Unleashing the Panther: A Validation Journey

Michael Pentella, PhD, D(ABMM)
Michael-pentella@uiowa.edu
Clinical Professor, College of Public Health
State Hygienic Laboratory, Director
University of Iowa

About the Panther Fusion

- True assay consolidation (PCR+TMA) on one platform
- Fully automated open-access PCR for LDTs
- High throughput with random access
- Reduced labor, waste, and operating costs
- Excellent clinical performance
- Scalable, future proof molecular diagnostics

Panther Fusion Overview

HSV 1&2/VZV
Mpox & NOV
Candida auris
Measles
Flu/SC2/RSV

- Why we chose Panther Fusion
- LDT Testing started in 2023
- Automation and reduced errors
- Staff time and ease of training
- Reliability

Panther Fusion Optimization

- Decision of singleplex or multiplex.
- Determine target Primer and Probe sequences
- Build protocols in myAccess software on Hologic Open Access laptop
- Optimize primer probe mix concentrations
- Optimization runs can be analyzed on the myAccess laptop

SHL Validation Process

- Start with an approved validation plan to determine:
  - Trueness – Accuracy Plan
  - Precision – repeatability, Intermediate Precision & Reproducibility
  - Reportable Range
  - Reference Intervals (normal values)
  - Analytical Sensitivity
  - Analytical Specificity and Interfering Substances
- Determine calibration & control procedures and proficiency testing
- Results are reviewed, and a determination is made whether the test is acceptable, or a repeat plan is developed

Mpox Clade II Validation


The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

IDSA Infectious Diseases Society of America hivma hiv medicine association OXFORD

A Laboratory-Developed Assay for Clade II Human Mpox Virus on the Panther Fusion Open Access System

Rebecca D. Villa,[1] Michael A. Pentella,[1] and Jeffrey L. Benfer[1]

[1]State Hygienic Laboratory, University of Iowa, Coralville

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- 10 positives and 10 negatives obtained from MI PHL
  - 95% accuracy and 100% precision
  - 100% Specificity
- Aptima Multitest Swab Transport Media, RTM4, UTM, and dry swabs validated
  - 98% accuracy and 100% precision
- LOD 1.475 copies/rxn, PCR efficiency 89.87%

Mpox clade II and Non-variola orthopox validation December 2024

1.3 WORKPLAN

Item	Run 1 (A)	Run 2 (B)	Run 3 (C)	Total
Day	1	1	2	2
Instrument: Panther#	1	2	1	2
Analyst	KE	MA	MA	2
Mpox Clade II Positive Specimens (Includes NVO)	5	5	5	15
Mpox Clade I Positive Specimens (Includes NVO)	5	5	5	15
Vaccinia Virus Positive Specimens (Includes NVO)	5	5	5	15
Negative Specimens	5	5	5	15
All Targets Positive Control (gBlock)	1	1	1	3
Negative Control	1	1	1	3
Specificity – Herpes1	1	1	1	3
Specificity – Herpes2	1	1	1	3
Specificity – VZV	1	1	1	3

3.1.1 Table for Acceptance Criteria

ID	Detail	Acceptance	Evaluation Pass / Fail
1.	5 Clade II Positive Specimens	90% concordance with Poxvirus Branch method (if not 100%, add report detail on discordance)	PASS
2.	5 Clade I Positive Specimens	90% concordance with LRN method (if not 100%, add report detail on discordance)	PASS
3.	5 Vaccinia Virus Positive Specimens	90% concordance with Poxvirus Branch method (if not 100%, add report detail on discordance)	PASS
4.	15 NVO Positive Specimens	90% concordance with Poxvirus Branch method (if not 100%, add report detail on discordance)	PASS
5.	Positive control	100%	PASS
6.	Panther1 vs Panther2	Within 3 Ct values (Evaluate %RSD for Ct values)	PASS
7.	Specificity Sample Herpes1	Negative for Mpox Clade II and NVO	PASS
8.	Specificity Sample Herpes2	Negative for Mpox Clade II and NVO	PASS
9.	Specificity Sample VZV	Negative for Mpox Clade II and NVO	PASS

Between Day Accuracy

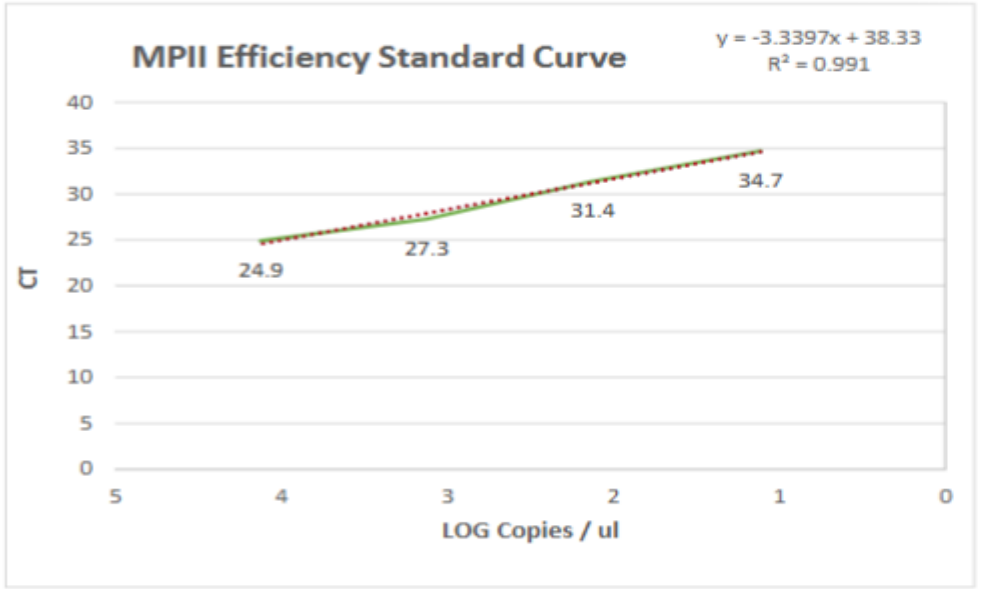
Day 1: 12-17-2024 (A) vs Day 2: 12-18-2024 (C)								
Specimen Type	Expected FAM	Observed FAM Run A Day 1	Observed FAM Run C Day 2	FAM Accuracy	Expected ROX	Observed ROX Run A Day 1	Observed ROX Run C Day 2	ROX Accuracy
Negative Specimen	neg	neg	neg	TRUE	neg	neg	neg	TRUE
Negative Specimen	neg	neg	neg	TRUE	neg	neg	neg	TRUE
Negative Specimen	neg	neg	neg	TRUE	neg	neg	neg	TRUE
Negative Specimen	neg	neg	neg	TRUE	neg	neg	neg	TRUE
Negative Specimen	neg	neg	neg	TRUE	neg	neg	neg	TRUE
MPII	POS	POS	POS	TRUE	POS	POS	POS	TRUE
MPII	POS	POS	POS	TRUE	POS	POS	POS	TRUE
MPII	POS	POS	POS	TRUE	POS	POS	POS	TRUE
MPII	POS	POS	POS	TRUE	POS	POS	POS	TRUE
MPII	POS	POS	POS	TRUE	POS	POS	POS	TRUE
MPI	neg	neg	neg	TRUE	POS	POS	POS	TRUE
MPI	neg	neg	neg	TRUE	POS	POS	POS	TRUE
MPI	neg	neg	neg	TRUE	POS	POS	POS	TRUE
MPI	neg	neg	neg	TRUE	POS	POS	POS	TRUE
MPI	neg	neg	neg	TRUE	POS	POS	POS	TRUE
Vaccinia	neg	neg	neg	TRUE	POS	POS	POS	TRUE
Vaccinia	neg	neg	neg	TRUE	POS	POS	POS	TRUE
Vaccinia	neg	neg	neg	TRUE	POS	POS	POS	TRUE
Vaccinia	neg	neg	neg	TRUE	POS	POS	POS	TRUE
Vaccinia	neg	neg	neg	TRUE	POS	POS	POS	TRUE
HSV1	neg	neg	neg	TRUE	neg	neg	neg	TRUE
HSV2	neg	neg	neg	TRUE	neg	neg	neg	TRUE
VZV	neg	neg	neg	TRUE	neg	neg	neg	TRUE
Positive Control	POS	POS	POS	TRUE	POS	POS	POS	TRUE
Negative Control	neg	neg	neg	TRUE	neg	neg	neg	TRUE

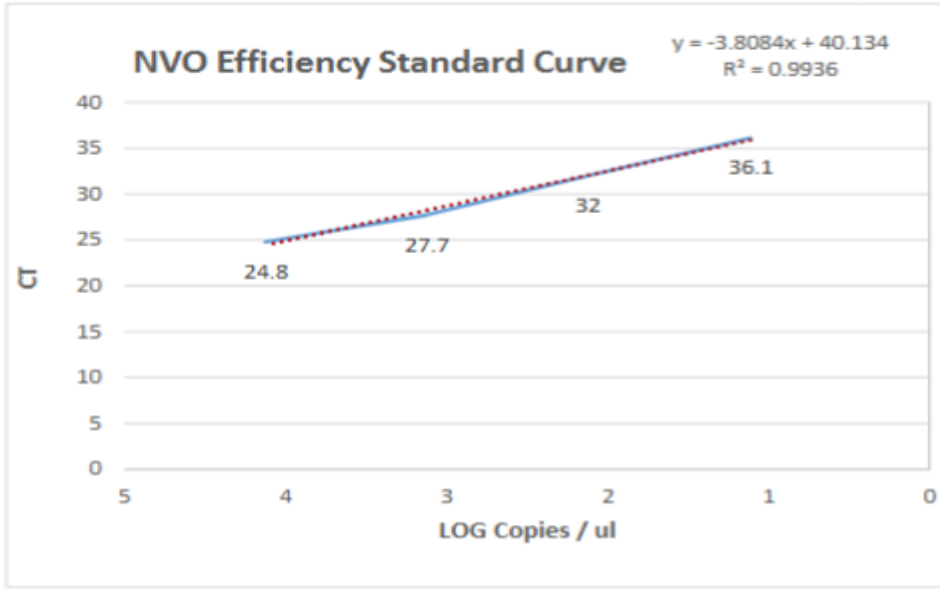
7.1.2 Analytical Sensitivity Result

- Limit of Detection (LOD): Phase 1 & 2

Phase 1: 10-fold Dilution Series of Positive Control				
Dilution	Sample ID	Copies / ul	MPII CT	NVO CT
Pos Control	A	13314	24.9	24.8
10^-1	B	1331	27.3	27.7
10^-2	C	133	31.4	32
10^-3	D	13	34.7	36.1
10^-4	E	1	0	0
10^-5	F	0.1	0	0

The limit of detection lies somewhere between 13 copies/ul and 1 copy/ul.





PCR Efficiency	1.992636551
% PCR Efficiency	99.26

PCR Efficiency	1.830532569
% PCR Efficiency	83.05

This assay demonstrated reasonable PCR efficiencies of 99.26% for the Mpox Clade II target and 83.05% for the NVO target.

Candida auris validation

- Modified the University of Texas Medical Branch (UTMB) method for Real-Time PCR *Candida auris* Using Panther Fusion and the CDC method for Real-Time PCR
- Ruled-out cross reaction with other Candida species (C. duobushaemulonii, haemulonii, glabrata, krusei, parapsilosis, tropicalis, kefyr, lusitaniae, pelliculosa, pararugosa, guilliermondii)

Candida auris workplan

3.1.1 Table for Acceptance Criteria

ID	Detail	Acceptance	Evaluation Pass / Fail
1.	16 positive specimens	90% w/ AR Isolate Bank MALDI-TOF MS (if not 100%, add report detail on discordance)	PASS
2.	10 negative specimens	90% w/ AR Isolate Bank MALDI-TOF MS (if not 100%, add report detail on discordance)	PASS
3.	Positive control	100%	PASS
4.	Panther1 vs Panther2	Within 3 Ct values (Evaluate %RSD for Ct values)	PASS
5.	Specificity Sample *Candida duobushaemulonii*	Negative for *Candida auris*	PASS
6.	Specificity Sample *Candida haemulonii*	Negative for *Candida auris*	PASS
7.	Specificity Sample *Candida glabrata*	Negative for *Candida auris*	PASS
8.	Specificity Sample *Candida krusei*	Negative for *Candida auris*	PASS
9.	Specificity Sample *Candida parapsilosis*	Negative for *Candida auris*	PASS
10.	Specificity Sample *Candida tropicalis*	Negative for *Candida auris*	PASS
11.	Specificity Sample *Candida kefyr*	Negative for *Candida auris*	PASS
12.	Specificity Sample *Candida lusitaniae*	Negative for *Candida auris*	PASS
13.	Specificity Sample *Candida pelliculosa*	Negative for *Candida auris*	PASS
14.	Specificity Sample *Candida pararugosa*	Negative for *Candida auris*	PASS
15.	Specificity Sample *Candida guilliermondii*	Negative for *Candida auris*	PASS
16.	Specificity Sample *Kodameae ohmeri*	Negative for *Candida auris*	PASS
17.	Specificity Sample *Saccharomyces cerevisiae*	Negative for *Candida auris*	PASS
18.	Specificity Sample *Corynebacterium xerosis*	Negative for *Candida auris*	PASS
19.	Specificity Sample *Streptococcus pnuemoniae*	Negative for *Candida auris*	PASS
20.	Specificity Sample *Staphylococcus aureus*	Negative for *Candida auris*	PASS
21.	Specificity Sample *Staphylococcus epidermidis*	Negative for *Candida auris*	PASS

3.2.1 Between Instrument Accuracy

Panther 1 (A) vs Panther 2 (B) - Accuracy					
Specimen Type	Isolate ID	Expected FAM	Observed FAM – Run A Panther 1	Observed FAM – Run B Panther 2	Accuracy
C. auris Positive Control	AR0382	POS	POS	POS	PASS
Negative Control	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS

3.2.2 Between Days Accuracy

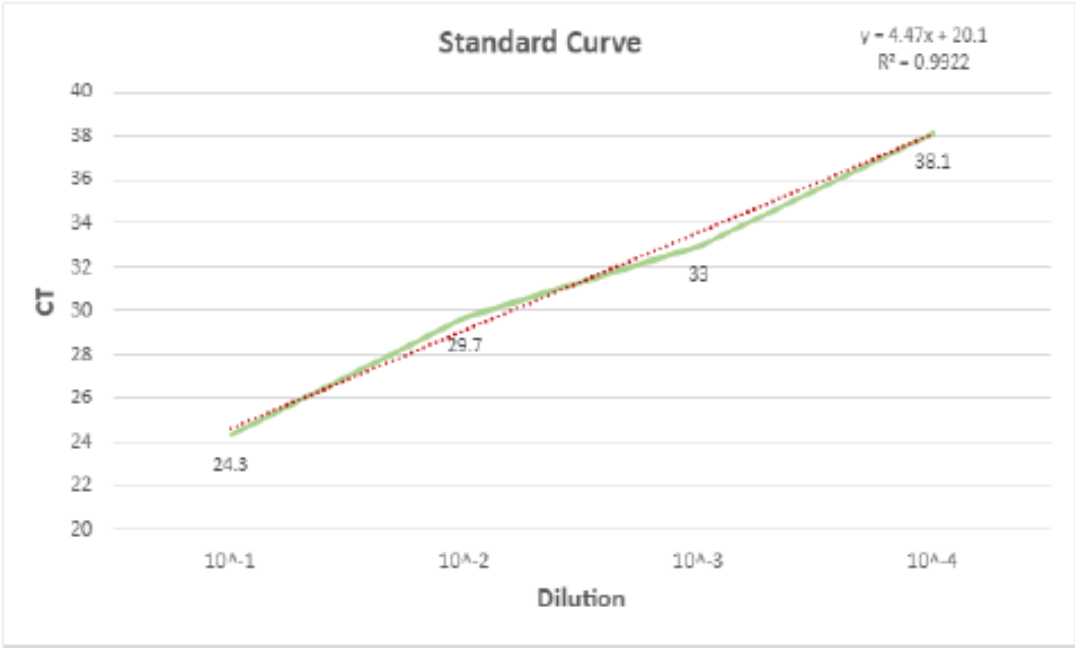
Day 1: 4-1-2024 (A) vs Day 2: 4-5-2024 (C) - Accuracy					
Specimen Type	Isolate ID	Expected FAM	Observed FAM Run A – Day 1	Observed FAM Run C – Day 2	Accuracy
C. auris Positive Control	AR0382	POS	POS	POS	PASS
Negative Control	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade I	AR0382	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade II	AR0381	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade III	AR0383	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS
C. auris Clade IV	AR0385	POS	POS	POS	PASS

Panther 1 (A) vs Panther 2 (B) - Precision				
Specimen Type	Isolate ID	FAM CT – Run A Panther 1	FAM CT – Run B Panther 2	Precision: Within 3 CT
C. auris Positive Control	AR0382	23.2	23.1	PASS
Negative Control	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
C. auris Clade I	AR0382	24.4	24.4	PASS
C. auris Clade I	AR0382	24.1	24.1	PASS
C. auris Clade I	AR0382	24	23.8	PASS
C. auris Clade I	AR0382	23.8	23.2	PASS
C. auris Clade II	AR0381	23.9	24.1	PASS
C. auris Clade II	AR0381	23.9	24.2	PASS
C. auris Clade II	AR0381	24.3	23.9	PASS
C. auris Clade II	AR0381	23.7	24.1	PASS
C. auris Clade III	AR0383	22.8	22.6	PASS
C. auris Clade III	AR0383	23.5	23	PASS
C. auris Clade III	AR0383	23.6	23.6	PASS
C. auris Clade III	AR0383	23.7	23.4	PASS
C. auris Clade IV	AR0385	23.4	23.8	PASS
C. auris Clade IV	AR0385	23.1	22.9	PASS
C. auris Clade IV	AR0385	23.5	23.1	PASS
C. auris Clade IV	AR0385	23.8	23.9	PASS

Day 1: 4-1-2024 (A) vs Day 2: 4-5-2024 (C) - Precision				
Specimen Type	Isolate ID	FAM CT – Run A	FAM CT – Run C	Precision: Within 3 CT
C. auris Positive Control	AR0382	23.2	24.3	PASS
Negative Control	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
C. auris Clade I	AR0382	24.4	24.4	PASS
C. auris Clade I	AR0382	24.1	25.1	PASS
C. auris Clade I	AR0382	24	25.1	PASS
C. auris Clade I	AR0382	23.8	25.4	PASS
C. auris Clade II	AR0381	23.9	25.6	PASS
C. auris Clade II	AR0381	23.9	24.6	PASS
C. auris Clade II	AR0381	24.3	25.2	PASS
C. auris Clade II	AR0381	23.7	25.4	PASS
C. auris Clade III	AR0383	22.8	25.2	PASS
C. auris Clade III	AR0383	23.5	25.1	PASS
C. auris Clade III	AR0383	23.6	24.7	PASS
C. auris Clade III	AR0383	23.7	25	PASS
C. auris Clade IV	AR0385	23.4	24.8	PASS
C. auris Clade IV	AR0385	23.1	25.5	PASS
C. auris Clade IV	AR0385	23.5	25.2	PASS
C. auris Clade IV	AR0385	23.8	24	PASS

7.1.2 Analytical Sensitivity Results

Phase 1: 10-fold Dilution Series of 0.5 McFarland			
Dilution	Sample ID	CFU (50 ul)	CT
10^-1	A	Too many to count	24.3
10^-2	B	Too many to count	29.7
10^-3	C	61	33
10^-4	D	1	38.1
10^-5	E	1	0
10^-6	F	1	0
10^-7	G	0	0
10^-8	H	0	0



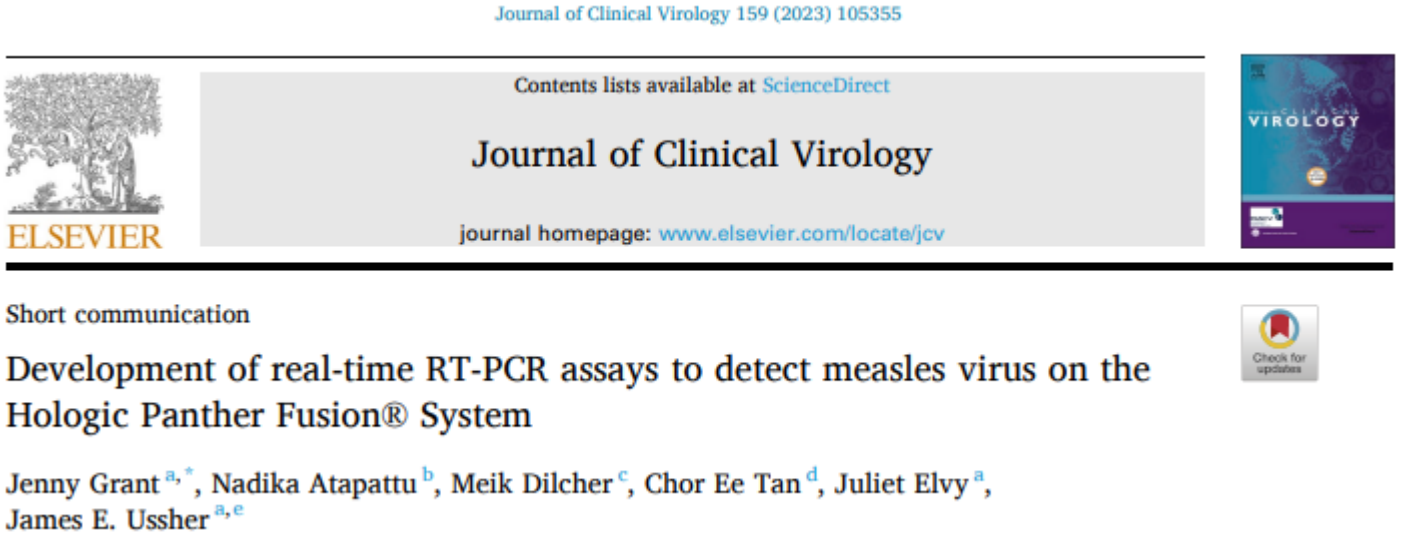
The limit of detection lies somewhere between the 10^{-4} and 10^{-5} dilution of the 0.5 McFarland Standard.

Phase 2: 3xLOD				Phase 2: 1xLOD (D from Phase 1)				Phase 2: 0xLOD			
CFU/mL	CT	Dilution	CT	Dilution	Sample ID	CFU/mL	CT	Dilution	Sample ID	CFU/mL	CT
207	36.40	1x	36.40	1x	1001	93	37.60	0x	0001	0	0.00
207	36.50	1x	36.50	1x	1002	93	39.40	0x	0002	0	0.00
207	35.20	1x	35.20	1x	1003	93	37.90	0x	0003	0	0.00
207	35.30	1x	35.30	1x	1004	93	0.00	0x	0004	0	0.00
207	35.00	1x	35.00	1x	1005	93	38.10	0x	0005	0	0.00
207	36.60	1x	36.60	1x	1006	93	40.10	0x	0006	0	0.00
207	35.20	1x	35.20	1x	1007	93	37.20	0x	0007	0	0.00
207	35.70	1x	35.70	1x	1008	93	38.50	0x	0008	0	0.00
207	35.30	1x	35.30	1x	1009	93	40.70	0x	0009	0	0.00
207	35.70	1x	35.70	1x	1010	93	42.20	0x	0010	0	0.00
207	34.40	1x	34.40	1x	1011	93	37.30	0x	0011	0	0.00
207	35.40	1x	35.40	1x	1012	93	35.30	0x	0012	0	0.00
207	35.30	1x	35.30	1x	1013	93	35.70	0x	0013	0	0.00
207	34.30	1x	34.30	1x	1014	93	35.70	0x	0014	0	0.00
207	36.10	1x	36.10	1x	1015	93	36.50	0x	0015	0	0.00
207	36.20	1x	36.20	1x	1016	93	35.90	0x	0016	0	0.00
207	36.20	1x	36.20	1x	1017	93	37.30	0x	0017	0	0.00
207	35.40	1x	35.40	1x	1018	93	35.60	0x	0018	0	0.00
207	34.70	1x	34.70	1x	1019	93	36.20	0x	0019	0	0.00
207	35.30	1x	35.30	1x	1020	93	37.20	0x	0020	0	0.00

The 1x solution corresponded to the 10^{-4} dilution with a concentration of ~93 CFU/mL, the 3x solution corresponded to the 10^{-4} dilution x 3 with a concentration of ~207 CFU/mL, and the 0x solution had a concentration of 0 CFU/mL. The concentration was determined by plating 50 ul of each solution in triplicate immediately before running PCR and taking the average of the CFUs. For the 1x solution, the CFU counts were 5, 6, and 3, averaging 4.67 CFU/50 ul, i.e., ~93 CFU/mL. For the 3x solution, the CFU counts were 13, 12, and 6, averaging 10.33 CFU/50 ul, i.e., ~207 CFU/mL. The were no CFUs for the 0x solution.

Measles Vaccine Strain Validation

- Measles real-time RT-PCR protocol1 and the real-time RT-PCR assay to detect Measles on the Hologic Panther Fusion System2 by Grant J, Atapattu N, Dilcher M, Tan CE, Elvy J, Ussher JE. Development of real-time RT-PCR assays to detect measles virus on the Hologic Panther Fusion System. *J Clin Virol*. 2023;159:105355. doi:10.1016/j.jcv.2022.105355



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Short communication

Development of real-time RT-PCR assays to detect measles virus on the Hologic Panther Fusion® System

Jenny Grant [a,*], Nadika Atapattu [b], Meik Dilcher [c], Chor Ee Tan [d], Juliet Elvy [a], James E. Ussher [a,e]

1.3 WORKPLAN

Item	Run 1 (A)	Run 2 (B)	Run 3 (C)	Total
Day	1	2	2	2
Instrument: Panther#	1	2	1	2
Analyst	KE	MA	MA	2
MeV Positive Throat Specimens	5	5	5	15
MeV Positive NP Specimens	5	5	5	15
MeV Positive Urine Specimens	5	5	5	15
Measles Vaccine Specimens	5	5	5	15
Negative Specimens	5	5	5	15
MeV Positive Control	1	1	1	3
Negative Control	1	1	1	3
Specificity – Flu A	1	1	1	3
Specificity – Flu B	1	1	1	3
Specificity – Mumps	1	1	1	3

3.1.1 Table for Acceptance Criteria

ID	Detail	Acceptance	Evaluation Pass / Fail
1.	5 MeV Positive Throat Specimens	90% concordance with CDC method (if not 100%, add report detail on discordance)	PASS
2.	5 MeV Positive NP Specimens	90% concordance with CDC method (if not 100%, add report detail on discordance)	PASS
3.	5 MeV Positive Urine Specimens	90% concordance with CDC method (if not 100%, add report detail on discordance)	PASS
4.	5 Measles Vaccine Specimens	90% concordance with CDC method (if not 100%, add report detail on discordance)	PASS
5.	5 Negative Specimens	90% concordance with LRN method (if not 100%, add report detail on discordance)	PASS
6.	Positive control	100%	PASS
7.	Panther1 vs Panther2	Within 3 Ct values (Evaluate %RSD for Ct values)	PASS
8.	Specificity Sample Flu A	Negative for Measles	PASS
9.	Specificity Sample Flu B	Negative for Measles	PASS
10.	Specificity Sample Mumps	Negative for Measles	PASS

3.2.1 Between Instrument Accuracy

Panther 1 (C) vs Panther 2 (B) - Accuracy					
Specimen Type	OE #	Expected FAM	Observed FAM Run C - Panther 1	Observed FAM Run B - Panther 2	Accuracy
MMR Positive Control	-	POS	POS	POS	PASS
PBS Negative Control	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
MeV Throat Swab	2641880	POS	POS	POS	PASS
MeV Throat Swab	2641880	POS	POS	POS	PASS
MeV Throat Swab	2651401	POS	POS	POS	PASS
MeV Throat Swab	2651401	POS	POS	POS	PASS
MeV Throat Swab	2651401	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeVac PT	2612439	POS	POS	POS	PASS
MeVac Throat Swab	2615922	POS	POS	POS	PASS
MeVac Throat Swab	2616356	POS	POS	POS	PASS
MeVac Nasopharyngeal Swab	2634539	POS	POS	POS	PASS
MeVac Throat Swab	2635212	POS	POS	POS	PASS
Flu A Nasopharyngeal Swab	2608148	neg	neg	neg	PASS
Flu B Nasopharyngeal Swab	2484149	neg	neg	neg	PASS
Mumps PT	2612441	neg	neg	neg	PASS

3.2.2 Between Days Accuracy

Day 1: 6-17-2025 (A) vs Day 2: 6-18-2025 (C) - Accuracy					
Specimen Type	OE #	Expected FAM	Observed FAM Run A - Day 1	Observed FAM Run C - Day 2	Accuracy
MMR Positive Control	-	POS	POS	POS	PASS
PBS Negative Control	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
Negative Specimen	-	neg	neg	neg	PASS
MeV Throat Swab	2641880	POS	POS	POS	PASS
MeV Throat Swab	2641880	POS	POS	POS	PASS
MeV Throat Swab	2651401	POS	POS	POS	PASS
MeV Throat Swab	2651401	POS	POS	POS	PASS
MeV Throat Swab	2651401	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Nasopharyngeal Swab	2641879	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeV Urine	2641881	POS	POS	POS	PASS
MeVac PT	2612439	POS	POS	POS	PASS
MeVac Throat Swab	2615922	POS	POS	POS	PASS
MeVac Throat Swab	2616356	POS	POS	POS	PASS
MeVac Nasopharyngeal Swab	2634539	POS	POS	POS	PASS
MeVac Throat Swab	2635212	POS	POS	POS	PASS
Flu A Nasopharyngeal Swab	2608148	neg	neg	neg	PASS
Flu B Nasopharyngeal Swab	2484149	neg	neg	neg	PASS
Mumps PT	2612441	neg	neg	neg	PASS

Day 1 (A) vs Day 2 (C) - Precision				
Specimen Type	OE #	FAM CT - Run A Day 1	FAM CT - Run C Day 2	Precision: Within 3 CT
MMR Positive Control	-	25.4	25.5	PASS
PBS Negative Control	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
Negative Specimen	-	0	0	PASS
MeV Throat Swab	2641880	27.4	27.8	PASS
MeV Throat Swab	2641880	27.8	27.5	PASS
MeV Throat Swab	2651401	28.6	27.1	PASS
MeV Throat Swab	2651401	29.1	28.3	PASS
MeV Throat Swab	2651401	29	28.8	PASS
MeV Nasopharyngeal Swab	2641879	28.5	28.5	PASS
MeV Nasopharyngeal Swab	2641879	28.5	28.3	PASS
MeV Nasopharyngeal Swab	2641879	28.7	28.3	PASS
MeV Nasopharyngeal Swab	2641879	29.4	28.6	PASS
MeV Nasopharyngeal Swab	2641879	28.5	28.6	PASS
MeV Urine	2641881	28.1	28.1	PASS
MeV Urine	2641881	28	27.6	PASS
MeV Urine	2641881	28.7	27.5	PASS
MeV Urine	2641881	28.7	27.7	PASS
MeV Urine	2641881	28.6	28.1	PASS
MeVac PT	2612439	30.2	29.9	PASS
MeVac Throat Swab	2615922	32.6	32.3	PASS
MeVac Throat Swab	2616356	36.1	35.5	PASS
MeVac Nasopharyngeal Swab	2634539	27.4	31.6	FAIL
MeVac Throat Swab	2635212	28.6	28.8	PASS
Flu A Nasopharyngeal Swab	2608148	0	0	PASS
Flu B Nasopharyngeal Swab	2484149	0	0	PASS
Mumps PT	2612441	0	0	PASS

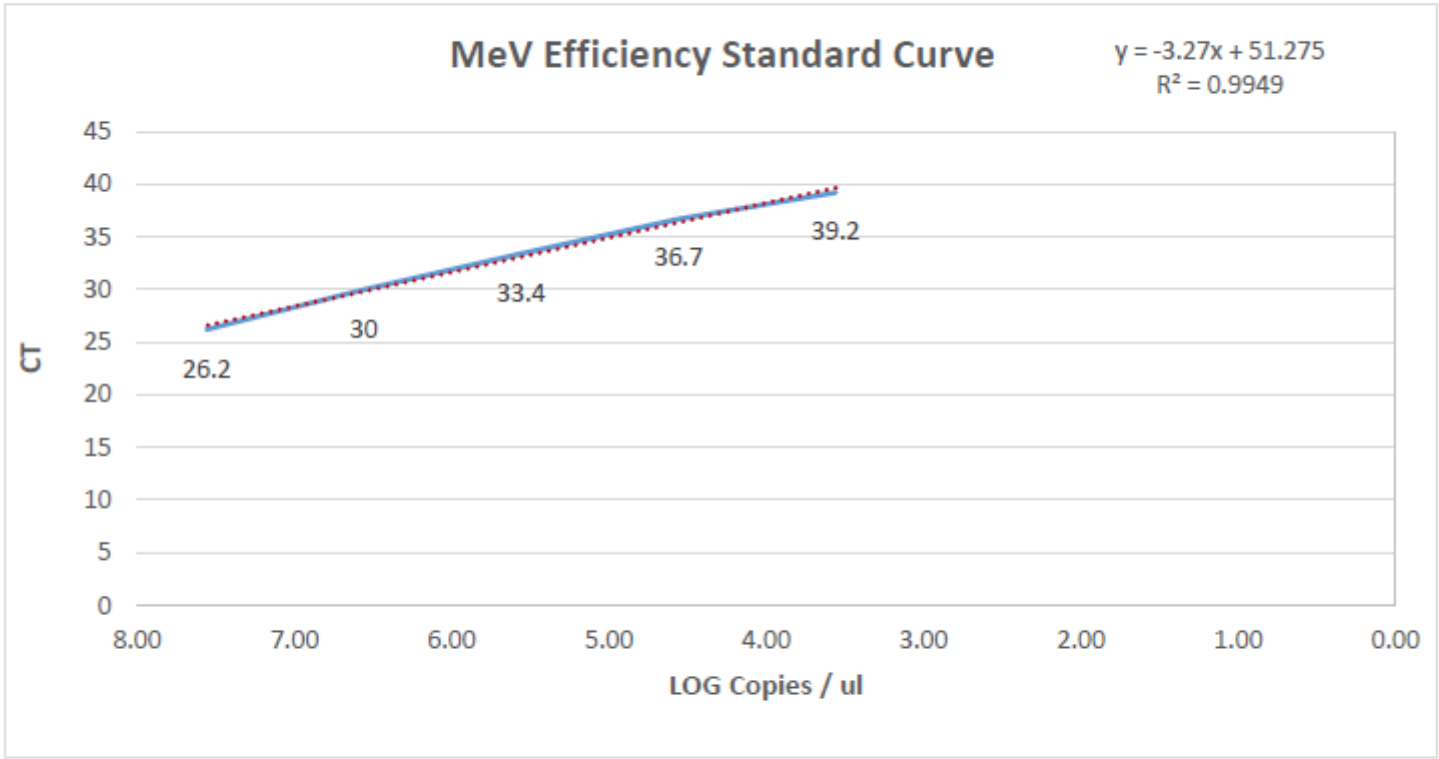
- LOD Phase 1 - 1:10 dilution series of gBlock positive control from IDT.

LOD Phase 1: 10-fold Dilution Series of gBlock				
Dilution	Sample ID	Copies / ul	Log Copies / ul	MeV CT
Diluent A	DilA	36,144,044	7.56	26.2
Diluent B	DilB	3,614,404	6.56	30
B^-1	0B-1	361,440	5.56	33.4
B^-2	0B-2	36,144	4.56	36.7
B^-3	0B-3	3,614	3.56	39.2
B^-4	0B-4	361	2.56	0
B^-5	0B-5	36	1.56	0
B^-6	0B-6	3	0.48	0

- LOD Phase 2 – 0x, 1x, and 3xLOD Replicates.

LOD Phase 2: 3xLOD				LOD Phase 2: 1xLOD				LOD Phase 2: 0xLOD			
Dilution	Sample ID	Copies / ul	MeV CT	Dilution	Sample ID	Copies / ul	MeV CT	Dilution	Sample ID	Copies / ul	MeV CT
3x	3x01	108,432	35.40	1x	1x01	36,144	36.80	0x	0x01	0	0
3x	3x02	108,432	34.90	1x	1x02	36,144	37.30	0x	0x02	0	0
3x	3x03	108,432	35.50	1x	1x03	36,144	36.50	0x	0x03	0	0
3x	3x04	108,432	35.70	1x	1x04	36,144	36.20	0x	0x04	0	0
3x	3x05	108,432	35.10	1x	1x05	36,144	35.50	0x	0x05	0	0
3x	3x06	108,432	34.50	1x	1x06	36,144	35.80	0x	0x06	0	0
3x	3x07	108,432	35.00	1x	1x07	36,144	35.50	0x	0x07	0	0
3x	3x08	108,432	35.20	1x	1x08	36,144	37.10	0x	0x08	0	0
3x	3x09	108,432	35.10	1x	1x09	36,144	37.00	0x	0x09	0	0
3x	3x10	108,432	35.10	1x	1x10	36,144	36.60	0x	0x10	0	0
3x	3x11	108,432	34.80	1x	1x11	36,144	36.60	0x	0x11	0	0
3x	3x12	108,432	34.90	1x	1x12	36,144	36.80	0x	0x12	0	0
3x	3x13	108,432	34.70	1x	1x13	36,144	37.30	0x	0x13	0	0
3x	3x14	108,432	35.40	1x	1x14	36,144	37.80	0x	0x14	0	0
3x	3x15	108,432	34.90	1x	1x15	36,144	37.30	0x	0x15	0	0
3x	3x16	108,432	35.30	1x	1x16	36,144	36.10	0x	0x16	0	0
3x	3x17	108,432	34.80	1x	1x17	36,144	37.50	0x	0x17	0	0
3x	3x18	108,432	34.70	1x	1x18	36,144	37.20	0x	0x18	0	0
3x	3x19	108,432	35.40	1x	1x19	36,144	36.80	0x	0x19	0	0
3x	3x20	108,432	34.60	1x	1x20	36,144	35.70	0x	0x20	0	0

- PCR Efficiency



PCR Efficiency	2.02213617
% PCR Efficiency	102.213617

This assay demonstrated a reasonable PCR efficiency of 102.21%.

Conclusion

- Each validation plan follows a standard format but is unique to the pathogen
- Costs vary but range between $7,500-$10,000 when considering reagents and staff time
- Future directions:
  - Flu A H5b and H7 validation in progress
  - Add Treponema pallidum to the HSV 1&2/VZV

Questions?