Department of Health & Social Care

Technical Validation Protocol for TaqPath™ COVID-19 CE-IVD RT-PCR Kit

Final: 29 April 2021 (Version 1.3)

Contents

Assay description and intended purpose

1. What is the principle and method of the assay (description of the assay according to the manufacturer's Instructions for Use (IFU)?

   The TaqPath™ COVID-19 CE-IVD RT-PCR assay is a multiplex real-time RT-PCR test for the qualitative detection of RNA from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19.

   Each kit includes the following components:

   - Multiplexed assays that contain three primer/probe sets specific to different SARS-CoV-2 genomic regions (ORF1ab, N gene, S gene) and primers/probes for bacteriophage MS2
   - MS2 Phage Control as an internal process control for nucleic acid extraction
   - TaqPath™ COVID-19 Control as a positive RNA control that contains targets specific to the SARSCoV-2 genomic regions targeted by the assays

2. What is the use for which the device is intended according to the data supplied by the manufacturer on the label, in the IFU, in promotional or sales materials or statements, or as specified by the manufacturer in the performance evaluation?

   Qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, and mid-turbinate swabs, and nasopharyngeal aspirate) and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider

3. Is the test a stand-alone device/test or to be used in conjunction with other equipment and is biosafety containment is required?

   Laboratory test kit intended for use with standard real time PCR instruments (evaluation used ABI7500) and appropriate software. In addition, the assay requires other laboratory equipment to be provided including RNA extraction platform/kits, centrifuge, vortex and micropipettes. Samples and reagents should be handled in a biosafety cabinet prior to inactivation, therefore conferring biosafety. Inactivation must be verified where samples are handled outside of CL3. I**nactivation methods alter the performance characteristics of an assay. Evaluation materials used in this study were gamma irradiated but the effect of heat inactivation compared to chemical inactivation and the effect on sensitivity of the assay as not been**

described. A report on inactivation methods is currently being written and the outcome will be reported in a future revision of the Instructions For Use.

Type of sample to be used in validation

1. Stipulate the sample type (for example, whole non-extracted virus, extracted RNA, synthetic RNA, plasmid DNA containing assay target regions) and any sample matrices (such as saliva, plasma, nasopharyngeal/oronasal swab [dry or in VTM] etc) in which the material is to be spiked.

   For Clinical evaluation the TaqPath™ RT-PCR COVID-19 kit was evaluated using contrived bronchoalveolar lavage (BAL) and nasopharyngeal swab (NP) specimens in support of a U.S. Food and Drug Administration Emergency Use Authorization submission. Contrived samples consisting of negative clinical specimens were spiked with purified SARS-CoV-2 viral RNA SARS-CoV-2 USA-WA1/2020 at varying concentrations for viral load. **These contrived samples were used in the LOD studies but were only of a single variant and may not be representative of the circulating variants. In addition, a post launch evaluation of Upper respiratory tract retrospective samples was undertaken. This did not differentiate between BAL and NP samples and therefore it is not clear whether the sensitivity and specificity of performance varies between sample types. The original LOD study and clinical evaluation were performed with contrived BAL and NP samples in March 2020. At that time, there was no variant of concern in question.**

   However, the manufacturer has provided assurance, based on in-silico analysis and melting temperature (Tm) analysis, that none of the mutations (except del69-70) in any of the other variants as of 29th April 2021 affects the Tm and assay performance. In case of the S-gene drop out caused by del69-70 the LOD would be unaffected because the assay design uses 3 targets with the Lod and the detection of Orf1ab and N gene are not affected by the del69-70.

   For reproducibility and repeatability contrived NP swabs of swab VTM pools were spiked with known concentration of gamma irradiated SARS-Related Coronavirus 2, Isolate USA-WA1/2020.

   For analytical sensitivity and linearity, a known concentration of specific and synthetic template from SARS CoV-2 was used for each of the assay targets.

   Precision, intra-assay and inter-assay testing were performed using nasopharyngeal swabs collected in viral transport material which were spiked with a known concentration of synthetic cDNA for each gene target. **These contrived samples were used in the LOD studies but were only of a single SARS-COV-2 variant and may not be representative of circulating variants (please see above).**

For analytical specificity and cross-reactivity, wet testing was performed on controls of known concentration alongside in silico analysis.

2. Stipulate if the material is required to be extracted for example, volume received, volume extracted, volume eluted, elution buffer to be used in the assay.

   This kit uses purified RNA as a sample for the analysis. The IFU states that extracted RNA can be prepared with any standard RNA extraction procedure or RNA extraction kit. The minimum recommended elution volume is 50 µL.

3. If possible, stipulate if any interfering substances such as preservatives are likely to be present. Shelf life and number of freeze thaw events should also be stated, if known. Where dry swabs are to be used, samples will need to be collected prospectively; two swabs per participant, one for a new test and one to be tested using reference method; or one swab for a new test collected within 24 hours of a positive reference method swab.

   The assay should be stored at –30°C to –10°C except for the COVID control that must be stored at ≤ –70°C. The shelf life of the product is 12 months. The company recommend aliquoting the test materials to minimise potential for contamination and to reduce freeze-thaw cycles. Freeze-thaw cycles should be kept to a minimum.

Equipment and reagents

1. List all the equipment required that are not supplied by the manufacturer with calibration/service requirements and dates where applicable.

- real-time PCR instrument (thermocycler) - manufacturer lists several compatible instruments
- RNA extraction kit
- centrifuge for 1.5 mL tubes and PCR-well strips or 96-well plate (if available)
- vortex
- micropipettes (0.5-20 µL, 20-200 µL)
- powder-free disposable gloves
- centrifuge, with a rotor for microplates if using 96 well or 484 plates
- microcentrifuge

- laboratory mixer, vortex or equivalent
- single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)
- cold block (96-well or 384-well) or ice
- nonstick, RNase-free microcentrifuge tubes (1.5 mL and 2.0 mL)
- sterile aerosol barrier (filtered) pipette tips

2. List all the reagents required that are not provided by the manufacturer with shelf-life expiry dates and storage conditions. Include positive and negative control materials.

   Nuclease-free Water (not DEPC-Treated). All other reagents to perform the assay are included in the kit.

Performance characteristics

1. Analytical Sensitivity and Linearity of SARS COV-2 targets

2. Dilution series:

   The manufacturer performed a dilution series of a synthetic sequence template as at that time no reference material was available.

   The data spans a range from 10 to 10^7 copies per reaction; each dilution was tested in four replicates, and data analysed for each target of the assay (ORF1ab-FAM, N-VIC, S-ABY), plus the internal control (MS2-JUN; constant level per well).

   Table 1. Analytical sensitivity was evaluated with synthetic gene specific cDNA and TaqPath™ COVID-19 CE-IVD RT-PCR Kit using ABI 7500

Sequence template	**ORF1AB**			**S Gene**			**N Gene**		
	X (Ct)	σ	CV %	X (Ct)	σ	CV %	X (Ct)	σ	CV %
10^1 rxn	32.83	0.95	0.03	32.34	1.45	0.04	32.66	0.32	0.01
10^2 rxn	29.31	0.12	0.00	27.88	1.38	0.05	29.38	0.21	0.00
10^3 rxn	26.18	0.15	0.01	25.53	0.30	0.01	26.53	0.03	0.00
10^4 rxn	22.23	0.20	0.01	22.03	0.19	0.01	22.70	0.09	0.00

Sequence template	ORF1AB			S Gene			N Gene		
10^5 rxn	18.80	0.48	0.03	18.59	0.24	0.01	19.16	0.29	0.02
10^6 rxn	14.42	1.85	0.13	14.32	1.69	0.12	15.18	1.30	0.09
10^7 rxn	11.66	0.12	0.01	11.37	0.32	0.03	12.12	0.18	0.01

rxn = reaction, (Ct) = threshold cycle, (X) = arithmetic mean Ct value, (σ) = standard deviation, (CV %) = coefficient of variation.

TaqPath™ COVID-19 CE-IVD RT-PCR Kit showed a tentative detection limit of ≥10 cDNA copies per reaction for all gene targets

3. Linearity and efficiency: Ideally for linearity, the use of a standardised reference panel should be used to ensure effective benchmarking and assure that dilutions are accurate. If an alternative route for establishing linearity is undertaken, the method will need to be documented For LAMP assays the linearity will need to be standardised via dilutions in appropriate matrixes using untreated virus. Plot the data from 4.1.1 and calculate linearity and efficiency. Compare the data with that supplied by the manufacturer, if applicable.

   The data spanned a range from 10 to 10^7 copies per reaction; each dilution was tested in four replicates, and data analysed for each target of the assay (ORF1ab-FAM, N-VIC, S-ABY), plus the internal control (MS2-JUN; constant level per well).

Table 2. Linearity and efficiency of TaqPath

Target	Slope	R-Squared	PCR Efficiency
MS2_JUN	N/A	N/A	N/A
N_VIC	-3.4782	0.9983	0.9387
Orf1ab_fam	-3.5954	0.9983	0.8973
S_ABY	-3.4625	0.9964	0.9445

TaqPath real-time PCR assays showed an acceptable efficiency and linearity, (R2) were >0.99 in all the target reactions tested.

Lowest Limits of Detection (LLOD): The Limit of Detection (LoD) of the TaqPath™ RT-PCR COVID-19 kit was determined for both bronchoalveolar lavage (BAL) and nasopharyngeal swab (NP) specimens. Contrived samples consisting of negative clinical specimens were spiked with purified SARS-CoV-2 viral RNA at varying levels. The testing was performed in three phases according to a study plan recommended by the U.S. Food and Drug Administration for an Emergency Use Authorization submission. The first two phases of testing produced a preliminary and refined LoD, and the LoD was confirmed in the third phase. **For both BAL and NP specimen types, the LoD was determined to be 250 genomic copy equivalents (GCE) per 1 mL of sample, corresponding to 10 GCE/reaction. This LLOD was for each of the gene targets. Data is summarised in the tables below.**

Table 3. LoD Phase III Data Summary – BAL

Dilution ID	Effective Concentration	Replicate	Mean CT				Interpretation	% Positive
			ORF1ab	N	S	MS2		
1	250 GCE/ml	1	29.91	29.06	28.49	23.14	Positive	100%
1	250 GCE/ml	2	30.14	29.27	29.71	23.96	Positive	100%
1	250 GCE/ml	3	30.00	29.65	29.30	24.01	Positive	100%
1	250 GCE/ml	4	30.31	29.68	29.07	23.80	Positive	100%
1	250 GCE/ml	5	30.17	29.63	29.64	23.70	Positive	100%
1	250 GCE/ml	6	30.33	29.27	29.70	23.45	Positive	100%
1	250 GCE/ml	7	29.90	29.61	32.81	23.42	Positive	100%
1	250 GCE/ml	8	30.19	29.78	29.24	23.77	Positive	100%
1	250 GCE/ml	9	30.07	29.38	28.55	23.75	Positive	100%
1	250 GCE/ml	10	30.11	29.39	29.06	23.97	Positive	100%
1	250 GCE/ml	11	29.81	29.54	29.35	24.26	Positive	100%
1	250 GCE/ml	12	30.13	29.74	29.08	24.63	Positive	100%
1	250 GCE/ml	13	30.70	30.06	28.42	25.12	Positive	100%
1	250 GCE/ml	14	30.35	29.80	29.12	24.83	Positive	100%
1	250 GCE/ml	15	30.15	29.83	29.68	24.89	Positive	100%

Dilution ID	Effective Concentration	Replicate	Mean CT				Interpretation	% Positive
1	250 GCE/ml	16	30.33	29.79	29.43	24.55	Positive	100%
1	250 GCE/ml	17	30.35	29.97	31.47	24.68	Positive	100%
1	250 GCE/ml	18	30.42	30.10	29.30	24.87	Positive	100%
1	250 GCE/ml	19	30.94	29.74	29.23	25.38	Positive	100%
1	250 GCE/ml	20	30.30	29.90	29.43	25.70	Positive	100%
NC	Negative Control	N/A	40.00	40.00	40.00	24.07	Valid	N/A
N/A	Positive Control	N/A	21.43	21.42	19.57	40.00	Valid	N/A

Table 4. LoD Phase III Data Summary – NP

Dilution ID	Effective Concentration	Replicate	Mean CT				Interpretation	% Positive
			ORF1ab	N	S	MS2		
1	250 GCE/ml	1	29.96	28.93	35.65	25.71	Positive	100%
1	250 GCE/ml	2	30.59	28.92	33.55	25.80	Positive	100%
1	250 GCE/ml	3	30.21	28.82	32.03	25.82	Positive	100%
1	250 GCE/ml	4	30.38	28.74	34.16	25.74	Positive	100%
1	250 GCE/ml	5	30.45	29.04	31.42	25.80	Positive	100%
1	250 GCE/ml	6	30.97	29.28	36.58	25.99	Positive	100%

Dilution ID	Effective Concentration	Replicate	Mean CT				Interpretation	% Positive
1	250 GCE/ml	7	30.31	29.17	31.10	25.82	Positive	100%
1	250 GCE/ml	8	31.05	29.15	31.81	26.47	Positive	100%
1	250 GCE/ml	9	30.46	28.88	32.95	26.15	Positive	100%
1	250 GCE/ml	10	30.31	28.80	34.73	26.78	Positive	100%
1	250 GCE/ml	11	30.49	29.80	38.74	27.35	Positive	100%
1	250 GCE/ml	12	31.56	29.67	35.02	27.61	Positive	100%
1	250 GCE/ml	13	30.71	29.28	36.37	27.41	Positive	100%
1	250 GCE/ml	14	31.55	28.82	31.26	27.15	Positive	100%
1	250 GCE/ml	15	30.95	29.30	35.96	26.96	Positive	100%
1	250 GCE/ml	16	30.50	29.12	35.70	26.97	Positive	100%
1	250 GCE/ml	17	30.72	29.44	34.75	27.40	Positive	100%
1	250 GCE/ml	18	30.73	29.30	34.56	27.54	Positive	100%
1	250 GCE/ml	19	30.99	29.30	35.89	28.67	Positive	100%
1	250 GCE/ml	20	30.35	29.21	32.74	28.39	Positive	100%
NC	Negative Control	N/A	40.00	40.00	40.00	26.83	Valid	N/A
N/A	Positive Control	N/A	21.21	21.42	19.01	40.00	Valid	N/A

Precision and robustness

1. To confirm the repeatability and reproducibility of the assay studies using contrived samples, prepared with pooled SARS-CoV-2-negative NP specimens obtained as remnant clinical samples, was undertaken. Contrived samples comprised one negative NP sample, one contrived low-positive NP sample formulated at 3X LoD (750 GCE/mL) and one contrived moderately positive NP sample formulated at 7X LoD (1,750 GCE/mL). NP was selected by the manufacturer as at the time they drafted their protocol this specimen type was the mostly widely used for COVID-19 testing. One replicate each of Negative Control and Positive Control were included in each run to determine run validity. The studies were undertaken at 3 sites, but the sites only performed the RT-PCR with all other steps pre-prepared. Sample were shipped as single use only to minimise any freeze-thaw effects.

   The manufacturer provided data for both the percent agreement (overall and for each assay) and the repeatability and reproducibility SDs for each assay. The response variables in this study are assay interpretation (positive or negative for each of the four assays), sample interpretation (SARS-CoV-2 Positive or SARS-CoV-2 Negative) and Ct.

   Repeatability is assessed with at least one operator using one instrument and one lot of reagents. Pooled extracted samples and Negative Control were tested with a 20 × 1 × 2 × 2 design (20 test days with 1 operator, 2 runs per day (morning and afternoon) and 2 replicates per run).

   Reproducibility is assessed using a multi-site study with three sites, one operator per site, one instrument per site and one lot of reagents (the same lot for all sites). Pooled extracted samples and Negative Control (the same for all sites) will be tested with a 5 × 3 × 1 × 5 design (5 non-consecutive test days, with 3 sites, 1 run per day alternating between morning and afternoon and 5 replicates per run).

2. Intra-assay precision: Use the data for 5 replicate values from a single day from 4.1.1 to calculate Standard Deviation & Coefficient of Variation measurement, with the values for the latter to be <10%. To include the use of inhibition controls.

   Data provided in company report (and in supplied raw data) for intra -assay precision to have CV <5% for all target genes for Neg, 3 x LOD for all three sites (document PCP0062570).

3. Inter-assay precision: Use the data for 5 replicate values from multiple days from 4.1.1 for Standard Deviation & Coefficient of Variation with the values for the latter to be <15%.

Data provided in company report (and in supplied raw data) for inter -assay precision to have CV <5% for all target genes for Neg, 3 x LOD for all three sites (document PCP0062570).

4. Repeatability: Spike 30 negative samples from different individuals with known amount of agent/positive material (suggested 3x the LLOD), all should be positive. **40 Samples at 3x LOD and 7xLOD were tested – all were positive.**

Note: 4 plates were retested during reproducibility studies due to inconclusive results in the negative control

Analytical specificity (interferences and cross-reactions)

1. Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related (for example other coronaviruses), syndromic diseases (such as other respiratory viruses and bacteria) and common diseases (such as HIV, HBV, HCV, VZV, EBV, CMV) should be tested.

   The manufacturer provided in silico analysis that was updated on June 3, 2020, using 25,998 complete SARS-CoV-2 genomes in GISAID and GenBank databases. Based upon BLAST analysis, the TaqPath™ COVID-19 CE-IVD RT-PCR Kit maps with 100% homology to >99.99% of known SARS-CoV-2 isolates in GISAID and 100% of known isolates in GenBank databases. Mapping was deemed successful for a given isolate if at least two of the three targets (ORF1ab, S gene, and N gene) showed 100% identity. **To ensure that this analysis remains current the company take part in the MHRA initiative and share, on a monthly basis, in silico analysis run on the most recent available sequences on GISAID, and any Variants of Concern (VOC) and Variants under Investigation (VUI) listed in the Public Health England document SARS-CoV-2 variants of concern and variants under investigation in England. The latest report was sent on April 13, 2021 to MHRA.**

   The analytical specificity (Cross-reactivity) of the TAQPATH COVID-19 assay targets (ORF1ab, S gene and N targets) were evaluated using both wet testing and in silico analysis. Results are shown in manufacturer document PCP0062573.

   The analytical specificity for this assay was confirmed by testing a panel of different microorganisms which represents the most common respiratory pathogens. No cross-reactivity of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit was found. These assays were run on ABI 7500 Fast l-Time PCR Detection System. Full report (PRJ0002222) available from manufacturer.

In silico analysis indicates that significant amplification of non-target sequences that result in cross-reactivity or potentially interfere with detection of SARS-CoV-2 is not likely to occur.

Table 8. Interfering substances

Interfering substance	Final concentration in sample	Agreement with expected results			
		Positive BAL samples	Positive NP samples	Negative BAL samples	Negative NP samples
None	N/A	100%[1]	100%	100%	100%
Mucin: bovine submaxillary gland, type I-S	0.1 mg/mL	100% [2]	100%	100%	100%
Blood (Human)	1% v/v	100%[3]	100%	100%	100%
Nasal sprays or drops—Nasacort™	10% v/v	100%[4]	100%[4]	100%	100%
Nasal corticosteroids—Dymista™	5 µg/mL	100% [2]	100%	100%	100%
NeilMed™ Nasogel™	1% w/v	100% [2]	100%	100%	100%
Influenza A H1N1 Brisbane/59/07	1 × 105 TCID50/mL	100% [2]	100%	100%	100%
Throat lozenges,	1% w/v	100% [3]	100%	100%	100%

Interfering substance	Final concentration in sample	Agreement with expected results			
		Positive BAL samples	Positive NP samples	Negative BAL samples	Negative NP samples
oral anaesthetic and analgesic—Chloraseptic™					
Oseltamivir phosphate	33 µg/mL	100% [2]	100%	100%	100%
Antibiotic, nasal ointment—Bactroban™	5 µg/mL	100% [2]	100%	100%	100%
Antibacterial, systemic—Tobramycin	0.6 mg/mL	100% [2]	100%	100%	100%
Homeopathic allergy relief medicine—Similasan™ Nasal	10% v/v	100%	100%	100%	100%

[1] Two of six replicates produced a Ct >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm.

[2] Two of three replicates produced a Ct >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm.

[3] All three replicates produced a Ct >37 or Undetermined for S Gene but were called Positive based on the interpretation algorithm.

[4] One of three replicates produced a Ct >37 or Undetermined for S Gene, but all replicates were called Positive based on the interpretation algorithm

The interference studies showed some reactivities in the S gene but these were at high CT and would not impact the final qualitative call as 2 gene targets need to be positive.

Diagnostic sensitivity and specificity (clinical validation with confirmed positives and negatives)

1. Samples selected for this validation will be appropriate to the assay. Low medium and high viral load samples will be equally distributed to avoid increasing or lowering Sensitivity and Specificity.

Table 9. breakdown of TaqPath positive results by comparator assay ct value

Ct	Comparator target 1	Proportion of total (%)	Comparator target 2	Proportion of total (%)
<25	71	41.3	67	38.9
25 to 30	58	33.7	54	31.4
30 to 35	42	24.4	44	25.6
>35	1	0.6	7	4.1

Diagnostic sensitivity:

RNA extraction was performed using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit for the COVID-19 Test. The comparator method assessed was Roche cobas® SARS-CoV-2 Assay (CE-IVD authorized). The Roche Cobas Assay which detects ORF1ab (target 1) and E gene (target 2) targets. The TaqPath™ COVID-19 Test was performed on the Applied Biosystems™ 7500 Fast Real-Time PCR instrument.

The data set comprised 172 positive specimens. These were retrospective upper respiratory specimens

Table 10. Breakdown of TaqPath positive results by comparator assay ct value

Ct	Comparator target 1	Proportion of total (%)	Comparator target 2	Proportion of total (%)	TaqPath % concordance
<25	71	41.3	67	38.9	100
25-30	58	33.7	54	31.4	100
30-35	42	24.4	44	25.6	100
>35	1	0.6	7	4.1	100

2. Diagnostic specificity:

278 specimens upper respiratory samples that had previously tested negative in Cobas assay were analysed.

20/278 of specimens showed reactivity in 1 or more gene targets in the TaqPath™ COVID-19 CE-IVD RT-PCR Kit – However, 2 gene targets needed for specimen to be called positive.

15/278 specimens showed reactivity in 2 or more gene targets in TaqPath™ COVID-19 CE-IVD RT-PCR Kit 1 of which showed reactivity in cobas assay and was excluded from analysis.

In 4 out of 278 samples were reactive in only 1 target of the Roche Cobas assay and were therefore labelled inconclusive and excluded.

Therefore, in total 274 specimens used for specificity analysis.

14 out of 446 discordant results were analysed by sequencing, of theses 8 yielded no result (Sequencing failed) and 6 were shown to be true positives. Of the 8 sequencing samples that yielded no result 7 were positive by TaqPath.

Table 11. Agreement between Taqpath and Comparator assay

COVID-19	**Comparator: cobas SARS-CoV-2 Assay**		
TaqPath™ COVID-19 Kit (CE-IVD)	Positive	Negative	Total
Positive	171	13	184
Negative	1	261	262
Total	172	274	446
Agreement Calculations			**95% CI**
Positive Percent Agreement (PPA)	171/172	99.4%	96.8% to 100.0%
Negative Percent Agreement (NPA)	261/274	95.3%	92.0% to 97.4%

Table 12. Sensitivity and specificity of TaqPath assay with reference standard

TaqPath™ COVID-19 Kit (CE-IVD)	Reference Standard		
	Positive	Negative	Total
Positive	177	0	177
Negative	0	261	261
Total	177	261	438
Clinical Sensitivity and Specificity Calculations			**95% CI**
Sensitivity	177/177	100.0%	97.9% to 100.0%
Specificity	261/261	100.0%	98.6% to 100.0%

Conclusions

The validation demonstrates that when used with the stated sample type, equipment and extraction method, the TaqPath assay reaches the standards for laboratory TPP assay for both sensitivity and specificity.

The evaluation was performed using the ABI 7500 therefore the performance on other platforms may vary.

Contrived and retrospective samples were used for evaluations rather than fresh specimens and it was not possible to determine the exact sample type used in the evaluation.

Extraction and inactivation methods alters the performance characteristics of an assay. Evaluation materials used in this study were gamma irradiated but the effect of heat inactivation compared to chemical inactivation and the effect on sensitivity of the assay as not been described.

TVG uses a wide range of sites in order to validate new technologies/tests. These independent sites use a range of RT-qPCR assays against different genomic regions and it is recognised that for some assay comparisons the sensitivity of RT-qPCR assay(s) may subtly differ from the true sensitivity of the test if compared to the same genomic region.

Published to GOV.UK in pdf format only.

www.gov.uk/dhsc