P0378 ViraQ SARS-CoV-2 Check 125

REF P0378

The kit insert contains a detailed protocol and should be read carefully before testing the run control to ensure optimal performance

Table of contents

Intended use 3
Key to symbols used 3
Principle of method 3
Traceability to standards calibrated in RNA copies and IUs 4
Stability of SARS-CoV-2 standards and run control 7
Kit contents (materials provided) 7
Materials required but not supplied 7
Storage instructions 7
Warning and precautions 7
Reagent preparation 8
Test procedure 8
Interpretation of results 8
Limitations 9
References 10

Intended use

P0378 ViraQ SARS-CoV-2 Check 125 is intended to be used as external run control for SARS-CoV-2 RNA amplification tests in combination with the assays on the platforms defined in Table 1. The run control helps laboratories to ensure sufficient analytical sensitivity and consistent performance of qualitative SARS-CoV-2 nucleic acid amplification technology (NAT) assays.

Table 1. Assays and platforms covered by P0378 ViraQ SARS-CoV-2 Check 125 run control

Assays (manufacturer)	Platform	Test environment
Cobas SARS-CoV-2 assay® (Roche)	cobas 6800/8800	Public health and clinical virology
Aptima SARS-CoV-2 assay® (Hologic)	Hologic Panther®	

P0378 ViraQ SARS-CoV-2 Check 125 should not be used to replace the internal controls or calibrators in the test kits. The test result on the run control should not be used to reject the run or delay the release of test results.

Key to symbols used

Manufacturer

Lot number

Catalogue number

Store below -30°C

For performance evaluation only

Caution

In vitro diagnostic medical device

Expiry date

Number of controls

Biological substance Category B

Read instructions for use

Principle of method

P0378 ViraQ SARS CoV-2 Check 125 control has been formulated to mimic swab specimens in viral transport medium with a low SARS-CoV-2 concentration. After thawing the run control tubes are ready for use and can be placed at random positions in sample racks on the NAT platforms. The run control contains 125 copies/mL of inactivated SARS-CoV-2 RNA and has been designed to ensure sufficient analytical sensitivity of real time polymerase chain reaction (PCR) and transcription mediated amplification (TMA) tests in public health and hospital laboratories. The SARS-CoV-2 RNA concentration in the run control has been set at 4-5 times the 95% lower limit of detection (LOD) of the Hologic Aptima assay (table 2a and 2b). The positioning of P0378 ViraQ SARS-CoV-2 125 control ensures reactivity rates above 99.5% in the NAT systems as listed in table 1. The run control enables laboratories to be alerted in case of a significant reduction of analytical sensitivity of NAT systems. The run control is a dilution of the S0203 SARS-CoV-2 standard in 2% human plasma/phosphate buffered saline (PBS) solution and is inactivated by betapropiolactone[1,2]. The S0203 standard was prepared from a pool swab fluid samples (in GLY medium) kindly provided by the Public Health Laboratory (GGD) in Amsterdam. Remnants of SARS-CoV-2 positive swab fluid samples were collected in August to November 2020 in the Netherlands and contain B.1 (Wuhan) type virus strains.

The 2% human plasma matrix in the run control was made from plasma units collected before the SARS-CoV-2 pandemic and tested negative for all relevant markers of blood borne viruses. The S0203 inactivated SARS-CoV-2 standard has been calibrated in PCR detectable RNA copies/mL and in International Units (IU)/mL traceable to the first WHO International Standard[3]. A positive result on the run control indicates that the NAT method has been performed with sufficient analytical sensitivity. A non-reactive result is indicative of reduced sensitivity of the NAT system and should trigger investigation of the technical performance of the assay. The run control generates RLU levels or sample to cut-off (S/CO) ratios in the Hologic TMA assay[4] and Ct values in the Roche cobas real time PCR[5] assay. Statistical analysis of these assay response values generated over a certain period of time allows for comparison of analytical performance of NAT reagent batches and laboratory instruments.

Table 2a. Proportion reactive and average assay response values on P0356 SARS-CoV-2 inactivated standard dilution panel in Roche cobas and Hologic Aptima assays.

S0203 standard^ dilutions		Roche cobas target 1 ORFa/b gene		Roche cobas target 2 E gene		Hologic Aptima	
member	PCR detectable copies/mL	r/n (%)	mean Ct	r/n (%)	mean Ct	r/n (%)	mean RLU
1	33784,4	4/4 (100%)	26.92	4/4 (100%)	27.71	2/2 (100%)	1082
2	11249,6	4/4 (100%	28.64	4/4 (100%)	29.46	2/2 (100%)	1090
3	3378,4	4/4 (100%)	30.40	4/4 (100%)	31.32	4/4 (100%)	1095
4	1125,0	8/8 (100%)	31.85	8/8 (100%)	32.87	4/4 (100%)	1093
5	337,8	8/8 (100%)	33.15	8/8 (100%)	34.21	14/14 (100%)	1104
6	112,5	8/8 (100%	34.69	8/8 (100%)	35.87	14/14 (100%)	1083
7	33,78	8/8 (100%)	35.26	8/8 (100%)	36.45	13/14 (93%)	883
8	11,25	8/8 (100%)	36.87	7/8 (88%)	38.06	9/14 (64%)	684
9	3,38	7/8 (88%)	37.55	4/8 (50%)	37.89	4/14 (290%)	429
10	1,12	1/8 (13%)		1/8 (13%)		1/14 (7%)	323

Table 2b. Detection limits using the P0356 SARS-CoV-2 inactivated standard dilution panel in Roche cobas and Hologic Aptima assays.

standard	Reference panel	NAT method	n	50% LOD (CI) copies/mL	95% LOD (CI) copies/mL
S0203 SARS-CoV-2 RNA inact.^	P0356 SARS-CoV-2 inact.	cobas [ORFa/b]	8	1.8 (1.0-3.3)	8.3 (4.5-18.6)
		cobas [E]		3.5 (2.0-6.0	15.5 (8.7-34.6)
		Aptima	14	6.6 (4.4-9.9)	29.7 (18.4-60.1)

^inactivated standard also used for preparation of P0378 SARS- CoV-2 Check 125 control

Traceability to standards calibrated in RNA copies and IUs

Ten-fold dilutions of native and inactivated SARS-CoV-2 standards were tested in duplicate in the Roche cobas SARS-CoV-2 assay (table 3). With parallel line assay the distance between Ct values (95% confidence interval (CI)) was 1.96 (1.79-2.12) for ORF1a/b and 2.67 (2.57-2.75) for E genes respectively. The potency (95% CI) of the native standard was $2^{1.96\ (1.79\text{-}2.12)}$ = 3.88 (3.46-4.35) fold higher than the inactivated standard based on ORFa/b gene PCR and 6.34 (5.97-6.79) fold higher based on E gene PCR. Hence the recovery after treatment of the pool of swab fluid with beta-propiolactone was 25.8 (23.0-28.9)% for the ORFa/b gene and 15.7 (14.8-16.8)% for the E gene. For estimation of the viral load in the native standard we combined the delta Ct values of 1.96 and 2.67 for ORFa/b and E targets respectively and from the average values it follows that the beta-

propiolactone inactivation had reduced the viral load 4.96 (3.88-6.35) fold and hence had destroyed 79.8 (74.3-84.3)% of SARS-CoV-2 RNA

Table 3. Ct values in Roche cobas SARS-CoV-2 RNA test on standard dilutions before and after inactivation by beta-propiolactone.

Standard		A-series		B-series	
		ORFa/b	E	ORFa/b	E
Viral state	Dilution	Ct	Ct	Ct	Ct
native	10	21.95	22.10	21.78	21.96
native	100	25.05	25.13	24.91	25.18
native	1000	27.87	28.20	28.09	28.32
inactivated	10	23.54	24.55	23.77	24.67
inactivated	100	26.93	27.87	26.95	27.86
inactivated	1000	30.15	30.99	30.04	30.95

From the different yields for the two targets it seems that beta-propiolactone destroyed 1.64 (1.55-1.72) fold more of the E gene than of the ORF gene. This significant difference in recovery may be caused by presence of unequal amounts of subgenomic RNA of the ORF and E genes derived from human cells that were present in the swab fluid pool before inactivation. Another explanation – although less likely – is that beta-propiolactone renders more ORFa/b than E gene targets undetectable in the Roche cobas assay.

At the time of development of the P0378 SARS-CoV-2 Check 125 control a WHO International Standard was not yet available. The concentration in the inactivated S0203 SARS-CoV-2 standard was first determined by limiting dilution probit analysis and expressed in PCR detectable units or copies/mL. From the proportions of reactive results in different NAT assays the 63% LOD (and 95% confidence interval (CI)) was calculated by parallel line probit analysis. We assumed that the NAT method with the highest sensitivity (the Roche cobas assay for ORFa/b target) reached 100% NAT efficiency and thus reached a 63% LOD of 1 RNA copy per amplification reaction as follows from Poisson distribution statistics. Since for each replicate test a volume of 400 uL was used as input in the Roche cobas amplification reaction the concentration at the 63% LOD was set at 2.5 copies/mL. The initially assigned concentrations needed to be adjusted with a recalibration factor so that the viral load in the inactivated S0203 standards was calculated to be 3.38×10^6 PCR detectable RNA copies/mL. Since the viral load in the standard before inactivation was estimated to be 4.96 (3.88-6.35) fold higher (see above) the concentration in the native S0202 standard was calculated to be 1.68×10^7 copies/mL. With the assigned unitage in copies/mL to the inactivated SARS-CoV-2 standard the calculated NAT efficiency was 100 (53-187)% and 54 (24-94)% in the Roche cobas ORF a/b and E PCR tests respectively, whereas it would be 67 (42-102)% in the Hologic Aptima TMA assay. The concentration of 125 copies/mL in P0378 ViraQ SARS CoV-2 control is ensured by gravimetrically recorded dilutions of the S0203 inactivated SARS-CoV-2 standard that are snap frozen in liquid nitrogen.

For calibration of the inactivated S0203 SARS-CoV-2 standard against the WHO 20/146 standard we tested dilution series in the Roche cobas SARS-CoV-2 assay in duplicate (table 4a) and analyzed the Ct values using parallel line analysis with exception of the lowest concentration to improve parallelism. The conversion factor from PCR detectable RNA copies to IUs appeared to be different for the two gene targets (ORF a/b and E) in the Roche cobas assay (table 4b). The amount of IUs per detectable RNA copy was 4,3 (3.4-

5.4) for the ORFa/b gene and 2.7 (2.2-3.3 for the E gene. Hence the conversion factor from PCR detectable copies to IUs was 1.60 (1.57-1.63) fold higher for the ORF gene than for the E gene when comparing the inactivated BioQ S0203 standard with the WHO 20/146 standard. By contrast the amount of E genes was reduced 1.64 (1.55-1.72) fold more than the amount of ORFa/b genes by treatment with beta-propiolactone (see above). It may be that the inactivation of the cell culture-derived WHO standard by acid and heat treatment acts differently than the chemical inactivation of the BioQ standard. However the relative amount of detectable genomic and subgenomic RNA of the ORFa/b and E genes in the WHO standard preparation before inactivation is unknown.

Table 4a. Ct values on WHO and BioQ SARS-CoV-2 standard dilution series in Roche cobas assay

	A-series		B-series	
WHO 20/146 IU/mL	ORFa/b Ct1	E Ct2	ORFa/b Ct1	E Ct2
100000	27.63	27.78	27.31	27.38
30000	29.16	29.26	29.43	29.61
10000	30.71	30.82	30.39	30.61
3000	32.20	32.61	33.07	33.05
1000	32.94	33.37	33.27	33.77
BioQ S0203 inactivated copies/mL	ORF Ct1	E Ct2	ORF Ct1	E Ct2
33784	26.94	27.75	26.54	27.30
11249	28.74	29.60	28.78	29.68
3378	30.34	31.25	30.09	31.02
1125	31.75	32.75	31.92	32.56
338	33.17	34.03	33.26	34.21

Table 4b. Potency of BioQ S0203 inactivated standard against WHO 20/146 standard in Roche cobas SARS-CoV-2 assay

Target	parameter	Value (95% CI)
ORFa/b	delta Ct	2.10 (1.78-2.42)
	IU/copy	4.29 (3.44-5.36)
E	delta Ct	1.42 (1.13-1.72)
	IU/copy	2.68 (2.19-3.29)

It must be noted that the difference in Ct value between target 1 [ORFa/b] and 2 [E] on the USA-WAI1-2020 standard standard used for determining the analytical sensitivity in the Roche cobas assay (data in Roche cobas package insert) was on average 2.42 as compared to a delta of 0.92 on the S0203 inactivated standard. In terms of a potency (or relative detectability) this is a factor 5.37 versus 1.88 for the two SARS-CoV-2 reference mateirials. This suggests that there is still 2.8 fold more of E gene RNA relative to the ORF a/b gene in the BioQ S0203 inactivated standard than in the cultured USA-WA1-2020 standard. Hence there are significant differences in the potency of ORFa/b and E genes in different standards according to quantification in the Roche cobas SARS-CoV-2 assay. The calibration of the BioQ S0203 standard in detectable RNA copies/mL was based on

assuming 100 (53-187)% NAT efficiency of the ORFa/b gene in the Roche cobas assay and 67 (42-102)% in the Hologic Aptima test. If the amount of IUs in the WHO standard would be equal to the true amount of RNA copies the efficiency of the two assays would be 23 (12-44)% and 16 (10-24)% respectively. When we use the IU/copy conversion factor of the ORFa/b gene target in table 4b as the basis for estimation of the concentration in IU/mL the P0378 ViraQ SARS-CoV-2 Check 125 run control contains 125 detectable RNA copies/mL in the cobas assay and 536 (430-670) IU/mL. It must however be emphasized that the conversion factor in other assays than the Roche cobas PCR assay may be different.

Stability of SARS-CoV-2 standards and run control

The long term stability of liquid frozen viral RNA standards stored below -65˚C has been firmly established for blood borne viruses[6] but not yet for SARS-CoV-2. Real time stability experiments using quantitative NAT assays showed less than 5-10% degradation of viral RNA per annum when stored at -30˚C[6]. Stability studies on P0378 SARS-CoV-2 Check 125 Control are ongoing to ensure that the run control generates a reactivity rate greater than 99.5% when stored at -30˚C and used before the expiration date (set at two years after preparation of the run control batch).

Kit contents (materials provided)

The run control contains human plasma without preservatives and is provided in one format as detailed in Table 5.

P0378 is intended to accommodate public health and hospital laboratories. To facilitate automation the run control is presented in a polypropylene vial with screw cap. The tube label has a unique code identifying the product, sequential batch number and viral marker.

Table 5. Description of kit formats and contents

Cat. Code	UDI code	Quantity run control	Size vials	packing
P0378	8718719830174	10 x 0.6 mL	2 mL	Plastic zip bag

Materials required but not supplied

The test kits and liquid handling devices provided by the NAT manufacturer as specified in Table 1.

Storage instructions

The run controls should be stored at or below -30°C for a maximum of two years[6]. Thawing should be done quickly in a water bath of 37°C to avoid formation of cryoprecipitates or matrix effects. Once thawed the run control samples should be used within 8 hours. During this period, when not in use, store sample at 2-8°C[4]. Do not refreeze the controls after thawing. Any control sample that appears cloudy or contains precipitates after thawing and mixing should be discarded.

Warning and precautions

Although P0378 ViraQ SARS-CoV-2 Check 125 contains inactivated SARS-CoV-2 particles the matrix may still be potentially bio-hazardous. The 2% human plasma matrix is prepared from plasma units that tested negative for blood borne viruses (HBV-DNA, HCV-RNA, HIV-RNA, HBsAg, anti-HBc, anti-HIV, anti-HCV and anti-Treponema *pallidum*). No test method can offer complete assurance that products derived from human plasma cannot transmit (unknown) infectious agents. The run control should only be used by trained laboratory workers who are aware of the potential risk of infectious agents in human swab fluid samples and take the necessary precautions. Observe the universal

precautions for prevention of transmission of infectious agents when handling these materials[7,8].

- Do not pipette by mouth.
- Use personal protective equipment, including lab coats, gloves and safety glasses.
- Do not eat, drink or smoke in areas where the run controls is handled.
- Disinfect spills using a 0.5% hypochlorite solution (1:10 v/v household bleach) or equivalent disinfectant.
- Dispose unused or spilled materials according to the normal practices for biological waste disposal in your institution.
- If precipitates are visible, mix the run controls for 2 minutes thoroughly.
- Once thawed, do not re-freeze and thaw the run control samples.
- Store thawed run controls in an upright position.

Reagent preparation

- Thaw the run control quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed.
- Immediately after thawing remove the run control tube from the water bath.
- Vortex the run control.
- Give a short spin in a centrifuge to remove liquid before releasing screw cap from vial.
- Minimise the time period from thawing until usage of the control samples.
- Use within 8 hours after thawing
- After thawing when not in use: store at 2-8°C

Test procedure

The run control should be tested in a manner identical to that of clinical swab specimens in viral transport medium and the result be calculated according to the instructions for use of the NAT procedure.

Interpretation of results

Roche cobas SARS CoV-2 assay

The cobas SARS-CoV-2 assay gives qualitative results (reactive, nonreactive or invalid) for two targets (OFRa/b and E) and if reactive a Ct value is generated. P0378 ViraQ SARS-CoV-2 Check 125 Control should react positive in more than 99.5% of test runs.

The positioning of P0378 ViraQ SARS-CoV-2 Check 125 control is near the Poisson detection endpoint range of the cobas MPX assay but at enough distance to guarantee reproducibility of Ct values. At the concentration of 125 copies/mL the Ct values in the cobas SARS CoV-2 assay are likely normally distributed. Only limited data are available on P0378 ViraQ SARS-CoV-2 Check control with Ct values expected to be in the range between 33.0 and 35.0 for target 1 [ORFa/b gene] and between 34.0 and 36.0 for targer 2 [E gene]. In one cobas test run the average Ct (and range) of values on P0378 ViraQ SARS-CoV-2 Check 125 control (n=9) were 34.0 (33.7-34.4) and 34.9 (34.5-35.2) on target 1 [ORFa/b] and target 2 [E] respectively. Hence for monitoring the performance of the Roche cobas SARS-CoV-2 assay one can use untransformed Ct values in a Levey-Jennings QC chart for trend analysis and apply the Westgard rules[9].

Hologic Aptima SARS-CoV-2 assay

The results of the Hologic SARS-CoV-2 TMA assay are expressed RLU levels or a sample to cut-off ratio (S/CO). P0378 ViraQ SARS-CoV-2 Check 125 Control should react positive in more than 99.5% of TMA test runs. Only limited data are available on P0378 ViraQ SARS-

CoV-2 Check 125 Control. In two test runs of 10 run control samples the average (and range) of RLU values were 1039 (806-1090) and 1240 (1113-1271) respectively.

The RLU or S/CO responses on P0378 ViraQ SARS-CoV-2 Check 125 in the Aptima TMA assay may not be normally distributed. A Gumbel distribution may be more suitable to describe the data. From this type of extreme value distribution it follows that the difference between the median and the average RLU or S/CO values is an indicator of the skewness of the distribution curve. Hence, the value of this parameter $\Delta(S/CO_{M-A})$ becomes higher with lower analytical sensitivity of the NAT system and may be used for trend analysis[10].

Limitations

- P0378 ViraQ SARS-CoV-2 Check 125 Control cannot be used to evaluate the analytical or diagnostic sensitivity of PCR or TMA assays (although a significant reduction of analytical sensitivity of the NAT system can become apparent with repeated occurrence of non-reactive results).
- P0378 ViraQ SARS-CoV-2 Check 125 Control must not be substituted for the mandatory controls or calibrators provided with NAT test kits for calculating the cut-off and/or criteria for releasing test results.
- The Poisson distribution in samples with low SARS-CoV-2 concentrations cannot guarantee that 100% reactive results will be found on P0378 ViraQ SARS-CoV-2 Check 125 Control in different NAT test runs. Therefore the response values on the run controls should not be used for a decision to accept or reject the test run.
- So far only limited data are available on P0378 ViraQ SARS-CoV-2 Check 125 Control. Therefore it cannot be guaranteed that different results will be found than those expected with the Roche cobas and Hologic Aptima SARS-CoV-2 assays with other NAT reagent lots.
- It cannot be guaranteed that the concentration of 536 (430-670) IU/mL in the run control that was estimated to be equivalent to 125 PCR detectable RNA copies/mL of the ORFa/b gene in the Roche cobas assay will also be found with other NAT assays

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