P0247
ViraQ WNV Check 125

CE

REF P0247

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

P0247 ViraQ WNV Check 125 is intended to be used as external run control for West Nile Virus (WNV)-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 WNV nucleic acid amplification technology (NAT) assays for blood screening or diagnostic testing.

Table 1. Assays and platforms covered by P0247 ViraQ WNV Check 125 run control

Assays (manufacturer)	Platform	Test environment
Cobas WNV assay® (Roche)	Cobas 6800/8800	Blood screening or diagnostic testing
Procleix WNV assay® (Grifols)	Procleix Panther®	

P0247 ViraQ WNV 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 on donor or patient samples.

Key to symbols used

Manufacturer

Lot number

Catalogue number

Store below -30°C

CE mark without Notified Body number

Caution

In vitro diagnostic medical device

Expiry date

Number of controls

Biological substance Category B

Read instructions for use

Principle of method

P0247 ViraQ WNV Check 125 Control has been formulated to mimic natural plasma specimens with a low WNV-RNA 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 WNV-RNA Lineage 2 and has been designed to ensure sufficient analytical sensitivity of real time polymerase chain reaction (PCR) and transcription mediated amplification (TMA) tests in blood screening laboratories. The WNV-RNA concentration in the run control has been set at 8-16 times the 95% lower limit of detection (LOD) of the Grifols Procleix and Roche cobas WNV assays (table 2). The positioning of P0247 ViraQ WNV Check 125 control ensures reactivity rates above 99.5% in the NAT systems 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 S0169 WNV-RNA Lineage 2 standard in plasma and is inactivated by betapropiolactone[1,2]. The S0169 standard was prepared from WNV Lineage 2 tissue culture supernatant obtained during the 2010 outbreak in Macedonia (Nea Santa strain kindly provided by Prof. A Papa, Aristotle University of Thessaloniki)[3,4]. The plasma matrix in which the run control is diluted is manufactured from plasma units that tested negative for all relevant markers of blood borne viruses. The S0169 WNV Lineage 2 standard has been calibrated in copies/mL against the ISS 0109 (Roche) and ISS 0410

standards[5,6] at the time World Health Organization (WHO) International Standards were not yet available. The low concentration of WNV Lineage 2 in the run control is also representative for WNV Lineage 1, which is detected with similar analytical sensitivity by the above mentioned commercial NAT assays[7-10]. 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 sample to cut-off (S/CO) ratios in the Procleix WNV TMA assay and Ct values in cobas real time PCR assays. Statistical analysis of these assay response values generated over a certain period of time allows for comparison of analytical performance of NAT reagent lots and laboratory instruments.

Table 2. Detection limits on P0346 WNV Lineage 2 standard dilution panel in Grifols Procleix and Roche cobas assays.

standard	Reference panel	NAT method	n	50% LOD (CI) copies/mL	95% LOD (CI) copies/mL
S0169 WNV-RNA Lineage 2 inact.^	P0346 WNV Lineage inact. 2	Cobas	10	0.9 (0.5-1.7)	7.8 (3.6-39.7)
		Procleix	12	1.7 (0.8-3.0)	15.1 (5.7-340)

^inactivated standard also used for preparation of the P0247 WNV Check 125 control

Traceability to WNV standards

At the time of development of the P0247 WNV Check 125 control a WHO International Standard for WNV-RNA was not yet available. The inactivated S0169 WNV Lineage 2 standard, used for preparation of the run control, has been prepared from a tissue culture isolate (Nea Santa strain) obtained from a patient who became infected during an outbreak in Macedonia[3,4]. The S0169 WNV Lineage 2 standard was calibrated against the ISS 0410 and ISS 0109 WNV Lineage 1 standards kindly provided by Dr G. Pisani and Dr. K. Cristiano (Instituto Superiore di Sanità (ISS), Rome). These ISS standards have been calibrated in copies/mL and were directly traceable to the Roche standard and the Health Canada standard[11]. The calibration of the Macedonian WNV Lineage 2 standard against the ISS standards was performed by three laboratories using different real time PCR assays in which dilutions of the WNV Lineage 1 and 2 standards were tested in multiple replicates (table 3a and b). Since one assay was not sensitive for WNV Lineage 2, the calibration of the S0169 standard was based on the data reported by the Roche TaqScreen and GFE Blut PCR assays.

Table 3a. Calibration of WNV standards against ISS 0410 standard in three real time PCR says.

Standard	BioQ code	GFE blut		TaqScreen		In house	
		n	cp/mL	n	cp/mL	n	cp/mL
WNV-RNA lineage 1 (=ISS 0410)^	S0166	7	1,00E+05	4	1,00E+05	27	1.00E+05
WNV-RNA lineage 2 (Macedonia)	S0167	8	3,18E+09	6	6,00E+09	18	5.15E+06
WNV-RNA lineage 2 inact. (Macedonia)	S0169	7	3,38E+07	6	9,55E+06	18	1.23E+05
WNV-RNA lineage 1 (ISS 0109)#	S0165	2	1,31E+03			18	9.47E+02

^reference standard for calibration # calibrated by ISS at 1000 copies/mL

Table 3b. Final calibration result based multiple replicate tests with three WNV-RNA assays (table 3a)

Standard	BioQ code	Final assigned WNV-RNA concentration to standard	
		Labs#	Geomean cp/mL
WNV-RNA lineage 1 (=ISS 0410)^	S0166	3	1,00E+05
WNV-RNA lineage 2 (Macedonia)	S0167	2	4,37E+09
WNV-RNA lineage 2 inact. (Macedonia)	S0169	2	1,80E+07
WNV-RNA lineage 1 (ISS 0109)	S0165	2	1,01E+03

^reference standard for calibration

In house assay was excluded for calibration of WNV L2 standards

Stability of WNV standards and run control

The long term stability of liquid frozen viral RNA standards stored below -65˚C has been firmly established[12]. Real time stability experiments using quantitative NAT assays showed less than 5-10% degradation of viral RNA per annum when stored at -30˚C[12]. Stability studies on P0247 WNV 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 two formats as detailed in Table 4.

P0247/01 and P0247/02 are intended to accommodate blood screening laboratories. To facilitate automation the run control is presented in a polypropylene tube with screw cap comparable in size to vacutainer tubes used for donor sample collection. The tube label has a unique barcode identifying the product, sequential batch number, sequential tube number and marker WNV. The barcode can be read by the automated NAT systems, also in replicate tests within the same test run.

Table 4. Description of kit formats and contents

Cat. Code	UDI code	Quantity run control	Size vials	packing
P0247/01	8718719833971	60 x 1.6 mL	10 mL	60 vials in rack/box
P0247/02	8718719832141	10 x 1.6 mL	10 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[12]. 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[12]. 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 P0247 ViraQ WNV Check 125 contains inactivated WNV particles the plasma may still be potentially bio-hazardous. The matrix is prepared from human blood plasma 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 blood 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 plasma samples and take the necessary precautions. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[13,14].

- 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 to avoid formation of cryoprecipitates that could alter reactivity or cause pipetting errors in the automated sampling systems.
- Store 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 specimens and the result be calculated according to the instructions for use of the NAT procedure.

Performance data on run control and interpretation of results

Procleix WNV assay

The results of the Procleix WNV assays are expressed as a sample to cut-off ratio (S/CO). P0247 ViraQ WNV Check 125 Control should react positive in more than 99.5% of TMA test runs.

Figure 1 presents the S/CO values found during two months by one laboratory using 5 Panther instruments. The average (and range) of S/CO values was 13.63 (11.03-17.89) and similar for the 5 Panther instruments (table 5). A similar variation of S/CO values as seen in 179 test runs was also seen when the P0247 WNV Check 125 Control was tested 10 times in the same test run (orange dots in Figure 1).

Figure 1. Scatter plot of S/CO ratios on P0247 WNV Check 125 Control in Grifols Procleix WNV assay in 179 test runs on 5 Panther instruments. Orange dots represent 10 S/CO values obtained in one test run on one Panther instrument

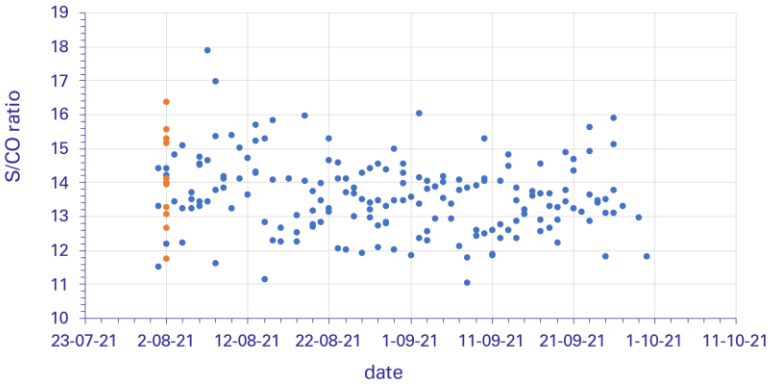
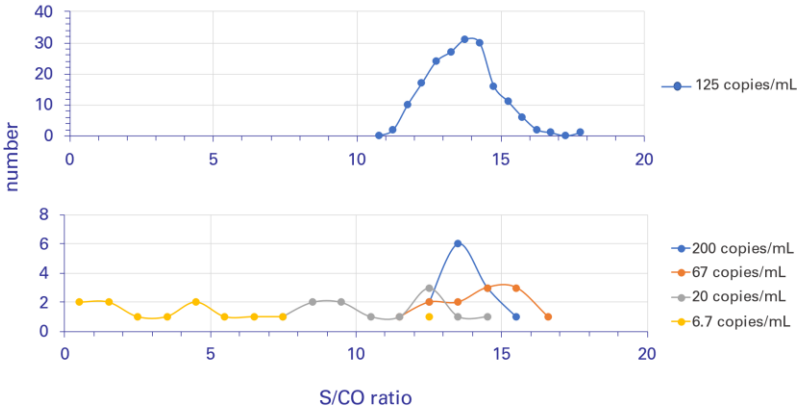


Table 5. Average (and range) of S/CO values found on P0247 WNV Check 125 Control with 5 Panther instruments

Panther	n runs	Average S/CO (range)
1	23	13,80 (11,75-16,37)
2	33	13,37 (11,13-17,89)
3	42	13,52 (11,03-15,3)
4	40	13,96 (11,51-16,04)
5	50	13,54 (11,79-15,83)

Figure 2 shows the distribution of 188 S/CO values on the P0247 ViraQ WNV Check run control of 125 copies/mL in comparison with those on concentrations of 200, 67, 20 and 6.7 copies/mL tested in 12 replicates. It can be seen that 200 and 125 copies/mL concentrations generate S/CO values in the saturation range of the assay but that lower concentrations closer to the 95% LOD of the Procleix assay generate values below the saturation point of the assay. It is expected that >98% of test results on the P0247 WNV Check Control will fall in the saturated range of the TMA assay with S/CO values varying between 11.0 and 17.0. Less than 2% of results are expected in the dynamic range of the TMA assay with S/CO ratios below 10.0.

Figure 2. Distribution of S/CO values on P0274 ViraQ WNV Check 125 Control (upper graph) as compared to 4 members of P0346 WNV L2 standard dilution panel (lower graph)



The S/CO responses on ViraQ WNV Check 125 in the Procleix assay are not normally distributed. A Gumbel distribution is likely more suitable to describe the data. From this type of extreme value distribution it follows that the difference between the median and the average of S/CO values is an indicator of the skewness of the distribution curve. Hence, the value of this parameter $\Delta(S/CO_{M-A})$ is expected to become higher with lower analytical sensitivity of the NAT system and may be used for trend analysis[15].

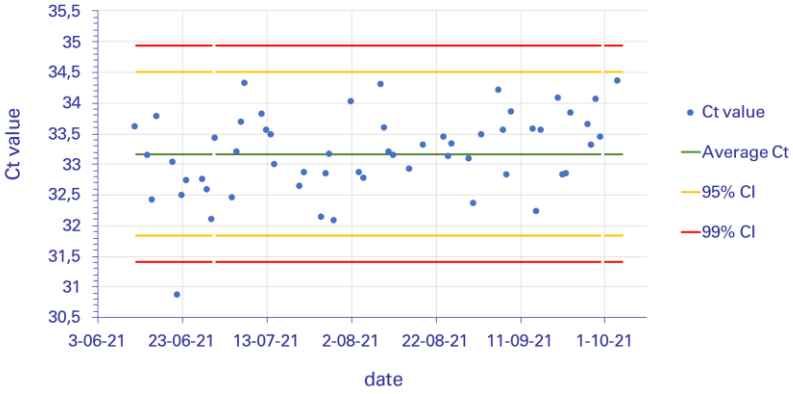
Cobas WNV assay

The cobas WNV assay gives qualitative results (reactive, nonreactive or invalid) and if reactive a Ct value is generated. P0247 ViraQ WNV 125 Control should react positive in more than 99.5% of test runs.

The positioning of P0247 ViraQ WNV 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 in the run control the Ct values in the cobas WNV assay are still normally distributed. Figure 3 shows performance data of this assay on the P0247 ViraQ WNV Check 125 Control in a Levey-Jennings chart with the following parameters:

- Number of test runs: 57
- Average Ct value: 33.17
- Standard deviation Ct: 0.66
- Student t value 95% CI: 2.0040
- Student t value 99% CI: 2.6682
- 95% CI of Ct: 31.84 – 34.51
- 99% CI of Ct: 31.40 – 34.95

Figure 3. Levey-Jennings chart of Ct values in Roche cobas WNV assay on P0247 WNV Check 125 Control observed in 57 test runs on the cobas 8800 instrument during three months



Hence for monitoring the performance of the cobas WNV assay one can use untransformed Ct values in a Levey-Jennings QC chart for trend analysis and apply the Westgard rules[16].

The expected average Ct value from standard dilution data was 32.97 or 2^0.2 = 1.15 fold different (in terms of a dilution factor) from the observed average Ct value of 33,17 on the run control. A 15% variation in the relative sensitivity of PCR reagent lots is not uncommon. Therefore we expanded the observed 99% CI of Ct values on the run control to an expected range between 31.2 and 35.15.

Limitations

- P0247 ViraQ WNV Check 125 Control cannot be used to evaluate the analytical or diagnostic sensitivity of NAT blood screening assays (although a significant reduction of analytical sensitivity of the NAT system can become apparent with occurrence of non-reactive and weak reactive results).
- P0247 ViraQ WNV 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 WNV concentrations cannot guarantee that 100% reactive results will be found on P0247 ViraQ HCV Check 125 Control in NAT blood screening assays. 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 P0247 ViraQ WNV Check 125 Control. Therefore it cannot be guaranteed that results will always be found within the expected ranges in the Procleix and cobas WNV assays when different reagent lots are used.

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Biologicals Quality Control B.V.
De Droogmakerij 31h
1851 LX, Heiloo
The Netherlands

Telephone : +31-72-2020730
E-mail: info@bioQControl.com
Internet: www.bioQControl.com

KI4247
V2.1 October 2021