



Reduction of AgPath Reagent Used in FMDV Multiplex Real-Time TaqMan Assay

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AAHL Molecular Diagnostic Service

- Real-Time TaqMan PCR is a frontline molecular test for detecting viral pathogens
- AgPath reagent kit is the kit of choice at Molecular Diagnostic at AAHL (AUD2,325/1,000 Rxns)
- Increasing number of TaqMan PCR assays requested for disease confirmation and disease exclusion
 - 2014 - 30,718 reactions
 - 2015 - 36,119 reactions
 - 2016 - 38,035 reactions
 - 2017 - 48,029 reactions
 - 2018 - 50,695 reactions
- Prepare for any future outbreak of exotic diseases

Objectives

- 1. To compare Ct values generated from**
 - a standard FMDV TaqMan assay (1.0X, total volume 25 μ L)
 - a modified FMDV TaqMan assay (0.6X, total volume 15 μ L)
- 2. To determine the feasibility of adoption of 0.6X assay as the new standard for FMDV TaqMan PCR assay**

As Australia is FMD-free, testing was conducted at the RRL in Pakchong, Thailand

FMDV Real-Time TaqMan Reaction

Component	1X	0.6X
Nuclease-free water	4.2	0.5
2X RT-Buffer	12.5	7.5
25X RT Enzyme	1.0	0.6
Primer-Probe MIX	2.3	1.4
RNA Template	5.0	5.0
TOTAL	25 μ l	15 μ l

40% reduction of reagent and primer-probe mix

Methods

1. Individual comparison

➤ 120 samples

- 77 FMDV infected samples (OIE-RRL, Pakchong)
- 43 exclusion samples (AAHL, Geelong)

2. Repeatability test

➤ Two sets (a and b) of 11 identical samples

- Set “a” samples were tested at day 1 and
- Set “b” samples were tested at day 3 after being stored at -80C

3. Limit of detection test

➤ Two FMDV-infected samples

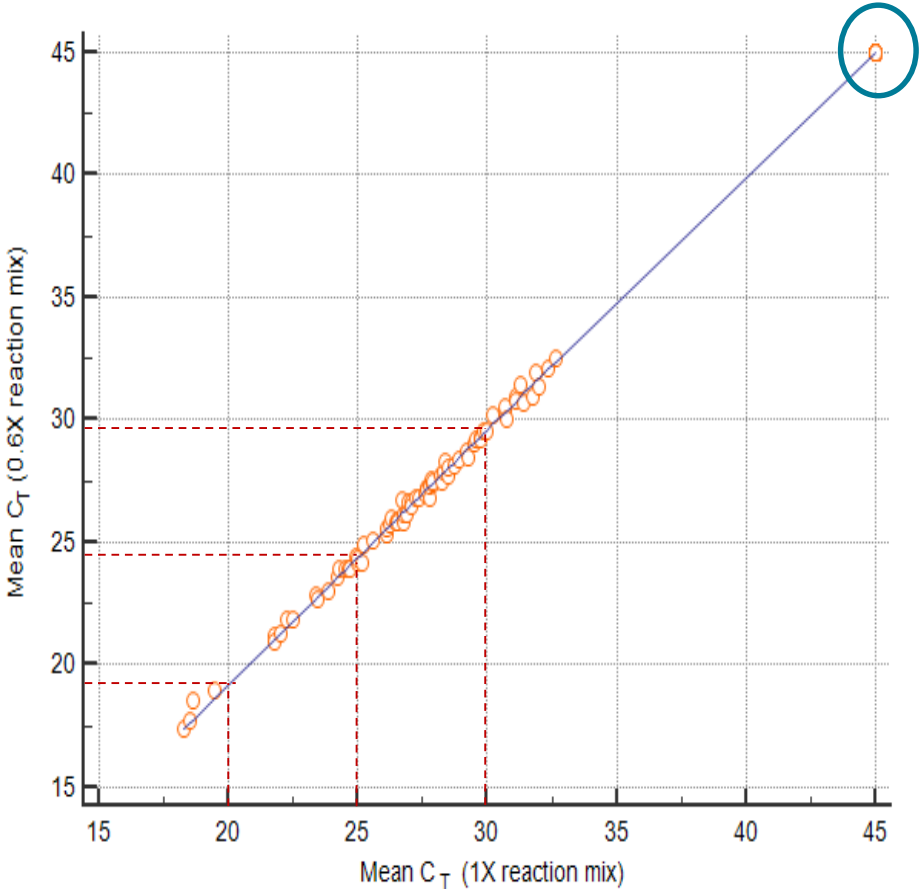
- strong positive ($Ct < 25$) and
- moderate positive ($Ct > 25$)

Results: Individual Comparison

- Tested in duplicate format for both standard 1X and 0.6X FMDV assays
- All 43 samples submitted for exclusion testing were negative in both assays
- All 77 FMDV positive samples (OIE-RRL Pakchong) were positive in both assays
- Ct value ranges were between 18.12 and 33.86
- Of 77 samples
 - 75 samples gave mean Ct values lower in 0.6X than 1X standard assay
 - 2 samples gave mean Ct values higher in 0.6X than 1X standard assay

sample	FMDV 1X		FMDV 0.6X	
	1	2	1	2
11a	31.84	31.91	31.91	32.02
36a	31.35	31.24	31.45	31.46

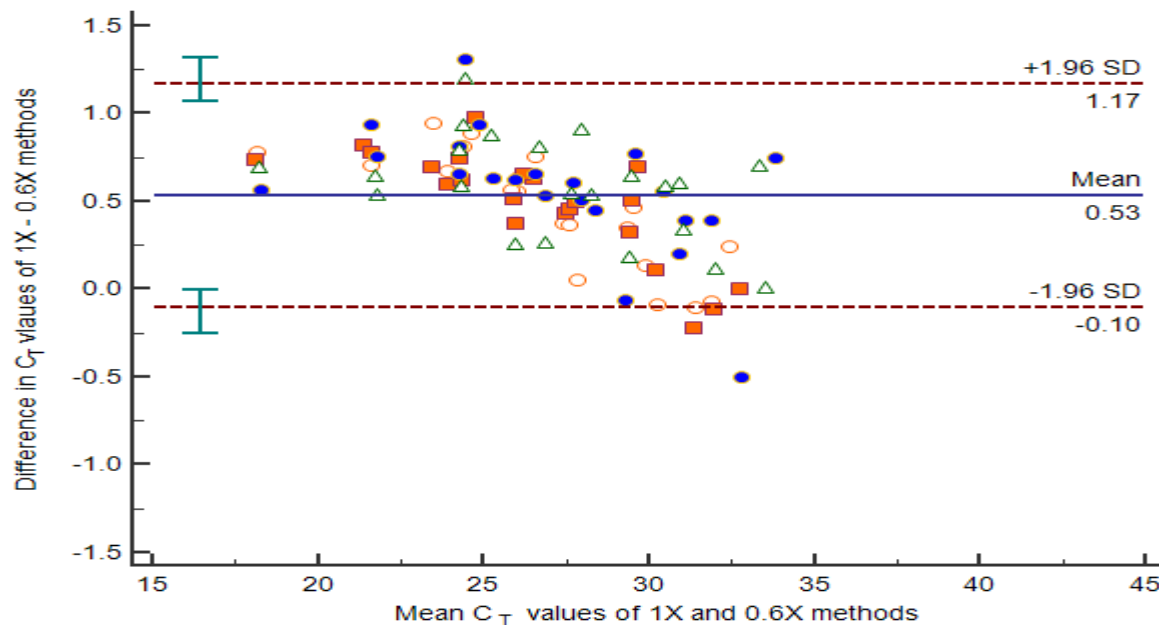
Scatter Plot comparing Ct values of 120 samples by the two methods (77 Positives and 43 Negatives)



43 negative samples

Repeatability of 1X and 0.6X Assays

- 22 samples were run four times on two different occasions
- The Ct values were compared for repeatability by Bland-Altman plot (1.96*SD with 95% CI)
- Both the assays have high repeatability



Results: Limit of Detection

- LoD was determined using a standard curve obtained using the Ct values of FMDV RNA standards.
- Two FMDV positive RNA samples (Low Ct and Moderate Ct) were diluted and run to compare the Ct values.

Copy numbers/ml	Strong Positive Sample					
	1X Reaction Mix			0.6X Reaction Mix		
	R1	R2	R3	R1	R2	R3
1.56E+07	19.09	19.20	19.09	18.34	18.24	18.32
1.56E+06	24.18	24.14	24.19	23.49	23.49	23.46
1.56E+05	27.02	26.98	27.02	26.30	26.36	26.36
1.56E+04	29.00	29.00	29.01	28.31	28.31	28.38
1.56E+03	31.47	31.62	31.50	31.04	30.97	31.10
1.56E+02	33.59	33.10	33.40	33.18	33.25	33.08
1.56E+01	45.00	45.00	34.98	45.00	34.22	34.47

Copy numbers/ml	Moderate Positive Sample					
	1X Reaction Mix			0.6X Reaction Mix		
	R1	R2	R3	R1	R2	R3
2.98E+04	27.57	27.53	27.56	27.05	27.08	27.02
2.98E+03	30.81	30.85	30.84	30.59	30.59	30.51
2.98E+02	32.13	32.14	32.05	31.92	31.78	31.64
2.98E+01	35.23	45.00	34.28	34.54	35.47	34.79

Conclusions

- **The FMDV reduced volume (0.6X)**
 - showed similar or marginally lower Ct values compared to the standard method
 - showed no loss in sensitivity compared to the standard method
 - has been routinely used in the Molecular Diagnostic Service since 2018
 - helps reduction of reagent and primer-probe mix by 40%
- **The reduced 0.6X method has also been applied to several other TaqMan assays such as internal control, influenza virus, Bluetongue virus, CSFV and recently ASFV assays**

Thank you



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