by an *F* test. CV ratio, formic acid over water. The two solvents were run on different dates.

Discussion and conclusion

Across 768 measurements and six independent runs, with no missing value and no outlier, the automated platform took the microsamples through to quantification. Intermediate precision landed between 9.7 and 11.3% for all four analytes, on a tenth of the usual blood volume. Tacrolimus met both acceptance criteria at every level, sirolimus at three of four.

Precision is homogeneous, so the sampling step itself is under control. What separates the four analytes is trueness, and the calibrators and controls used here came from two different manufacturers, a documented source of disagreement for immunosuppressants⁶. Repeating the controls with material from the calibrator supplier is a single experiment, and it will tell us how much of the bias belongs to the method.

Limitations

- Analytical evaluation only: no patient samples, no paired venous whole blood, no haematocrit or stability study^{7,8}.
- Bias is measured against control material from a manufacturer other than the calibrator supplier.
- The two extraction solvents were run on different dates.

For tacrolimus, the most prescribed of the four, a 10 µL dried tip on our routine line already delivers the required analytical performance. The remaining three are within reach of a few analytical adjustments. Next step is patient samples, paired with venous whole blood.