

Bacteriological Analytical Manual (BAM) 19b: Molecular Detection of *Cyclospora cayetanensis* in Fresh Produce Using Real-Time PCR

Method modification due to the discontinuation of the current/original QuantiFast Multiplex PCR kit and replacement with TaqMan™ Fast Advanced Master Mix

Verification Study

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1. Background and Overview of the Verification Study.

The validated qPCR method for detecting *Cyclospora cayetanensis* in food and water uses the Qiagen QuantiFast Multiplex PCR Kit. This kit will be discontinued by the manufacturer, Qiagen. The QuantiNova Multiplex PCR kit was suggested by Qiagen as a replacement kit. However, use of the suggested replacement kit resulted in cross-reactivity with *Eimeria* species and *Toxoplasma gondii*. A new replacement kit was advised, the TaqMan™ Fast Advanced Master Mix manufactured by Applied Biosystems. Analyses revealed only subtle differences in performance between the two kits using the recommended cycling parameters.

The purpose of this verification study was to determine if the replacement TaqMan™ Fast Advanced Master Mix is equivalent in performance to the current/original QuantiFast Multiplex PCR Kit in food.

According to the FDA Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, Edition 3.0 section 5.0, a verification study “against the original method according to criteria detailed in Section 4.2.1.1” is needed for this method modification, due to the availability/substitution of reagents.

Previous verification study protocols for *C. cayetanensis* proficiency tests for ORA labs, required the analysis of six replicates (per lab), which included two un-inoculated matrix replicates, and four replicates of the inoculated matrix [two replicates at low seeding level (5-10 oocysts) and two replicates at high seeding level (200 oocysts)]. In the present verification study, three different commodities/matrices (e.g. romaine lettuce, salsa/pico de gallo and blackberries) were included for comparison. Each commodity/matrix tested included replicates from un-inoculated (four samples) and inoculated samples [six replicates at low seeding level (5-10 oocysts) and four replicates at high seeding level (200 oocysts)]. Both the TaqMan™ Fast Advanced Master Mix and the QuantiFast Multiplex PCR were tested and compared.

2. Methodology for the evaluation of the method modification of the BAM Chapter 19b method due to the substitution of the current/original QuantiFast Multiplex PCR kit by the replacement TaqMan™ Fast Advanced Master Mix.

The present verification study was performed using DNA extracts from seeded and unseeded samples used in previous matrix extension studies for *C. cayetanensis* detection following the BAM chapter 19b method (1). The real time qPCR amplification was compared using the new TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit. Each run included a negative control (NTC) and a positive control (gBlock135m). Each control and experimental sample were run in triplicate on the ABI 7500 Fast Instrument. The software analysis settings applied were the same as described in the BAM chapter 19b: i.e., manual threshold values and manual baseline settings. The reaction setup and cycling conditions for the QuantiFast Multiplex PCR was the same as described in the BAM Chapter 19b. The modifications required for the TaqMan™ Fast Advanced Master Mix are indicated below:

1. The replacement TaqMan™ Fast Advanced Master Mix uses an initial activation step of 95°C for 2 minutes. The current/original QuantiFast Multiplex kit has an initial activation step of 95°C for 5 minutes.
2. The replacement TaqMan™ Fast Advanced Master Mix is stored at 4°C.

In both, the replacement TaqMan™ Fast Advanced Master Mix and in the current/original QuantiFast Multiplex PCR kit used in the BAM chapter 19b, the master mix includes the ROX Reference Dye in the commercial mix. Both, the replacement TaqMan™ Fast Advanced Master Mix and the current/original

QuantiFast Multiplex kit uses a 2X master mix. Primer and probe concentrations, as well as annealing/extension temperatures, remained the same as those indicated in the BAM Chapter 19b.

As a first step, a previously used exclusivity panel comprised of 11 parasite species (*Giardia intestinalis*, *Blastocystis hominis*, *Cryptosporidium parvum*, *Cryptosporidium hominis*, *Entamoeba histolytica*, *Plasmodium falciparum*, *Trypanosoma cruzi*, *Eimeria acervulina*, *Eimeria tenella*, *Eimeria maxima* and *Toxoplasma gondii*) (2), and additional parasite species not included in that panel (*Cyclospora macacae*, *Cyclospora papionis*, *Entamoeba dispar A*, *Entamoeba dispar B*, *Plasmodium vivax*, *Entamoeba invadens* and *Neospora caninum*) were used to evaluate the replacement TaqMan™ Fast Advanced Master Mix. The same threshold and cycle conditions were followed as those indicated in the BAM Chapter 19b.

In a second step, the assay performance of the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit was assessed and compared over serial dilutions of the positive control target separately for each kit (generation of standard curves) (3). Performance characteristics evaluated included assay specificity, repeatability, linearity, sensitivity, and efficiency.

In a third step, the DNA extracts from spiked and unspiked romaine lettuce, salsa/pico de gallo and blackberries (used as matrices for previous validated matrix extension studies for *C. cayetanensis*) were used as templates for the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit. Results were compared.

3. Results.

3.1. Exclusivity panel analysis.

A previously used exclusivity panel comprised of 11 parasite species (2) and several other species were used to evaluate the replacement TaqMan™ Fast Advanced Master Mix. The same threshold and cycle conditions were followed as those indicated in the BAM Chapter 19b. No cross-reactivity was observed with any of the protozoan parasites: *Cyclospora macacae*, *Cyclospora papionis*, *Giardia intestinalis*, *Blastocystis hominis*, *Cryptosporidium parvum*, *Cryptosporidium hominis*, *Entamoeba histolytica*, *Entamoeba dispar A*, *Entamoeba dispar B*, *Plasmodium falciparum*, *Plasmodium vivax*, *Entamoeba invadens*, *Trypanosoma cruzi*, *Eimeria acervulina*, *Eimeria tenella*, *Eimeria maxima*, *Toxoplasma gondii* and *Neospora caninum*.

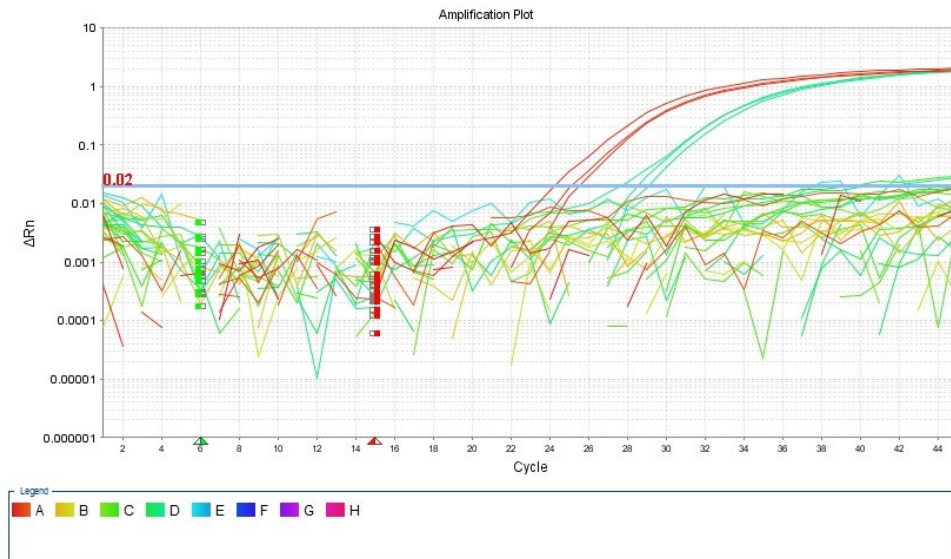


Figure 1. Amplification plot for the exclusivity panel of representative protozoan parasites for specific amplification of the *C. cayetanensis* 18S rRNA gene using the replacement TaqMan™ Fast Advanced Master Mix. The red line represents the positive control (synthetic standard of 1000 copies *C. cayetanensis* 18S rRNA gene). The light blue (cyan) line represents spiked blackberry samples (seeded with 200 oocysts *C. cayetanensis*). All other parasite species analyzed did not produce amplicons.

3.2. Assessment of reagent performance for the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit.

Reagent specificity, repeatability, linearity, sensitivity, and efficiency of each qPCR performance for the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit was assessed independently by amplification of serial dilutions from 10^4 to one copy of *C. cayetanensis* positive control to generate separate standard curves for each kit (Figure 2).

Baseline cycles and threshold manual values targeting the *C. cayetanensis* 18S rRNA gene and for the Internal Amplification Control (IAC) target were those indicated in the BAM chapter 19b for both kits (Baseline cycles: 6 to 15 and threshold manual values of 0.02 for *C. cayetanensis* 18S rRNA target, and baseline cycles: 6 to 15 and threshold manual values of 0.01 for IAC target). A linear relationship between target concentration and C_T values over six orders of magnitude was seen for both the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit, the *C. cayetanensis* 18S rRNA gene; the correlation coefficient was $R^2 > 0.99$ (Figure 2). Efficiencies were 98.6% for the replacement TaqMan™ Fast Advanced Master Mix and 106% for the current/original QuantiFast Multiplex PCR kit.

C_T values were not determined for the non-template control (NTC) reactions. Similar C_T values were observed for triplicates for each copy number of target gene (Table 1); additionally, both kits showed a sensitivity of one copy of template as the lowest template concentration detectable with a reliable C_T value. The C_T value for the lowest copy number of template detected was $C_T = 36.1$ for the replacement TaqMan™ Fast Advanced Master Mix and $C_T = 37.5$ for the current/original QuantiFast kit (Figure 1). Based on the performance of the internal amplification control (IAC), no inhibited qPCR reactions were identified in any of the kits (Table 1).

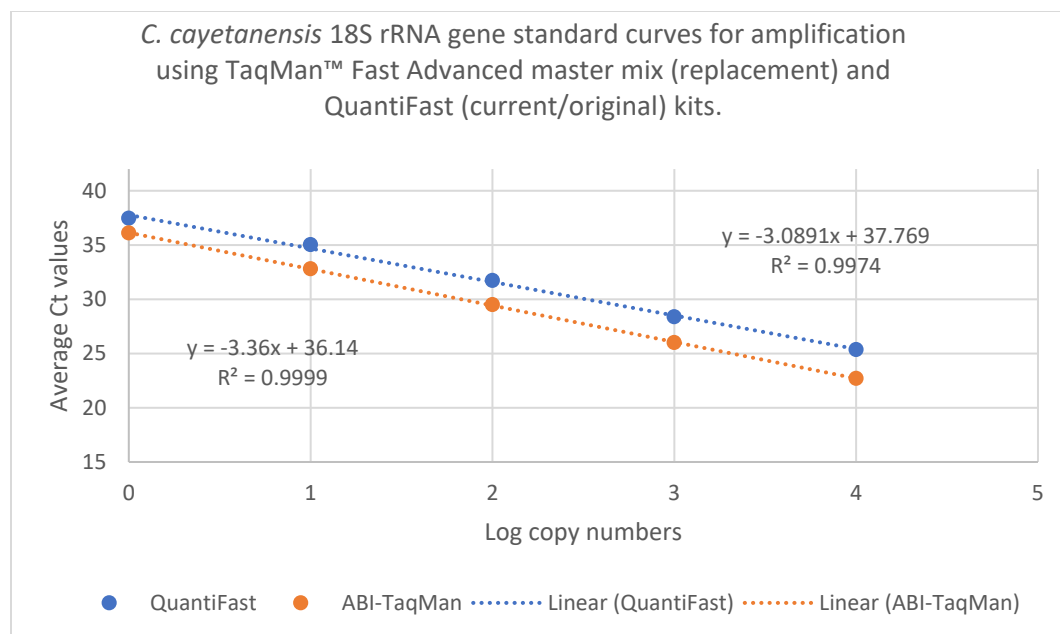


Figure 2. Comparison of the linear regression for detection of the *Cyclospora cayetanensis* 18S rRNA gene using the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit. Log DNA copies: 0=log 1 copy number; 1=log 10 copy numbers; 3=log 100 copy numbers, 4=log 10E3 copy numbers; 5=log 10E4 copy numbers.

Table 1. qPCR data from standard curve analysis using the replacement TaqMan™ Fast Advanced Master Mix and using the current QuantiFast Multiplex PCR kit.

TaqMan™ Fast Advanced Master Mix (replacement)				QuantiFast Multiplex PCR Kit (current/original)			
DNA copy number	18S sRNA		IAC*	DNA copy number	18S sRNA		IAC*
	No. positive replicates [#]	Mean C _T value± sd	Mean C _T value± sd		No. positive replicates [#]	Mean C _T value± sd	Mean C _T value± sd
10000	3	22.7±0.1	27.6±0.7	10000	3	25.4±0.0	25.3±0.2
1000	3	26.0±0.1	27.2±0.2	1000	3	28.4±0.1	25.0±0.4
100	3	29.5±0.3	26.8±0.3	100	3	31.7±0.2	24.9±0.4
10	3	32.8±0.4	24.6±0.2	10	3	35.0±0.9	24.5±0.4
1	1	36.1 [§]	25.2±1.0	1	1	37.5 [§]	24.7±0.3
NTC	3	Und**	25.6±0.7	NTC	0	Und**	24.6±0.4

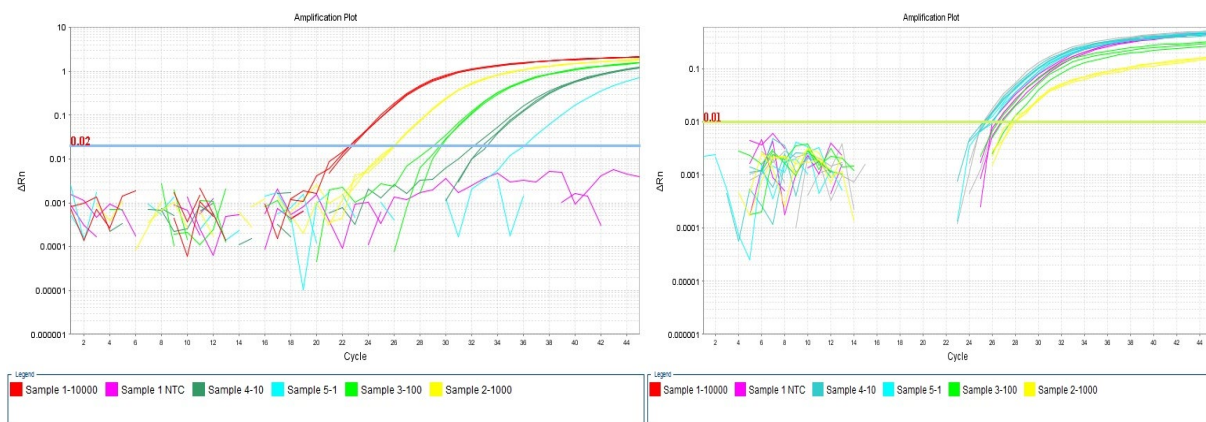
* All positive Internal Amplification Control (IAC) qPCR reactions (out of 3 replicates)

Out of 3 replicates by DNA copy number

** Und: Not detected

§ Standard deviation not indicated if only one replicate was positive.

Figure 3 shows amplification plots for the *C. cayetanensis* 18S rRNA gene and IAC on serial dilutions (from 10^4 to one copy of *C. cayetanensis*) of DNA copies. Amplification plots were independently assessed for the replacement TaqMan™ Fast Advanced Master Mix and for the current/original QuantiFast kit.



TaqMan™ Fast (replacement) 18S rRNA amplification B) TaqMan™ Fast (replacement) IAC amplification

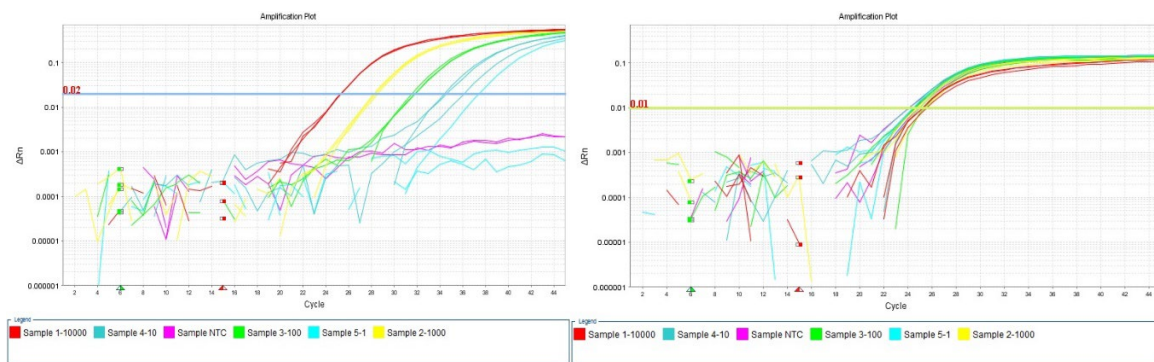


Figure 3. Amplification plots for the *C. cayetanensis* 18S rRNA gene using the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit (3A and 3C) and for the IAC control using the replacement TaqMan™ Fast Advanced Master Mix and the current/original QuantiFast Multiplex PCR kit (3B and 3D). Amplification plots colors: A (red)=

10,000 copy numbers; B (orange)=1,000 copy numbers; C (light green)=100 copy numbers, D (dark green)=100 copy numbers; E (light blue)= 10 copy numbers; F (dark blue)= 1 copy number, G (purple)=NTC.

3.2. Verification of the replacement TaqMan™ Fast Advanced Master Mix compared to the current/original QuantiFast Multiplex PCR kit using DNA extracts from selected samples from previously validated matrix extensions.

3.2.1. Verification in romaine lettuce.

Table 2 shows a summary of the results in romaine lettuce obtained for the verification evaluation using the replacement TaqMan™ Fast Advanced Master Mix compared to the current/original QuantiFast Multiplex PCR kit. The detection rate for romaine lettuce samples seeded with 5 oocysts (the only seeded low level performed in the matrix extension for this commodity) was 83.3% for both kits. All romaine lettuce samples seeded with 200 oocysts were positive, and all unseeded romaine lettuce samples were negative. No inhibited qPCR reactions were identified based on the performance of the internal amplification control (IAC) in any of the kits. See Table 3 for detailed qPCR detection data including the number of positive qPCR replicates and C_T values for the *C. cayetanensis* and IAC targets for each sample.

Results for detection of *C. cayetanensis* in romaine lettuce using the replacement TaqMan™ Fast Advanced Master Mix were equivalent in performance to the current/original QuantiFast Multiplex PCR Kit, with 5 *C. cayetanensis* oocysts identified as the limit of detection.

Table 2. Summary of verification evaluation results for the replacement TaqMan™ Fast Advanced Master Mix compared to QuantiFast Multiplex PCR kit based on the BAM chapter 19b method for romaine lettuce.

Matrix	TaqMan™ Fast Advanced Master Mix (replacement)			QuantiFast (current/original) Multiplex PCR kit		
	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)
Romaine Lettuce	0	4	0 (0.0)	0	4	0 (0.0)
	5	6	5 (83.3)	5	6	5 (83.3)
	200	4	4 (100.0)	200	4	4 (100.0)

Table 3. Romaine lettuce qPCR data for the verification evaluation for the replacement TaqMan™ Fast Advanced Master Mix compared to the current QuantiFast Multiplex PCR kit using baseline and threshold conditions indicated in the BAM chapter 19b.

Romaine lettuce							
TaqMan™ Fast Advanced Master Mix				QuantiFast Multiplex PCR kit			
No. of oocysts seeded	18S sRNA		IAC*	No. of oocysts seeded	18S sRNA		IAC*
	No. positive replicates	Mean C _T value± sd	Mean C _T value± sd		No. positive replicates	Mean C _T value± sd	Mean C _T value± sd
0	0	Und**	24.9±0.4	0	0	Und**	25.0±0.4
0	0	Und	24.9±1.3	0	0	Und	25.1±0.0
0	0	Und	24.7±1.4	0	0	Und	24.8±0.2
0	0	Und	23.8±1.5	0	0	Und	24.7±0.0
5	1	35.0 [§]	24.4±0.6	5	2	36.9±0.6	24.4±0.2
5	3	33.1±0.9	23.8±0.6	5	2	36.1±0.1	24.6±0.2
5	1	34.7 [§]	23.9±0.5	5	2	35.9±0.4	24.5±0.3
5	3	34.9±0.8	23.0±0.3	5	2	36.0±0.4	24.6±0.2
5	1	37.3 [§]	23.0±0.1	5	1	36.3 [§]	24.8±0.3
5	0	Und ^{&}	21.5±0.3	5	1	Und ^{&}	24.8±0.2
200	3	29.6±0.4	22.4±0.4	200	3	32.8±0.7	25.5±0.5
200	3	30.3±0.6	22.3±0.6	200	3	33.4±0.4	25.5±0.4
200	3	29.2±0.3	22.3±0.5	200	3	32.1±0.1	25.0±0.0
200	3	28.9±0.2	21.7±0.2	200	3	31.6±0.0	25.6±0.4

* All positive IAC qPCR reactions (out of 3 replicates)

** Und: Not detected

§ Standard deviation not indicated if only one replicate positive.

& Undetermined (not detected) when DNA diluted 1/4

3.2.2. Verification in salsa/pico de gallo.

Table 4 shows a summary of the results obtained in salsa/pico de gallo for verification evaluation of the replacement TaqMan™ Fast Advanced Master Mix compared to the current QuantiFast Multiplex PCR kit,

. The detection rate for the salsa/pico de gallo samples seeded with 5 oocysts or 10 oocysts was 100% and 83.3% respectively for the replacement TaqMan™ Fast Advanced Master Mix and the current QuantiFast Multiplex PCR kit. Salsa/pico de gallo samples seeded with 200 oocysts were all positive; all unseeded salsa/pico de gallo samples were negative. No inhibited qPCR reactions were identified based on the performance of the internal amplification control (IAC) for either kit. See Table 5 for detailed qPCR detection data including the number of positive qPCR replicates and C_T values for the *C. cayetanensis* and IAC targets for each sample.

Results for detection of *C. cayetanensis* in salsa/pico de gallo using the replacement TaqMan™ Fast Advanced Master Mix were equivalent (or even superior) in performance to the current/original QuantiFast Multiplex PCR Kit, with 5 *C. cayetanensis* oocysts identified as the limit of detection.

Table 4. Summary of verification evaluation results for the replacement TaqMan™ Fast Advanced Master Mix compared to the current QuantiFast Multiplex PCR kit based on the BAM chapter 19b method in salsa/pico de gallo.

Matrix	TaqMan™ Fast Advanced Master Mix (replacement)			QuantiFast (current/original) Multiplex PCR kit		
	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)
Salsa/pico de gallo	0	4	0 (0.0)	0	4	0 (0.0)
	5-10	6	6 (100.0)	5	6	5 (83.3)
	200	4	4 (100.0)	200	4	4 (100.0)

Table 5. Salsa/pico de gallo qPCR data for the verification evaluation by the replacement TaqMan™ Fast Advanced Master Mix compared to the current/original QuantiFast Multiplex PCR kit using baseline and threshold conditions indicated in the BAM chapter 19b.

Salsa/pico de gallo							
TaqMan™ Fast Advanced Master Mix (replacement)				QuantiFast Multiplex PCR kit (current/original)			
No. of oocysts seeded	18S sRNA		IAC*	No. of oocysts seeded	18S sRNA		IAC*
	No. positive replicates	Mean C _T value± sd	Mean C _T value± sd		No. positive replicates	Mean C _T value± sd	Mean C _T value± sd
0	0	Und**	24.6±0.3	0	0	Und**	25.1±0.3

0	0	Und	24.7±1.1	0	0	Und	24.8± 0.1
0	0	Und	24.5±0.2	0	0	Und	24.7±0.2
0	0	Und	24.8±0.8	0	0	Und	24.5±0.2
5-10	1	35.5 [§]	23.9±1.0	5-10	1	37.3 [§]	24.6±0.2
5-10	3	33.8±0.7	24.7±0.6	5-10	3	36.6±1.1	24.6±0.1
5-10	1	32.8 [§]	24.1±0.6	5-10	3	36.4±1.1	25.6±0.4
5-10	3	31.9±0.4	25.1±0.8	5-10	0	Und ^{&}	24.1±0.0
5-10	3	34.1±1.7	23.9±0.0	5-10	3	34.2±0.4	24.9±0.2
5-10	3	34.1±0.6	23.8±0.7	5-10	1 [§]	36.4±0.7	24.4±0.1
200	3	28.5±0.5	24.4±0.4	200	3	31.7±0.6	25.1±0.3
200	3	28.3±1.3	24.4±0.8	200	3	31.5±0.2	24.8±0.1
200	3	29.9±0.3	26.2±0.9	200	3	31.8±0.2	26.2±0.2
200	3	30.2±0.2	25.2±0.3	200	3	32.6±0.3	25.5±0.2

* All positive IAC qPCR reactions (out of 3 replicates)

** Und: Not detected

§ Standard deviation not indicated if only one replicate positive.

& Undetermined (not detected) when DNA diluted 1/4

3.2.3. Verification in blackberries.

Table 6 shows a summary of the results obtained for verification evaluation in blackberries for the replacement TaqMan™ Fast Advanced Master Mix using DNA extracts compared to the current QuantiFast Multiplex PCR kit. The detection rate for the blackberry samples seeded with 5 oocysts or 10 oocysts was 100% for both the replacement TaqMan™ Fast Advanced Master Mix and the current QuantiFast Multiplex PCR kits. Blackberry samples seeded with 200 oocysts were positive and all unseeded blackberries samples were negative. No inhibited qPCR reactions were identified based on the performance of the internal amplification control (IAC) in any of the kits. See Table 7 for detailed qPCR detection data including the number of positive qPCR replicates and C_T values for the *C. cayetanensis* and IAC targets for each sample.

Results for detection of *C. cayetanensis* in blackberries using the replacement TaqMan™ Fast Advanced Master Mix were equivalent in performance to the current QuantiFast Multiplex PCR Kit, with 5 *C. cayetanensis* oocysts identified as the limit of detection.

Table 6. Summary of verification evaluation results for the replacement TaqMan™ Fast Advanced Master Mix compared to the current QuantiFast Multiplex PCR kit in blackberries.

Matrix	TaqMan™ Fast Advanced Master Mix (replacement)			QuantiFast (current/original) Multiplex PCR kit		
	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)
Blackberries	0	4	0 (0.0)	0	4	0 (0.0)
	5-10	6	6 (100.0)	5-10	6	6 (100.0)
	200	4	4 (100.0)	200	4	4 (100.0)

Table 7. Real-time qPCR data for the verification evaluation in blackberries for the replacement TaqMan™ Fast Advanced Master Mix compared to the current QuantiFast Multiplex PCR kit using baseline and threshold conditions indicated in the BAM chapter 19b.

Blackberries							
TaqMan™ Fast Advanced Master Mix (replacement)				QuantiFast Multiplex PCR kit (current/original)			
No. of oocysts seeded	18S sRNA		IAC*	No. of oocysts seeded	18S sRNA		IAC*
	No. positive replicates	Mean C _T value± sd	Mean C _T value± sd		No. positive replicates	Mean C _T value± sd	Mean C _T value± sd
0	0	Und**	26.7±0.4	0	0	Und**	25.0±0.1
0	0	Und	26.2±1.2	0	0	Und	25.5±0.1
0	0	Und	25.8±1.0	0	0	Und	24.7±0.1
0	0	Und	21.6±1.0	0	0	Und	24.9±0.1
5-10	2	34.3±0.5	23.6±1.6	5	1	37.3 [§]	24.8±0.0
5-10	3	33.9±1.2	25.1±0.7	5	3	36.6±1.1	25.0±0.1
5-10	2	32.9±0.1	23.9±1.1	5	3	35.7±0.4	24.7±0.1
5-10	2	35.2±0.1	23.0±0.2	5	2	36.9±0.2	24.6±0.1
5-10	2	34.4±0.8	23.8±1.1	5	2	35.7±0.6	24.8±0.3

5-10	3	33.9±1.7	23.8±0.2	5	2	35.2±0.8	24.9±0.4
200	3	28.9±0.3	22.5±0.7	200	3	31.1±0.1	24.8±0.0
200	3	29.5±0.1	23.8±1.2	200	3	31.2±0.2	24.8±0.2
200	3	28.3±0.0	24.1±0.4	200	3	30.9±0.3	25.3±0.2
200	3	28.4±0.5	24.7±0.3	200	3	31.2±0.2	25.4±0.0

* All positive IAC qPCR reactions (out of 3 replicates)

** Und: Not detected

§ Standard deviation not indicated if only one replicate positive.

Table 8. Summary of verification evaluation results for the replacement TaqMan™ Fast Advanced Master Mix compared to the current QuantiFast Multiplex PCR kit in the total samples analyzed from three commodities.

Matrix	TaqMan™ Fast Advanced Master Mix (replacement)			QuantiFast Multiplex PCR kit (current/original)		
	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)	No. of oocysts seeded	No. of Samples tested	No. of samples positive by qPCR (%)
Romaine lettuce, salsa/pico de gallo and blackberries	0	12	0 (0.0)	0	12	0 (0.0)
	5-10	18	17 (94.4)	5	18	16 (88.9)
	200	12	12 (100.0)	200	12	12 (100.0)

4. Conclusion.

Based on the criteria for verification for this study (accepted by MMVS in 05/12/2021):

- All unseeded samples must be found negative for acceptance.
- All samples seeded at the high level must be found positive for acceptance.
- Samples seeded at the low level may be either positive or negative for acceptance.

No false positive or false negative results were obtained in the verification study for either of the kits. The overall detection rate for samples of the three commodities seeded with 5 oocysts or 10 oocysts was 94.4% for the replacement TaqMan™ Fast Advanced Master Mix and 88.9% for the current/original QuantiFast Multiplex PCR kits (Table 8), with a similar or a higher number of seeded samples found positive by the

replacement TaqMan™ Fast Advanced Master Mix. Therefore, the alternate method (TaqMan™ Fast Advanced Master Mix) can be considered verified to function in the user's laboratory on any matrix included in the scope of the method.

In conclusion, the replacement TaqMan™ Fast Advanced Master Mix can perform the BAM Chapter 19b method adequately, and based on this verification, the TaqMan™ Fast Advanced Master Mix can replace QuantiFast Multiplex PCR kit for the Chapter 19b method in the food matrices for which the method has been validated.

References:

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