

Flexible, automated sample-to-result opportunistic virus monitoring for transplant care on the myCROBE™ system

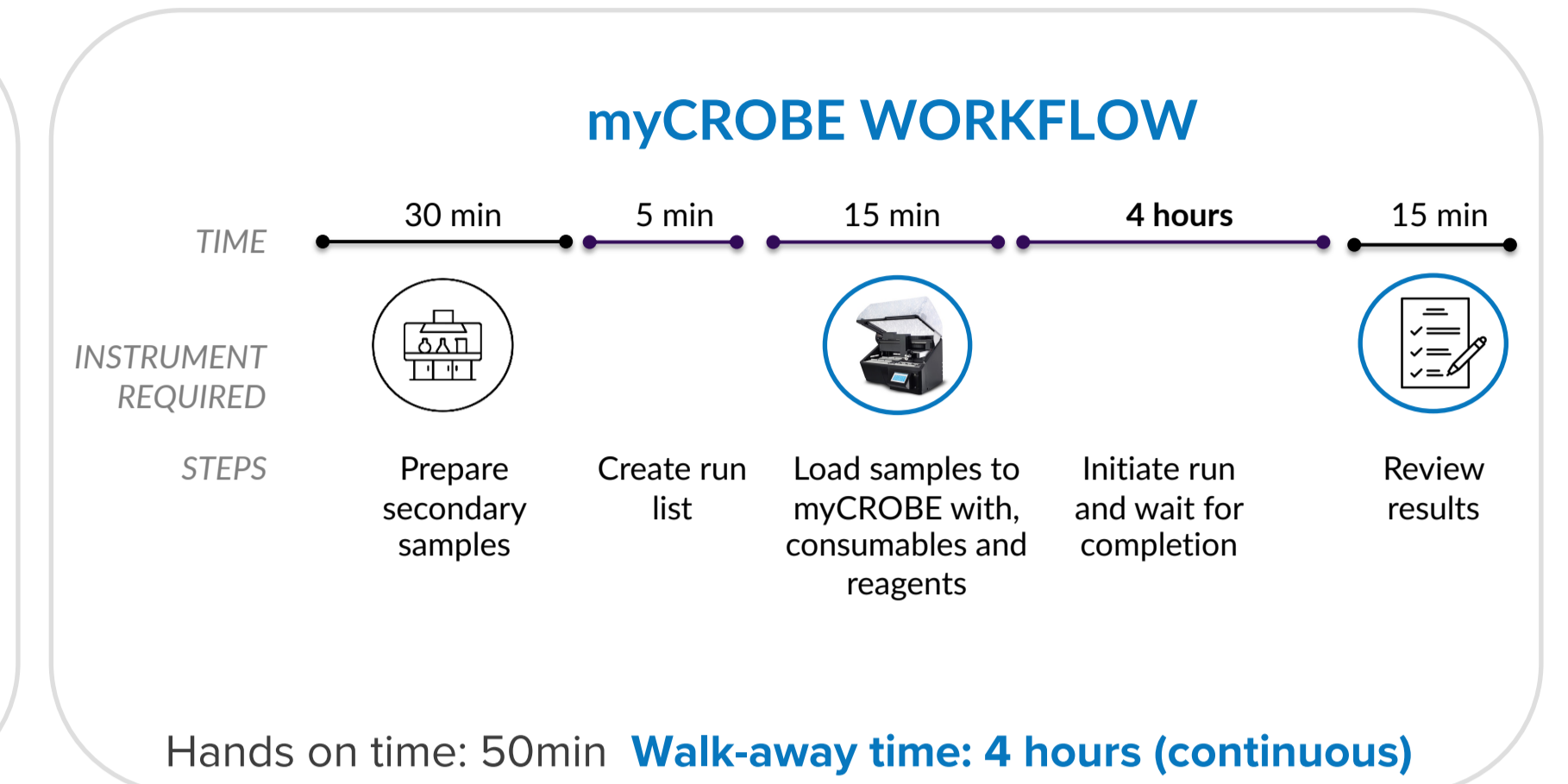
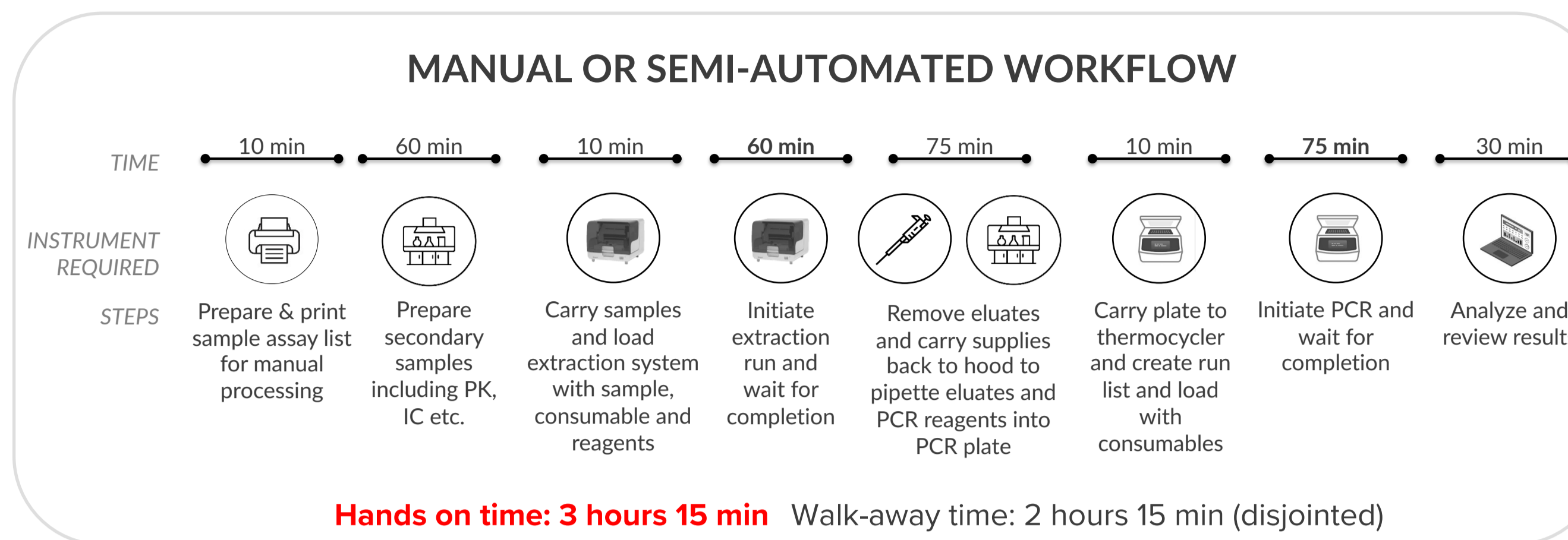
Horlitz Martin, Sapáková Kateřina, Skopalová Natálie, Pořízková Kateřina
GeneProof a.s., Vídeňská 101/119, Dolní Heršpice, CZ - 619 00 Brno

Background Need

Immunocompromised patients, including solid organ and hematopoietic stem cell transplant recipients, are at high risk for opportunistic viral infections, with severe courses associated with substantial morbidity and reported mortality up to 40%. Serial molecular monitoring supports early detection, timely treatment decisions, and longitudinal management.

myCROBE Introduction

Most sample-to-result systems offer limited viral breadth and do not support flexible, patient-specific target selection within a single automated workflow. We implemented and validated a broad opportunistic virus PCR menu on the myCROBE Fully Automated Sample-to-Result platform, enabling per-sample assay selection without changing the overall workflow.



Assays and Tests

Assay	Quantitative parameters	Whole system failure test	Cross-reactivity	On-board stability	Interfering substances
CMV	✓	✓	✓	✓*	✓
EBV	✓	✓	✓		✓
BKV	✓	✓	✓		✓
JCV	✓	✓	✓		✓
HSV 1/2	✓	✓	✓		✓
HHV 6/7	✓	✓	✓		✓
HHV 8	✓	✓	✓		✓
VZV	✓	✓	✓		✓

*On-board stability for all kits was assessed based on data obtained from the CMV PCR Kit.

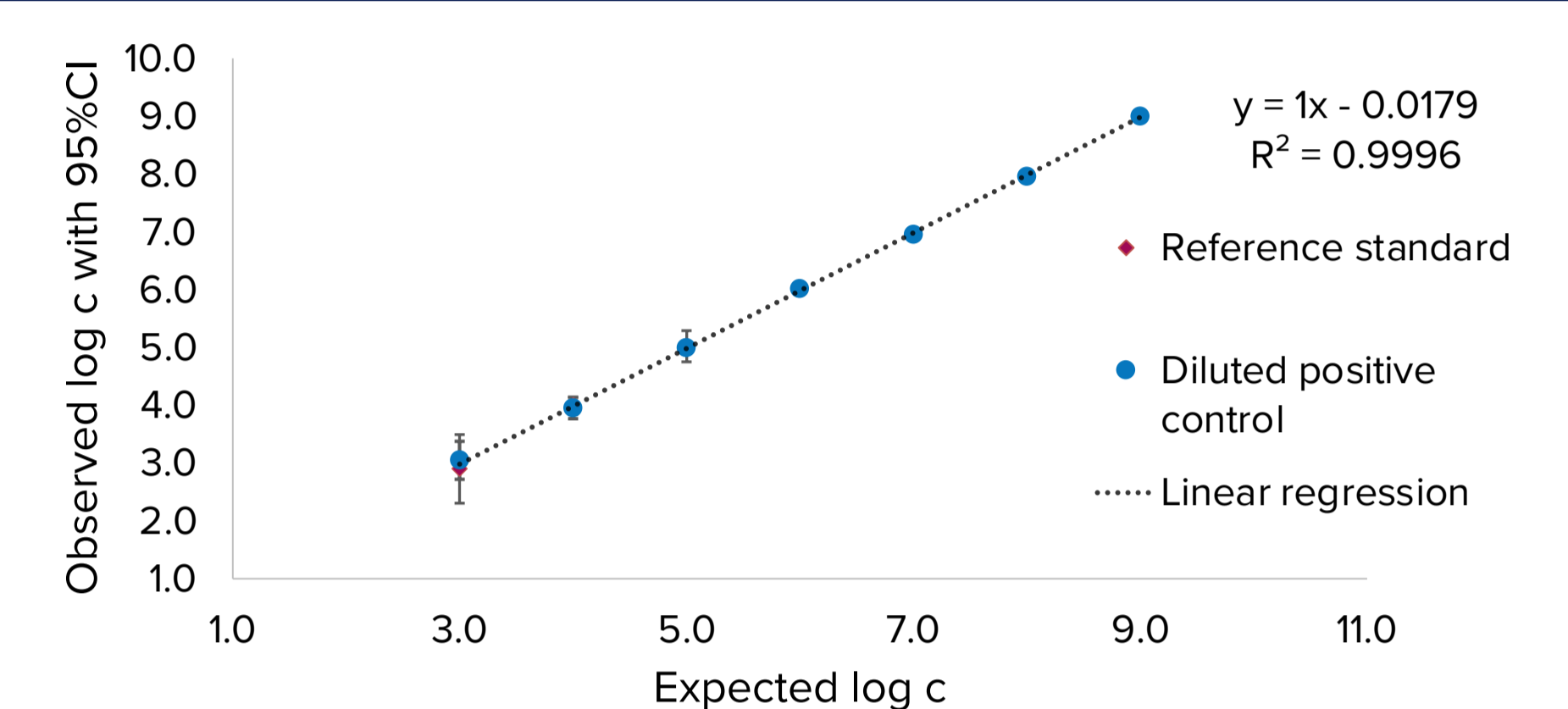
The myCROBE transplant panel includes the assays shown above. Comprehensive analytical performance validation was performed as indicated in the table.

Precision

Variability of concentration	SD (log Concentration)	95% CI (lower)	95% CI (upper)
Intra-assay	0.131	0.093	0.222
Inter-assay	0.140	0.073	0.875
Inter-operator	0.122	0.064	0.765
Inter-device	0.129	0.067	0.810
Total	0.138	0.072	0.862

High precision was demonstrated with a total SD of 0.138 log, consistent across days, operators and devices.

Linear Range

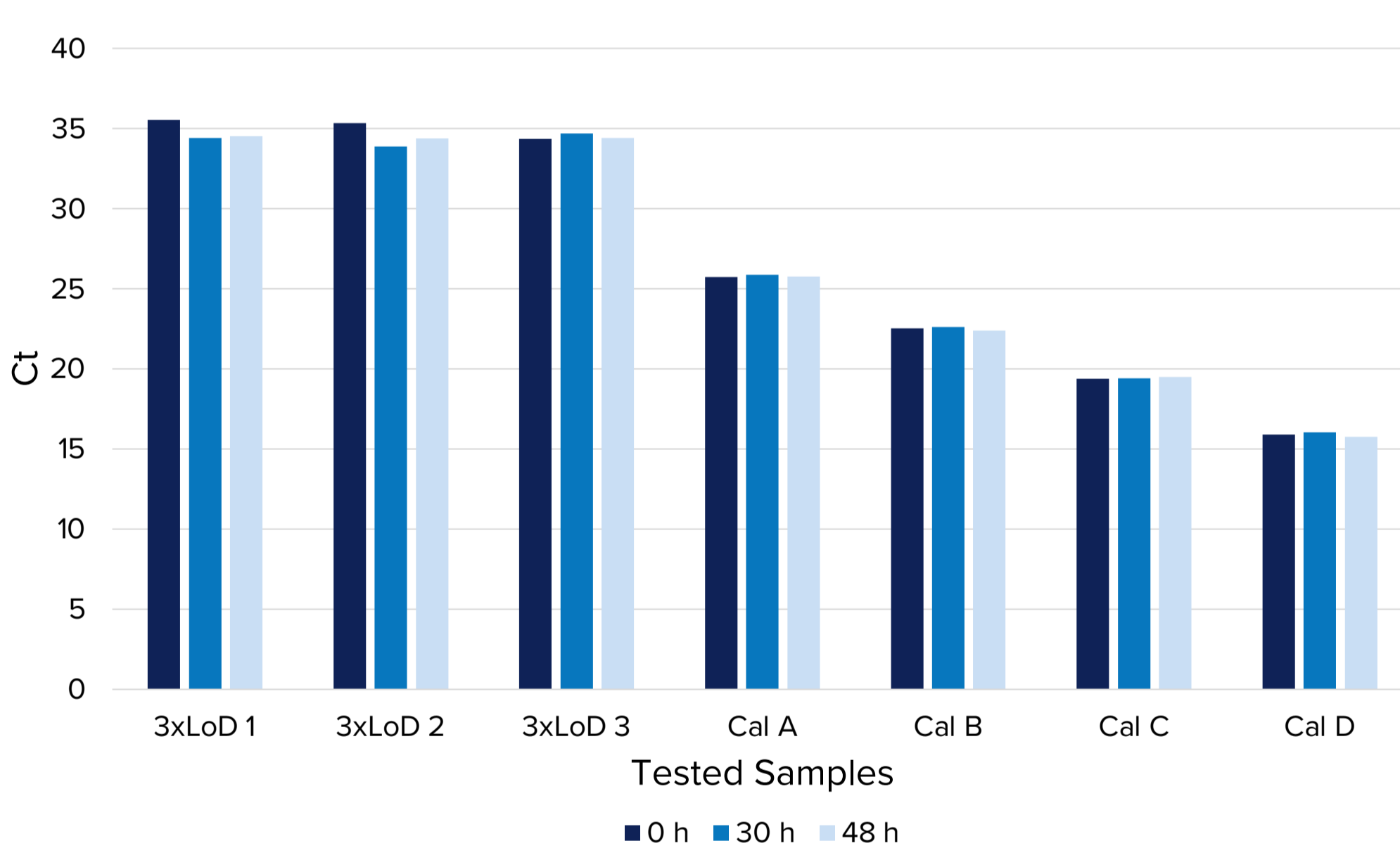


myCROBE CMV testing in triplicate across the linear range over multiple days and devices vs:

- CMV NIBSC 09/162 standard
- Positive control diluted in plasma

The GeneProof Cytomegalovirus (CMV) MC PCR Kit demonstrated a linear range of 10^9 – 10^3 IU/mL with trueness -0.02 log (95% CI: 0.05 - 0.01) in plasma.

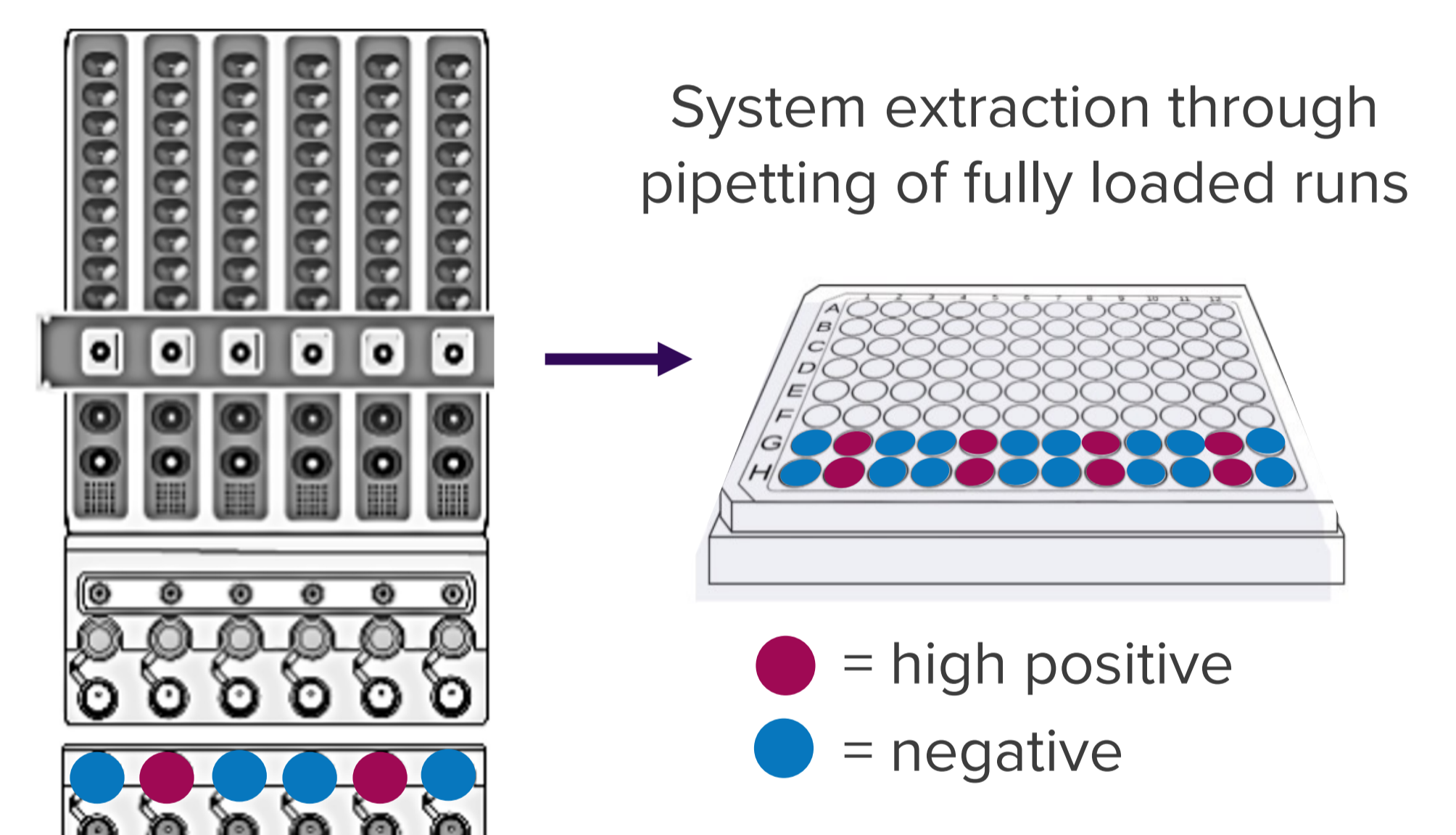
On-board Stability



On-board stability was evaluated using 3 replicates of reference material (3xLoD) and calibrator (Cal) reagents. myCROBE PCR kits were tested during 48 hours within a 30-day period, with freezing of assay reagents between runs.



Cross-contamination



Cross-contamination testing was performed using alternating highly positive (Ct ≈ 15) and negative samples across extraction and PCR plate positions under full load conditions. No cross-contamination was detected in six PCR runs across three myCROBE instruments.

Interference

Specimen	Interferents					
Endogenous plasma	Bilirubin 342 µmol/L	Albumin 60 g/L	Urea 42.9 mmol/L	Glucose 55 mmol/L	Uric Acid 1.4 mmol/L	Citrate 19 g/L
Exogenous plasma	Caffeine 308 µmol/L	Vancomycin 69 µmol/L	Ibuprofen 2425 µmol/L	Fluconazole 245 µmol/L	Prednisone 0.84 µmol/L	
Whole blood	EDTA 2.2 g/L	Heparin 30 IU/mL				
CSF	Albumin 60 g/L	Glucose 55 mmol/L	Lactic Acid 16.5 mmol/L			
Urine	Bilirubin 20 mg/dL	Albumin 5 %	Urea 600 mmol/L	Glucose 1 %	Uric Acid 1.4 mmol/L	pH 4 pH 9
Saliva	Whole blood 2 % (v/v)	Mucin 60 µg/mL				
Swab	Whole blood 2 % (v/v)	Mucin 60 µg/mL	Clotrimazole cream 0.25 % (w/v)	Gel lubricant 0.25 % (w/v)		

Assay performance was evaluated in the presence of potential interfering substances in validated sample materials at defined concentrations.

Note: Data presented are derived from the GeneProof Cytomegalovirus (CMV) MC PCR Kit and are representative of the myCROBE Immunocompromised/Transplant PCR kit panel performance.

Summary

- Reliable detection of all 8 target viruses across 7 clinically relevant specimen types.
- No cross-contamination observed across multiple runs and instruments, demonstrating high analytical reliability.
- High assay precision (total SD 0.138 log) maintained consistently across days, operators and devices.
- Stable reagent performance throughout the entire on-board working period
- Robust assay performance confirmed across a broad panel of potential interfering substances and sample materials.
- Overall, myCROBE PCR kits provide a reliable and robust solution for routine monitoring of opportunistic viral infections in transplant patients.