Bio QControl

P0264
ViraQ HEV Check 125

CE

REF P0264

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

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Intended Use

P0264 ViraQ HEV Check 125 is intended to be used as external run control for monitoring consistent performance and ensuring sufficient analytical sensitivity of nucleic acid test (NAT) blood screening assays for detection of hepatitis E virus (HEV)-RNA as defined in Table 1.

Table 1. Nucleic acid blood screening kits covered by this run control

Manufacturer	Equipment	Agent	Test kit
Grifols Diagnostic Solutions	PANTHER®	Hepatitis E virus	Procleix® HEV Assay
Roche Molecular Systems	Cobas® 6800/8800		Cobas® HEV Assay

P0264 ViraQ HEV Check 125 must not be used to replace the internal kit controls or calibrators required for the release of test results. 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

Biological substance Category B


CE marked device


Expiry date

Contents


Caution


Read instructions for use

Principle of method

P0264 ViraQ HEV Check 125 enables blood screening laboratories to monitor performance and ensure sufficient analytical sensitivity of transcription mediated amplification (TMA) assays and real time polymerase chain reaction (RT-PCR) assays for the qualitative detection of HEV-RNA in plasma or serum samples. The external run control is designed to mimic naturally occurring plasma specimens with a low concentration of HEV-RNA. The concentration of HEV-RNA in P0264 ViraQ HEV Check 125 control samples is set at 100 International Units (IU)/mL and assumed to be equivalent to approximately 125 copies(cp)/mL. The concentration is measured by Sanquin (Amsterdam, the Netherlands) using a realtime PCR assay which is calibrated in IU/mL[1,2]. A conversion of approximately 1.25 copies being equal to 1.00 IU (with wide confidence bounds) is derived from the WHO collaborative study[3] after exclusion of outlier test results and by using limiting dilution data on in vitro RNA standards[4]. The concentration of 100 IU/mL is more than 5 times the 95 % lower limit of detection (LOD) of the Procleix HEV assay[4] and the cobas HEV assay[5], and comparable to the viral concentration of 125 copies/mL in other ViraQ Check 125 Controls for blood screening. The external run control is expected to generate sample to cut-off (S/CO) ratios in the

range between 10.0 to 30.0 in the Procleix HEV assay and Ct values in the lower 'quantitative' range of the cobas HEV assay with Ct values between 35.0 and 38.0. Changes to lower S/CO values or higher Ct values to the run control over time may indicate suboptimal performance of the automated NAT screening systems, deterioration of assay reagents or variation in analytical sensitivity of NAT reagent batches. The external run control tubes are barcoded and comparable in size to blood donor collection tubes. The tubes are ready for use after thawing and can be placed at random positions in sample racks of test systems. The HEV standard used for manufacturing of the run control is not inactivated and diluted in EDTA plasma negative for infectious disease markers. At the concentration present in the run control the risk of viral transmission by parenteral exposure is low since the minimum infectious dose (MID) was estimated to be approximately 10,000 IU of HEV-RNA[6,7]. To guarantee consistent detection and quantification of the viral load the run control sample should not be re-used.

Traceability to nucleic acid copies and International Units

In the WHO collaborative study[3] the assays reported 1.19 copies per 1.00 IU (after exclusion of laboratories with outlier test results). When comparing the 50% LODs on the WHO PEI 6329/10 genotype 3a standard and *in vitro* HEV RNA transcripts in the package insert of the Procleix HEV assay[4], a conversion factor of 1.46 copies per 1.00 IU was estimated. Since a reference measurement method for quantification in copies/mL is lacking the calibration of P0264 ViraQ HEV Check 125 Control was based on IU values assigned to the WHO 6329/10 standard and the concentration in the P0264 ViraQ Check Control was set at 100 IU/mL. Traceability and securing continuous and stable performance of P0264 ViraQ HEV Check 125 control is thus based on calibration of a secondary HEV-RNA standard against the WHO 6329/10 standard[3] and consistent manufacturing. The secondary standard is a plasma unit interdicted by the HEV-RNA screening program of the Sanquin Blood Supply Foundation (Amsterdam, the Netherlands)[1,2]. The concentration in the plasma unit was measured by replicate real time PCR tests against the WHO 6329/10 standard using a quantitative in house developed assay[1,2] and was reported to be 255,000 IU/mL (S0176 standard). The secondary standard was later replaced by another plasma standard (S0181) which was available in larger volume and was quantified at 54,000 IU/mL. In a later experiment dilutions of S0176 and S0181 standard were compared to the WHO 6329/10 standard in 16 replicate real time PCR tests per dilution. According to this calibration experiment the concentrations in the S0176 and S0181 standard were estimated at 331,500 and 51,200 IU/mL respectively. However, the originally assigned values to the S0176 and S0181 standards had been used for preparation of the run control batches of 100 IU/mL by gravimetrically recorded dilution steps. Therefore, in hindsight, the run control batches derived from the S0176 standard were found to have a significantly higher concentration than those prepared from the S0181 standard with average (and 95% confidence interval (CI)) concentrations of 129 (122-138) versus 93 (87-100) IU/mL respectively. The consistency of P0264 ViraQ HEV Check Control batches is guaranteed by the manufacturing methods and batch release testing procedure; and in the event of replacement of the secondary S0181 standard by extensive calibration of a new HEV genotype 3 plasma stock against the current S0181 and WHO 6329/10 standards.

Stability of HEV plasma standards and run control

The BioQControl viral stock solutions are stored at -80°C at which temperature viral RNA standards are known to be stable[8]. The stability of P0264 ViraQ HEV Check Controls stored at -30°C can best be established by comparison against the same run control

batch stored at -80°C. After 66 months of storage at -30°C the potency of P0264 batch 4976-001 was estimated at 87 (61-122)% as compared to batch 4976-001 stored at -80°C by performing 16 replicate quantitative PCR tests per temperature. In the same experiment batch B4976-006 stored for 26 months at -30°C was quantified at 93 (72-119)% of the reference batch stored at -80°C. From this experiment it is claimed that the decay of HEV-RNA in the P0264 run control is less than 10% (and not significantly different from 0%) during 4 years of storage at -30°C. Therefore the shelf life of P0264 ViraQ Check Control has been extended from two years to four years when stored at -30°C.

Kit Contents

The run control contains human plasma without preservatives and is provided in two formats as detailed in Table 2.

Table 2. Description of kit formats and contents.

Cat. Code	UDI code	Quantity run control	Size vials	packing
P0264/01	8718719830098	60 x 1.5 mL	10 mL	60 vials in rack/box
P0264/02	8718719830304	10 x 1.5 mL	10 mL	Plastic zip bag

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. In addition, the label includes a barcode identifying the product, sequential batch number and marker. The barcode of each run control tube is unique and can be read by the Roche cobas 6800/8800 instrument.

Storage instructions

The run controls should be stored at or below -30°C for a maximum of four years[9]. 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. Do not refreeze the controls after thawing to prevent formation of cryoprecipitates. Any control sample that appears cloudy or contains precipitates after thawing and mixing should be discarded.

Warning and precautions

Although P0264 ViraQ HEV Check 125 control has viral concentration below the minimum infectious dose[6,7] 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, HEV-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[10,11].

- 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
- Allow run control to reach room temperature before testing.
- Use within 8 hours after thawing
- After thawing when not in use: store at 2-8°C

Interpretation of results

P0264 ViraQ HEV Check 125 Control should be reactive in more than 99.9% of NAT blood screening test runs, both with the Grifols Procleix HEV Assay and the Roche cobas HEV assay. The run control concentration of 100 IU/mL is positioned at approximately 5 times the 95% lower limit of detection (LOD) of the cobas MPX assay and at more than 10 times the 95% LOD of the Procleix HEV assay (table 2).

Table 3 Analytical sensitivity of the Procleix HEV Assay and cobas MPX assay and predicted reactivity on run control

Source WHO standard dilution panels	HEV assay	Replicate tests per dilution	50 % LOD (IU/mL)	95 % LOD (IU/mL)	Predicted reactivity on 100 IU/mL run control
Grifols^	Procleix	162	2.0 (1.7-2.3)	7.1 (6.6-9.8)	>99.9 %
BioQControl		18	2.5 (1.7-3.5)	8.6 (5.4-24.6)	>99.9%
Roche^	cobas	189	3.8 (2.6-5.1)	18.6 (15.9-22.6)	>99.9%

^data from package inserts

At the concentration of 100 IU/mL the S/CO values in the Procleix HEV assay and the Ct values in the cobas HEV assay are normally distributed. Therefore the S/CO values and Ct values can be plotted in a Levey-Jennings graph and interpreted according to the Westgard rules[13]. The expected results can be deduced from the performance evaluation data in the Procleix and cobas HEV assays presented below.

Performance evaluation data

Grifols Procleix HEV assay

Figure 1 and table 4 summarize four years of Procleix HEV test data on P0264 ViraQ HEV Check Control.

Figure 1. Performance of consecutive Procleix HEV assay reagent lots on P0264 ViraQ HEV Check 125 run control batches over a 4 year evaluation period. Each color represents a Procleix lot and ViraQ run control batch combination

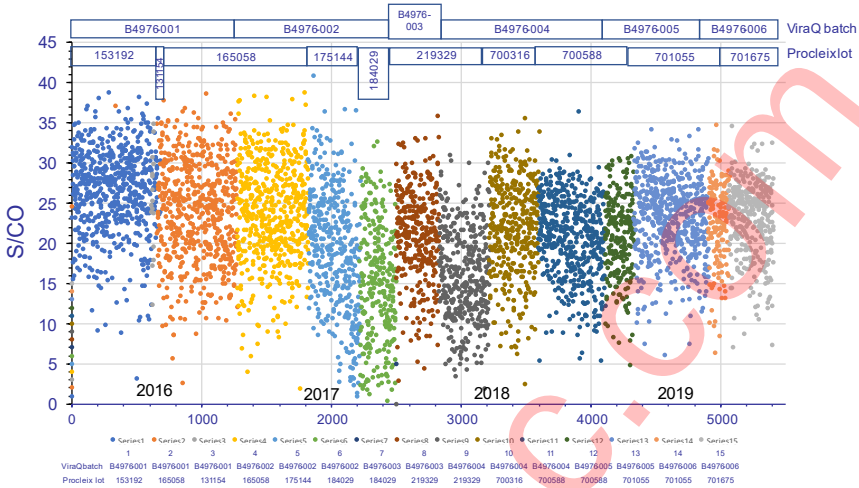


Table 4. Reproducibility of Procleix HEV assay on ViraQ run control: mean S/CO and 95% confidence limits over a 4 year period comparing 15 combinations of run control batches and Procleix reagent lots.

ViraQ batch	Procleix lot	n	Average S/CO (95% CI)	Min S/CO	Max S/CO
B4976-001	153192	643	26.46 (16.23-36.68)	3.2	38.77
B4976-001	165058	613	23.95 (11.88-36.03)	2.64	38.69
B4976-001	131154	16	25.13 (11.97-38.30)	12.31	34.88
B4976-002	165058	550	23.82 (11.79-38.30)#	1.92	38,73
B4976-002	175144	398	19.13 (4.69-33.56)#	0,87	40.92
B4976-002	184029	276	14.91 (0.87-28.96)	0.42	32.63
B4976-003	184029	1	4.93	4.93	4.93
B4976-003	219329	333	21.06 (9.97-32.15)^	2.87	35.93
B4976-004	219329	387	16.01 (4.87-27.15)^$	1.92	31.02
B4976-004	700316	378	21.76 (10.33-33.19)#$	2.49	35.58
B4976-004	700588	513	20.67 (10.86-30.49)	5.33	36.41
B4976-005	700588	224	20.74 (10.77-30.72)	4.79	30.85
B4976-005	701055	572	23.14 (13.77-32.51)	6.07	34.24
B4976-006	701055	158	22.46 (12.52-32.40)	6.33	34.71
B4976-006	701675	342	22.91 (13.94-31.88)	7.03	34.59

# p<0.0001, ^p<0.0001 $ p<0.0001 (Student t test)

ViraQ batches had overlapping S/CO distributions within one Procleix reagent lot (see example Figure 2) except when batch B4976-003 was compared with B4976-004. Here

significantly lower S/CO values were found on B4976-004 than on B4976-003 within the same reagent lot 219329 ($p<0.0001$, Student t-test). This could later be explained by a higher concentration (95% CI) of 129 (122-138) IU/mL in run control batch 003 (derived from standard S0176) than 93 (87-100) IU/mL in batch 004 (prepared from standard S0181) due to initial underestimation of the viral load in HEV standard S0176 (see traceability chapter above)[9]. There was no difference in S/CO value distribution between batches 004, 005 and 006 in 4 Procleix reagent lots (table 4).

Figure 2. Distribution of S/CO values on two batches of P0264 ViraQ HEV Control in 1163 test runs of Procleix Master Lot 165058

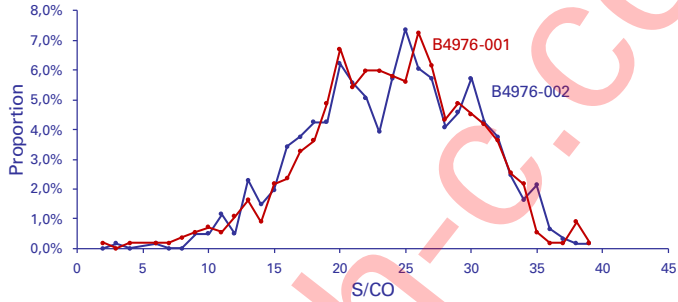


Figure 3. Distribution of S/CO values on P0264 ViraQ HEV 125 control in 4 consecutive Procleix HEV reagent lots of different analytical sensitivity

During one period nonreactive results were found in two Procleix lots (175144 and 184029) that generated significantly lower S/CO values on the run control than the previously used reagent lots (Figure 3). A customer investigation demonstrated significant lower analytical sensitivity of the reagent lot in use (table5).

Table 5. Customer investigation of reduced analytical sensitivity of Procleix HEV Master Lot 184029 by testing BioQ P0262 and P0274 HEV genotype 3 standard dilution panels

	% reactive in Grifols Procleix package insert	% reactive in customer investigation of Lot 184029	
IU/mL	WHO 6329/10 standard	WHO 6329/10 standard	BioQ S0181 standard
100			24/24 (100%)
90	162/162 (100%)	31/32 (97%)	
30	162/162 (100%)	28/32 (88%)	21/24 (88%)
10	159/162 (98%)	13/32 (41%)	14/24 (58%)
3	109/162 (67%)	5/32 (16%)	4/24 (17%)
1	44/162 (27%)	1/32 (3%)	1/24 (4%)
50% LOD	2.0 (1.7-2.3)	10.3 (7.6-14.0)	8.0 (5.6-11.3)
95% LOD	7.9 (6.6-9.8)	67.8 (42.7-137.8)	47.0 (28.8-103.7)

The 4 year performance evaluation of P0264 ViraQ HEV Check 125 Control in the Procleix HEV assay demonstrates that the run control is instrumental in recognizing deterioration of the HEV NAT test system or inconsistency of NAT reagent batches. Nonreactive results and increased proportions of S/CO values <10.0 are indicative of reduced analytical sensitivity of the Procleix HEV test system.

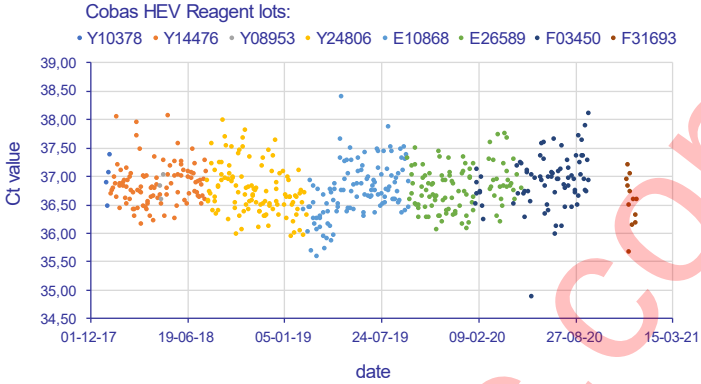
Roche cobas HEV assay

Figure 4 and table 6 summarize three years of cobas HEV test data on P0264 ViraQ HEV Check Control. Consistent mean Ct values and 95% confidence limits were generated with different cobas reagent lots except for one period in which Ct values became slightly lower, possibly due to an instrument calibration issue.

Table 6. Mean S/CO values (and 95% confidence intervals) found by testing of P0264 HEV Check 125 control (batch B4976-005 and 006) in consecutive test runs of the Roche cobas HEV assay

cobas HEV lot^	n	Ct value (95% CI)
Y10378	4	36.95 (35.33-38.57)
Y14476	101	36,85 (36,13-37,58)
Y08953	3	36.81 (34.08-39.54)
Y24806	113	36.75 (35.89-37.61)
E10868	113	36.73 (35.78-37.69)
E26589	102	36.79 (36.02-37.55)
F03450	76	36.91 (35.96-37.85)
F31693	12	36.56 (35.59-37.53)

Figure 4. Ct values in Roche cobas HEV assay on P0264 HEV Check 125 control obtained during three years of testing with consecutive cobas HEV reagent lots



From the performance evaluation of the run control it can be deduced that increased proportions Ct values above 37.5 or below 36.0 are indicative of inconsistencies in the cobas HEV NAT detection system.

Limitations

- P0264 ViraQ HEV Check 125 Control is not intended to be used for evaluation of 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 increased occurrence of low reactive or non-reactive results or an upward shift in Ct values).
- P0264 ViraQ HEV Check 125 Control must not be substituted for the mandatory controls or calibrators provided with IVD test kits for calculating the cut off and/or criteria for releasing test results.
- Due to possible variation in NAT reagent lots general applicable confidence bounds for a NAT system cannot be given. Laboratories should calculate their own mean and 95% CI for the NAT reagent lot in use and interpret results according to the Westgard rules[12]
- The run control product should not be used to determine the accuracy of quantitative results in IU/mL reported by real time PCR assays since the exact viral concentration of 100 IU/mL cannot be guaranteed and the variability of quantitative results at this low level is too high due to impact of Poisson distribution. Moreover the accuracy of different quantitative assays in reporting in IU/mL values may be affected by differences in calibration of the NAT systems

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