P0154
ViraQ HBV Trend 50

CE
0483

IVD

REF P0154

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

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Intended Use

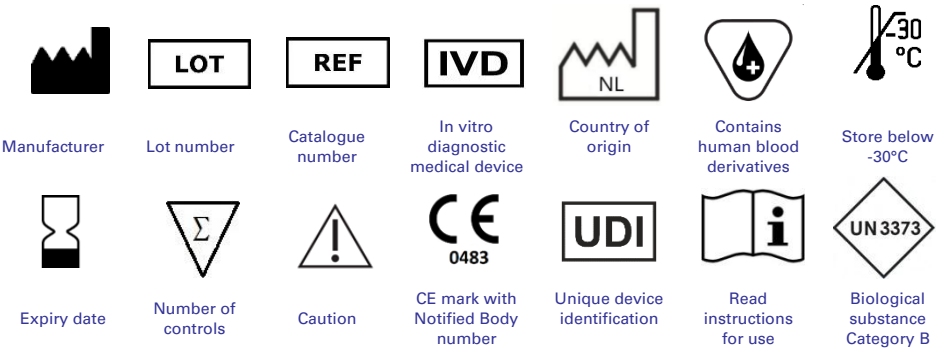
P0154 ViraQ HBV Trend 50 is intended to be used as external trend control for detection of hepatitis B virus (HBV)-DNA by the nucleic acid amplification technology (NAT) assays on the platform defined in Table 1. P0154 ViraQ HBV Trend 50 is a heat-inactivated HBV-DNA standard in plasma at a concentration of 50 copies/mL giving approximately 95% to 99% reactive results in the Procleix Ultrio assay versions. The trend control is intended to be used by trained laboratory workers. The trend control helps laboratories to ensure sufficient analytical sensitivity and consistent performance of the multiplex HBV/HCV/HIV transcription mediated amplification (TMA) assays. The trend control can be used in daily test runs to continuously monitor NAT performance over time or tested occasionally in multiple replicates in one test run for acceptance (transport integrity) testing of TMA reagent lots.

P0154 ViraQ HBV Trend 50 should not be used to replace the internal controls or calibrators in the test kits. The test result on the trend control should not be used to reject the run or delay the release of test results on donor samples.

Table 1. Assays and platform covered by P0154 ViraQ HBV Trend 50 control.

Assay (manufacturer)	Platform	Test environment
Procleix® Ultrio Elite (Grifols)	Procleix® Panther®	Blood screening
Procleix® UltrioPlex E (Grifols)		

Key to Symbols Used



Principle of method

P0154 ViraQ HBV Trend 50 control has been formulated to mimic natural plasma specimens with a low HBV-DNA concentration. After thawing the trend control tubes are ready for use and can be placed at random positions in sample racks on the NAT platform. The trend control contains 50 copies/mL of heat-inactivated HBV-DNA (equivalent to 9.4 IU/mL). The trend control has been designed to ensure sufficient analytical sensitivity of TMA tests in blood screening laboratories. The HBV-DNA concentration in the trend control has been set near the 95% lower limit of detection (LOD) of the Ultrio, Ultrio Plus, Ultrio Elite and UltrioPlex E assays (Table 2)[1-6]. The trend control enables laboratories to be alerted in case of a significant reduction of the analytical sensitivity of NAT test systems and to identify changes in TMA performance over time. The trend control is a

dilution of the S0043 HBV-RNA genotype A2 standard, prepared by heat-inactivation of a pool of HBV positive plasma units from the same donor[7-9]. The plasma matrix in which the trend control is diluted is manufactured from plasma units that tested negative for all relevant markers of blood borne viruses. The S0043 HBV standard has been calibrated in copies/mL and IU/mL against the Viral Quality Control (VQC)-Sanquin, Eurohep and World Health Organization (WHO) International Standards (Figure 1). The low concentration of HBV genotype A in the trend control is representative for HBV genotypes A to H that are prevalent in different geographical regions of the world (and that are detected with similar analytical sensitivity by the above mentioned commercial NAT assays)[1-6,10]. A positive result on the trend control indicates that the NAT method has been performed with sufficient analytical sensitivity. A higher-than-expected proportion of non-reactive or weakly reactive results is indicative of reduced analytical sensitivity of the NAT system and should trigger investigation of the technical performance of the assay. The trend control generates sample to cut-off (S/CO) ratios in the Procleix Ultrio assay versions. Statistical analysis of these assay response values generated over a certain period allows for comparison of analytical performance of NAT reagent lots and laboratory instruments. For this purpose, users of the Procleix assays in combination with the P0154 ViraQ HBV Trend 50 Control can generate a statistical report for post-market performance follow-up[11] using the DataQ Analytics system accessible via the website www.bioqcontrol.com. The trend control can also be used in multiple replicates in the same test run to ensure that TMA reagents fulfil the minimum requirements for analytical sensitivity before they are accepted for routine blood screening. A summary of the safety and performance of the P0154 ViraQ HBV Trend 50 control will be made available at the EUDAMED website of the European Union, and is currently available upon request[12].

Table 2. Detection limits on native and inactivated HBV standard dilution panels in Procleix Ultrio assay versions calculated by an alternative link function instead of probit analysis for a better fit of the dose response curve.

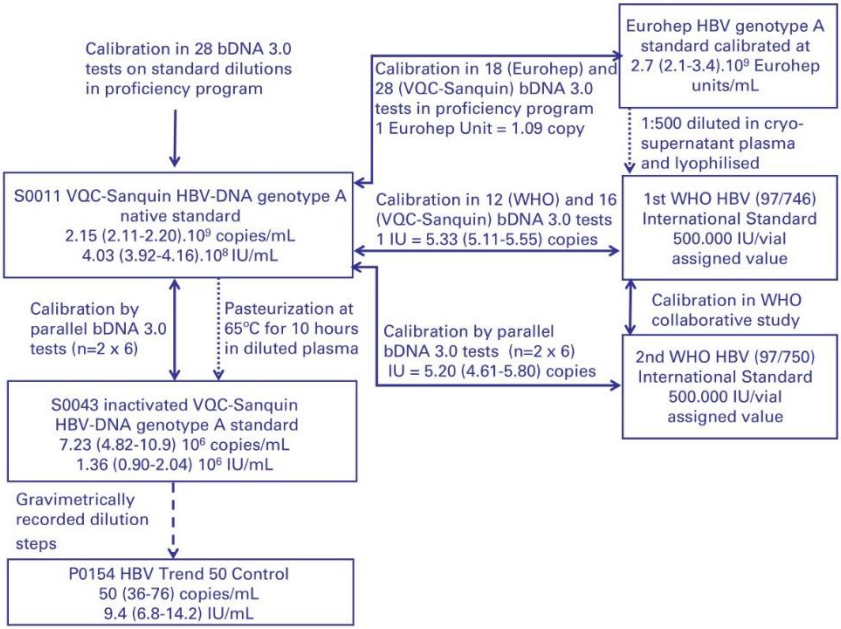
standard	BQC panel	NAT method	n	50% LOD (CI) copies/mL	95% LOD (CI) copies /mL
S0043 BioQ HBV-DNA genotype A inactivated	P0031	Ultrio Plus	24	8.4 (5.7-11.9)	38.1 (24.9-75.1)
	P0031	Ultrio Elite	25	7.2 (4.9-9.9)	30.3 (20.6-54.4)
	P0031	Ultrio Plus/Elite	49	7.8 (6.0-9.8)	33.9 (25.2-50.9)
S0011 VQC-Sanquin HBV-DNA genotype A	P0007	Ultrio Plus	48	6.1 (4.7-7.7)	25.2 (18.6-38.3)
	P0007	Ultrio Elite	74	4.4 (3.4-5.5)	28.5 (21.2-41.9)
	P0007	Ultrio Plus/Elite	122	5.0 (4.2-5.9)	27.4 (21.9-36.0)
	P0280	Ultrio Elite	48	6.6 (5.0-8.6)	38.4 (27.5-60.1)
	P0280	UltrioPlex E	98	7.4 (6.2-8.8)	36.9 (29.3-49.0)
S0010 Eurohep HBV-DNA genotype A	P0001	Ultrio Plus	96	4.7 (3.8-5.7)	28.8 (22.5-38.9)
	P0001	Ultrio Elite	24	9.6 (6.4-13.3)	38.9 (26.1-75.7)
WHO HBV-DNA 97/750#	P0023	Ultrio Plus	303	4.4 (3.3-5.9)	28.4 (18.0-57.7)
	P0023	Ultrio Elite	252	4.4 (3.6-5.4)	30.9 (22.4-47.4)

1 IU = 5.33 copies

Traceability to HBV-DNA copies and International Units

Figure 1 shows the traceability chain between the ViraQ trend control, the inactivated and native VQC-Sanquin standard, the Eurohep standard and the 1st and 2nd WHO 97/746 and 96/750 International Standards for HBV-DNA. The inactivated S0043 HBV-DNA standard (used for preparation of the P0154 ViraQ trend control) is derived from the S0011 VQC-Sanquin HBV-DNA genotype A standard and has been calibrated in copies/mL by replicate testing in the Siemens Versant bDNA 3.0 assay[13] against this primary standard[14]. The VQC-Sanquin HBV-DNA genotype A standard has been calibrated at 5.33 (5.11-5.55) and 5.20 (4.61-5.80) copies per IU against the first and second WHO HBV-DNA (97/746 and 97/750) standards respectively in two experiments[14]. It must be emphasized that this conversion factor from copies to IU values has not been confirmed for the later 3rd WHO 10/264 replacement standard. The copy number assigned to the VQC-Sanquin standard was found to be comparable to that of the Eurohep standard[15] used for preparation of the WHO standards[16]. The accurate calibration of the native and inactivated VQC-Sanquin standards against the WHO and Eurohep standards in IU/mL and in copies/mL has been confirmed in analytical sensitivity studies of the Grifols Procleix TMA and Roche cobas MPX assays[1-5,14]. The manufacturing and quality control procedures of BioQControl guarantee consistent virus concentrations in consecutive ViraQ HBV Trend 50 batches[11,17]. The inactivated VQC-Sanquin HBV genotype A standard is available in sufficient supply to ensure batch to batch consistency of ViraQ trend controls for a prolonged period.

Figure 1. Traceability chain between trend control, inactivated and native VQC-Sanquin, Eurohep and WHO HBV genotype A International Standards.



Stability of HBV standards and trend control

The long-term stability of the liquid frozen native S0011 and inactivated S0043 HBV standard stored at ≤65˚C has been firmly established[18]; hence the stock solution from which the trend control is prepared has shown to be stable in the BioQControl storage facilities. Real time stability experiments using quantitative NAT assays showed no degradation of HBV-DNA in in standard dilutions of higher concentration than in P0154 HBV Trend 50 control when stored at -30˚C for five years [18]. Since the relative degradation is independent of concentration the degradation estimate can be extrapolated to P0154 HBV Trend 50 control. Hence, it can be guaranteed that the trend control is still functional and should generate a reactivity rate greater near 95% when stored at -30˚C and used before the expiration date (five years after preparation of the trend control batch)[6,11,17].

Kit contents (materials provided)

The trend control contains human plasma without preservatives and is provided in two formats as detailed in Table 3.

Table 3. Description of P0154 ViraQ HBV Trend 50# kit formats and contents.

Cat. Code	GTIN/UDI-DI code^	Quantity	Tube size	Sample volume	Secondary packing
P0154/01	8718719831540	60 tubes	10 mL	1.4 mL (+overfill)	Rack in box
P0154/02	8718719832110	10 tubes	10 mL	1.4 mL (+overfill)	Plastic zip bag

^ Global Trade Item Number = Unique Device Identification - Device Identifier (UDI-DI) code,
# Global Model Number (GMN) = Basic UDI code for P0154 is 871871983P0154EP

P0154/01 and P0154/02 are intended to accommodate blood screening laboratories. To facilitate automation the trend 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: HBV. The barcode of each trend control tube can be read by the automated NAT systems.

Materials required but not supplied

The test kits and liquid handling devices by the NAT manufacturer as specified in Table 1, a water bath of 37°C for quickly thawing of trend control and a vortex instrument for thorough mixing of samples are not provided.

Storage instructions

The trend controls should be stored at or below -30°C for a maximum of five years after preparation of the trend control batch and not be used after the expiry date[18]. Once thawed the trend control samples should be used within 8 hours. During this period, when the trend control is not in use, store sample at 2-8°C[18]. 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 P0154 ViraQ HBV Trend 50 contains inactivated HBV particles[7,8] the plasma may still be potentially biohazardous. 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-HBs, anti-HIV, anti-HCV, anti-HTLV and anti-Treponema *pallidum*). No test method can offer complete assurance that products derived from human blood cannot transmit (unknown) infectious agents. The trend 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[19,20].

- 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 trend 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 trend controls for 2 minutes thoroughly.
- Once thawed, do not re-freeze and thaw the trend control samples to avoid formation of cryoprecipitates that could alter reactivity or cause pipetting errors in the automated sampling systems
- Store trend controls in an upright position.

Reagent preparation

- Thaw the trend control quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed.
- Immediately after the ice clot has disappeared remove the trend control tube from the water bath.
- Before testing allow the trend control tube to adapt to room temperature.
- Mix the trend control tube thoroughly prior to use with a vortex instrument.
- Give a short spin in a centrifuge to remove liquid before releasing screw cap from vial.
- Minimise the time from thawing until testing of the control samples.
- Test trend control sample within 8 hours after thawing.
- After thawing when not in use, store trend control tube at 2-8°C.

Analytical Performance Characteristics

The results of the Procleix Ultrio assays are expressed as a sample to cut-off ratio (S/CO). P0154 ViraQ HBV Trend 50 Control should be reactive in approximately 95% to 99% of TMA test runs.

The positioning of P0154 ViraQ HBV Trend 50 control is near the 95% lower limit of detection (LOD) of the Procleix Ultrio assays. This has been demonstrated by testing dilution panels of the native S0011 HBV genotype A standard (Figure 2) and of the inactivated S0043 HBV genotype A standard used for preparation of the trend control (Figure 3). At the concentration of 50 copies/mL S/CO ratios follow a non-parametric distribution.

Figure 2. Distributions of S/CO ratios on dilutions of native S0011 HBV standard dilutions in reference panel P0280 tested in 48 replicates by the UltrioPlex E assay with a 95% LOD (CI) of 36.9 (29.3-49.0) copies/mL.

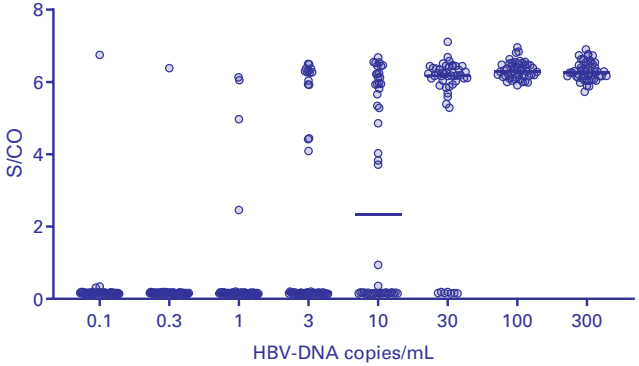
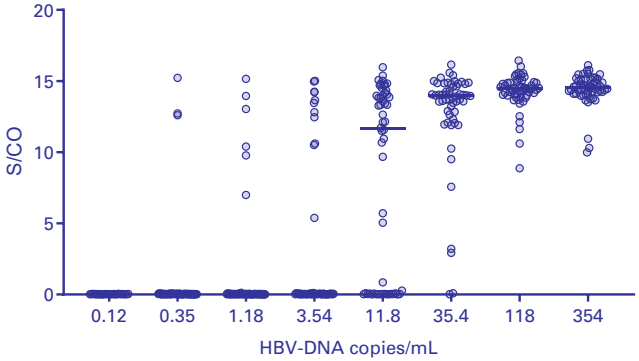


Figure 3. Distributions of S/CO ratios on dilutions of inactivated S0043 HBV standard dilutions in P0031 reference panel tested by the Ultrio Plus and Ultrio Elite assays with a 95% LOD (CI) of 33.9 (25.2-50.9) copies/mL being ~1.5-fold lower than the trend control concentration of 50 (36-76) copies/mL.



Expected assay response values on trend control

The expected results for the P0154 ViraQ HBV Trend 50 Control are as follows:

Procleix Ultrio Elite assay
HBV-DNA S/CO ratios range: 0.0 – 15.7

Procleix UltrioPlex E assay
HBV-DNA S/CO ratios range: 0.0 – 7.9

Approximately 95% to 99% of results in these assays are expected to be reactive, with approximately 85 to 90% of results in the saturation range of the assays. 5% to 10% of results are expected to be in the dynamic range of the assay, indicating incomplete TMA reactions. Each TMA assay and reagent lot has its own distribution of response values on

HBV standard dilutions and on P0154 ViraQ HBV Trend 50 Control. This depends on the design of the assay and the analytical sensitivity of the reagent lots that are in use. Thus, it cannot be guaranteed that the assay response values will always fall within these ranges.

Interpretation of results

The trend 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. Subsequent test runs can be analysed by appropriate statistical approaches on the response values obtained on the external control samples. A software system (DataQ Analytics) is available via the website www.bioqcontrol.com for entering response values and generating a statistical report with assay-specific calculations for preparing a Levey-Jennings Chart.

Calculations

Since the Procleix Ultrio Elite and UltrioPlex E assays return non-parametrically distributed S/CO ratios, predictive ranges are based on the percentiles of the data range. Calculate the 95% and 99% predictive limits as follows:

- 99% Lower limit: (interpolated) 0.005th percentile
- 95% Lower limit: (interpolated) 0.025th percentile
- 99% Upper limit: (interpolated) 0.995th percentile
- 95% Upper limit: (interpolated) 0.975th percentile

Test the trend control at least 30 times during the reference period. The Levey-Jennings chart (Figures 5 and 6) is designed to identify individual aberrant values outside the 95% and 99% ranges. By collecting additional data, the chart characteristics may be updated. Use Nelson rules[21] to identify deviations in the Levey-Jennings trend analysis.

The Procleix Ultrio Elite and UltrioPlex E test results on P0154 ViraQ HBV Trend 50 control are expected to be reactive in 95-99% of cases according to alternative link analysis on the inactivated S0043 HBV genotype A standard dilutions in the P0031 reference panel. A decrease in the proportion of reactive results or results in the saturated range of the assay is indicative of reduced analytical sensitivity of the NAT system.

Levey-Jennings Chart

The S/CO ratios obtained in consecutive Procleix Ultrio Elite and UlrioPlex E test runs for HBV-DNA can be plotted in a Levey-Jennings chart using the statistical report function of the DataQ Analytics system available on www.bioqcontrol.com as shown in the charts of Figure 5 and Figure 6.

Table 8 summarizes the Levey-Jennings chart parameters in Figure 5, which were deduced from the Ultrio Elite S/CO ratios reported on two P0154 ViraQ HBV Trend Control batches by one laboratory over a period of 15 months.

Table 8. Median S/CO ratios with 95% and 99% ranges as found on two batches of P0154 ViraQ HBV Trend 50 Control in 2541 Ultrio Elite test runs in the period from 16-07-2021 to 13-09-2023 (data in Figure 5).

Assay	n	Median S/CO	95% range S/CO	99% range S/CO
Ultrio Elite	2541	13.96	8.99 – 15.23	0.05 – 15.56

Table 9 summarizes the Levey-Jennings chart parameters in Figure 6, which were calculated from the UltrioPlex E S/CO ratios reported on one P0154 ViraQ HBV Trend Control batch by one laboratory over a period of 1 month.

Table 9. Median S/CO ratios with 95% and 100% ranges as found on one batch of P0154 ViraQ HBV Trend 50 Control in 90 UltrioPlex E test runs in the period from 22-08-2024 to 4-09-2024 (data in Figure 6).

Assay	n	Median S/CO	95% range S/CO	100% range S/CO
UltrioPlex E	90	7.21	0.09 – 7.82	0.08 – 7.88

Figure 5. Levey-Jennings QC chart of Ultrio Elite assay S/CO ratios for HBV-DNA on two batches of P0154 ViraQ HBV Trend Control.

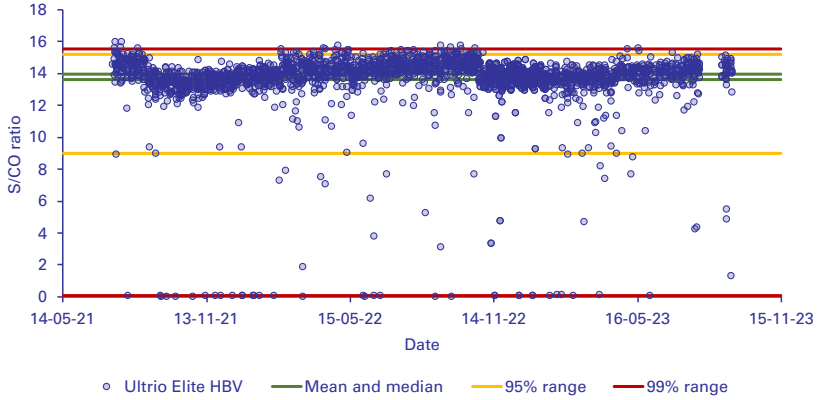
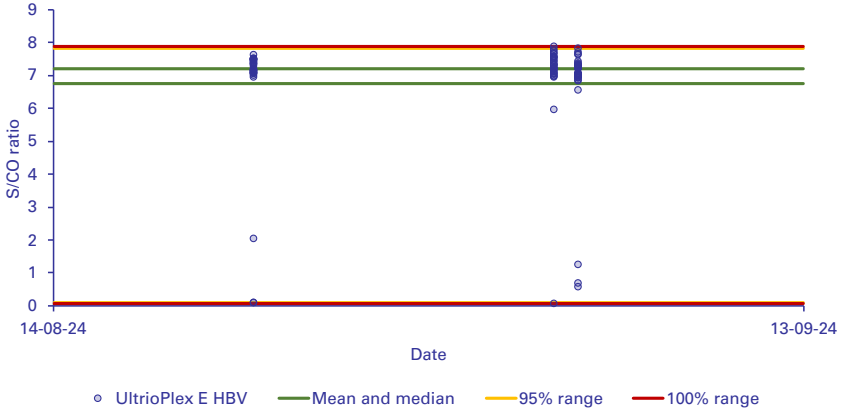


Figure 6. Levey-Jennings QC chart of UltrioPlex E assay S/CO ratios for HBV-DNA on one batch of P0154 ViraQ HBV Trend Control.



Post-market performance follow-up of trend control batches

One can use P0154 ViraQ HBV Trend 50 in combination with the DataQ Analytics system on www.bioqcontrol.com to compare the S/CO ratios on different trend control batches, Procleix Ultrio Elite and UltrioPlex E reagent lots or Procleix Panther instruments. Table 10 and Figure 7 compare the median S/CO ratios in the Ultrio Elite assay on P0154 ViraQ HBV batches and Procleix Ultrio reagent lots for HBV-DNA, showing moderate variability in analytical sensitivity. However, all ViraQ batch-reagent lot combinations had a reactivity rate of >90%, and 40 out of 41 combinations (98%) had a reactivity rate of >95%. The preliminary data of the UltrioPlex E assay is based on test runs with only one P0154 ViraQ batch (B4154-013) and one test reagent lot (707270). Therefore, no comparisons can be made for the UltrioPlex E assay. However, the reactivity rate of 94% in this limited data set indicates sufficient analytical sensitivity in the UltrioPlex E assay for HBV-DNA.

Table 10. Comparison of Ultrio Elite S/CO on P0154 ViraQ HBV Trend 50 Control batches and between consecutive Procleix Ultrio Elite reagent lots.

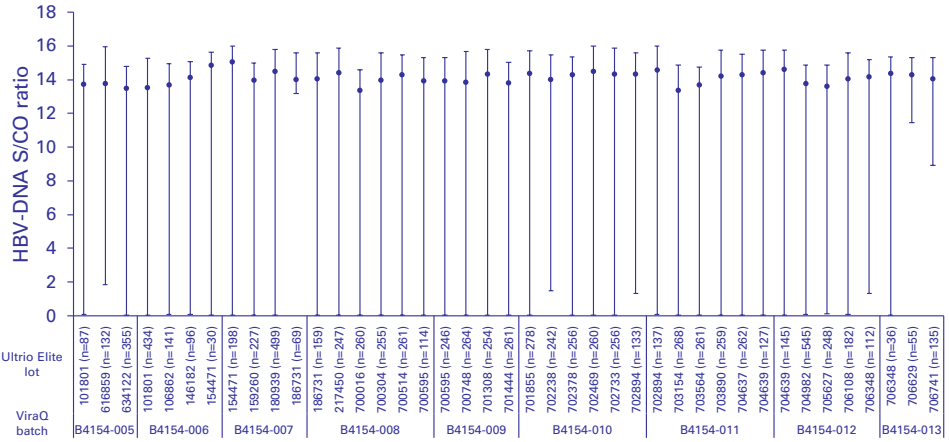
P0154 ViraQ batch	Ultrio Elite reagent lot	n	Median S/CO (range)	Avg. S/CO	$\Delta S/CO_{M-A}$	S/CO values ≥ 1
B4154-005	101801	87	13.75 (0.06 - 14.93)	13.30	0.45	85 (97.70%)
	616859	132	13.80 (1.86 - 15.96)	12.60	1.19	132 (100.00%)
	634122	355	13.49 (0.05 - 14.80)	12.90	0.59	345 (97.18%)
	All	**574**	**13.55 (0.05 - 15.96)**	**12.89**	**0.66**	**562 (97.91%)**
B4154-006	101801	434	13.55 (0.04 - 15.26)	13.17	0.38	427 (98.39%)
	106862	141	13.69 (0.07 - 14.94)	13.19	0.50	138 (97.87%)
	146182	96	14.16 (0.08 - 15.07)	13.76	0.39	95 (98.96%)
	154471	30	14.88 (0.04 - 15.65)	13.65	1.23	28 (93.33%)
	All	**701**	**13.72 (0.04 - 15.65)**	**13.27**	**0.45**	**688 (98.15%)**
B4154-007	154471	198	15.07 (0.04 - 15.98)	14.46	0.61	192 (96.97%)
	159260	227	13.97 (0.03 - 15.01)	13.31	0.66	219 (96.48%)
	180939	499	14.49 (0.03 - 15.81)	14.14	0.35	489 (98.00%)
	186731	69	14.03 (13.18 - 15.58)	14.11	-0.08	69 (100.00%)
	All	**993**	**14.43 (0.03 - 15.98)**	**14.01**	**0.42**	**969 (97.58%)**
B4154-008	186731	159	14.08 (0.02 - 15.59)	13.74	0.34	156 (98.11%)
	217450	247	14.42 (0.04 - 15.87)	14.19	0.23	243 (98.38%)
	700016	260	13.39 (0.04 - 14.57)	12.90	0.49	253 (97.31%)
	700304	255	14.00 (0.02 - 15.59)	13.70	0.30	250 (98.04%)
	700514	261	14.29 (0.05 - 15.46)	13.84	0.45	256 (98.08%)
	700595	114	13.96 (0.03 - 15.33)	13.27	0.69	111 (97.37%)
	All	**1296**	**14.05 (0.02 - 15.87)**	**13.63**	**0.42**	**1269 (97.92%)**
B4154-009	700595	246	13.94 (0.03 - 15.32)	13.29	0.64	236 (95.93%)
	700748	264	13.88 (0.03 - 15.66)	13.19	0.69	255 (96.59%)
	701308	254	14.34 (0.03 - 15.80)	13.75	0.59	249 (98.03%)
	701444	261	13.83 (0.05 - 15.02)	13.62	0.21	258 (98.85%)
	All	**1025**	**13.98 (0.03 - 15.80)**	**13.46**	**0.52**	**998 (97.37%)**

(continued)

Table 10 *(continued)*

P0154 ViraQ batch	Ultrio Elite reagent lot	n	Median S/CO (range)	Avg. S/CO	$\Delta S/CO_{M-A}$	S/CO values ≥ 1
	701855	278	14.41 (0.04 - 15.71)	14.26	0.14	277 (99.64%)
	702238	242	14.03 (1.48 - 15.46)	13.97	0.05	242 (100.00%)
	702378	256	14.32 (0.05 - 15.36)	13.96	0.36	252 (98.44%)
B4154-010	702469	260	14.51 (0.03 - 16.00)	14.14	0.37	255 (98.08%)
	702733	256	14.35 (0.05 - 15.88)	14.23	0.12	255 (99.61%)
	702894	133	14.33 (1.32 - 15.58)	14.12	0.21	133 (100.00%)
	All	**1425**	**14.34 (0.03 - 16.00)**	**14.12**	**0.22**	**1414 (99.23%)**
	702894	137	14.57 (0.06 - 15.99)	14.34	0.23	136 (99.27%)
	703154	268	13.38 (0.03 - 14.89)	13.05	0.33	262 (97.76%)
	703564	261	13.69 (0.05 - 14.76)	13.16	0.53	252 (96.55%)
B4154-011	703890	259	14.23 (0.03 - 15.76)	13.95	0.28	258 (99.61%)
	704637	262	14.30 (0.03 - 15.50)	13.96	0.34	258 (98.47%)
	704639	127	14.42 (0.04 - 15.77)	14.12	0.30	126 (99.21%)
	All	**1314**	**14.02 (0.03 - 15.99)**	**13.67**	**0.35**	**1292 (98.33%)**
B4154-012	704639	145	14.62 (0.04 - 15.76)	14.42	0.20	144 (99.31%)
	704982	545	13.77 (0.07 - 14.89)	13.48	0.29	538 (98.72%)
	705627	248	13.64 (0.10 - 14.89)	13.19	0.45	244 (98.39%)
	706108	182	14.07 (0.07 - 15.59)	13.84	0.23	181 (99.45%)
	706348	112	14.20 (1.33 - 15.21)	13.72	0.47	112 (100.00%)
	All	**1232**	**13.92 (0.04 - 15.76)**	**13.61**	**0.31**	**1219 (98.94%)**
B4154-013	706348	36	14.37 (0.05 - 15.37)	13.72	0.65	35 (97.22%)
	706629	55	14.29 (11.44 - 15.33)	14.17	0.12	55 (100.00%)
	706741	135	14.06 (8.93 - 15.33)	13.98	0.08	135 (100.00%)
	All	**226**	**14.14 (0.05 - 15.37)**	**13.98**	**0.15**	**225 (99.56%)**
All	**All**	**8786**	**14.05 (0.02 - 16.00)**	**13.67**	**0.38**	**8636 (98.29%)**

Figure 7. Median S/CO (and min – max range) ratios for HBV-DNA in Procleix Ultrio Elite assay reagent lots on P0154 ViraQ HBV Trend 50 control batches (data in Table 10).



The S/CO responses on P0154 ViraQ HBV Trend 50 in the Procleix Ultrio Elite and UltrioPlex E assays are not normally distributed, since >85% of S/CO ratios are in the saturation range of the assays. Therefore, an increase in the number of S/CO ratios below the saturation range will increase the difference between the median and the average of S/CO values ($\Delta S/CO_{M-A}$). Hence, $\Delta S/CO_{M-A}$ is expected to become higher with lower analytical sensitivity of the NAT system and may be used for trend analysis[11,18].

Acceptance testing of reagent lots

To estimate the analytical sensitivity of a new reagent lot, test the trend control in 20 replicates in a single test run and follow the guidelines in Table 11. The expected frequencies are based on the overall reactivity rate on P0154 ViraQ HBV Trend 50 Control in the Ultrio Elite assay only (98%), since the UltrioPlex E assay data is too limited.

Table 11. Decision algorithm for acceptance of reagent lots by replicate testing of 20 vials of P0154 ViraQ HBV Trend 50 Control in one Procleix Ultrio Elite or UltrioPlex E test run.

Phase	Number of reactives	Expected frequency	Decision
Initial test	≥19	>94%	Accept
	≤18	<6%	Repeat acceptance testing procedure
Repeat test	≤17	<0.7%	Initiate root cause analysis

Limitations

- P0154 ViraQ HBV Trend 50 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 repeated occurrence of non-reactive results and a higher frequency of weak reactive results).
- P0154 ViraQ HBV Trend 50 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.
- A single non-reactive test result on P0154 ViraQ HBV Trend 50 Control cannot be used to invalidate a test run. The Poisson distribution in samples with low HBV concentrations cannot guarantee that the response values are reproducible. Therefore, the trend control cannot be used for a decision to accept or reject a test run.
- The expected ranges of results in the Procleix Ultrio Elite and UltrioPlex E assays on P0154 ViraQ HBV Trend 50 Control that are presented in this package insert were based on performance evaluation studies involving a limited number of trend control batches and NAT reagent lots. It cannot be guaranteed that slightly different results will be found on other trend control batches, assay reagent lots or laboratory instruments.
- The parameter $\Delta(S/CO_{M-A})$ is an indicator of the skewness of the distribution curve of S/CO values and may be a performance indicator for the analytical sensitivity of Ultrio assay versions and reagent lots. However, a threshold value for $\Delta(S/CO_{M-A})$ above which a deterioration of the test system is predicted cannot be given.
- It is not guaranteed that reagent lots that pass the acceptance testing procedure always have sufficient analytical sensitivity. A small reduction in analytical sensitivity is less likely to be identified by the procedure than a large reduction in analytical sensitivity.

- It cannot be excluded that some variation exists between the performance of consecutive batches of the P0154 ViraQ HBV Trend 50 control due to matrix effects or other manufacturing variables.

References

1. Grabarczyk P, van Drimmelen H, Kopacz A, Gdowska J, Liszewski G, Piotrowski D, Górska J, Kuśmierczyk J, Candotti D, Lętowska M, Lelie N, Brojer E. Head-to-head comparison of two transcription-mediated amplification assay versions for detection of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus Type 1 in blood donors. Transfusion. 2013; 53:2512-2524.
2. Assal A, Barlet V, Deschaseaux M, Dupont I, Gallian P, Guitton C, Morel P, David B, and De Micco P. Comparison of the analytical and operational performance of two viral nucleic acid test blood screening systems: Procleix Tigris and cobas s 201. Transfusion 2009; 49:289-300.
3. Koppelman M, Assal A, Chudy M, Torres P, de Villaescusa RG, Reesink HW, Lelie PN, Cuypers HT. Multi-center performance evaluation of a transcription-mediated amplification assay for screening of human immunodeficiency virus-1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA in blood donations. Transfusion 2005; 45:1258-66.
4. Grabarczyk P, Koppelman M, Boland F, Sauleda S, Fabra C, Cambie G, O'Riordan K, Van Drimmelen H, Vermeulen M, O'Riordan J, Lelie N. Inclusion of human immunodeficiency virus Type 2 (HIV-2) in a multiplex transcription-mediated amplification assay does not affect detection of HIV-1 and hepatitis B and C virus genotypes: a multicenter performance evaluation study. Transfusion 2015; 55:2246-55.
5. Coleman C. Lelie N, Rademeyer R, Van Drimmelen H, Van den Berg K, Vermeulen M. Comparison of two nucleic acid amplification technology systems for detection of human immunodeficiency virus, hepatitis B virus and hepatitis C virus. Transfusion 2020;60:2929-2937
6. Lelie PN, Van Drimmelen AAJ. Positioning of ViraQ Check and Trend Controls relative to analytical sensitivity of HBV/HCV/HIV-NAT assays. VR4059. Technical documentation BioQControl
7. Lelie PN. Van Drimmelen AAJ. Preparation of inactivated viral standards: Safety assessment of quality control samples for viral serology and NAT assays in blood screening laboratories. CE4006. Technical Documentation BioQControl
8. Lelie PN, Reesink HW, Niessen J, Brotman B, Prince AM. Inactivation of 10^{15} chimpanzee-infectious doses of hepatitis B virus during preparation of a heat-inactivated hepatitis B vaccine. J Med Virol. 1987; 23(3):289-95
9. Lelie PN, Reesink HW, Lucas CJ. Inactivation of 12 viruses by heating steps applied during manufacture of a hepatitis B vaccine. J Med Virol. 1987;23:297-301.
10. Chudy M, Hanschmann KM, Kress J, Nick S, Campos R, Wend U, Gerlich W, Nübling CM. First WHO International Reference Panel containing hepatitis B virus genotypes A-G for assays of the viral DNA. J Clin Virol. 2012;55:303-9
11. Kerst S, Van Drimmelen AAJ, Lelie PN. Post-market performance follow-up report of ViraQ HCV/HIV-1/HBV Trend Controls. VR4115. Technical Documentation BioQControl.
12. Summary of safety and performance (SSP) of P0154 ViraQ HBV Trend 50 control. CE4667. Technical documentation BioQControl.
13. Collins ML, Zayati C, Detmer JJ, Daly B, Kolberg JA, Cha TA, Irvine BD, Tucker J, Urdea MS. Preparation and characterization of RNA standards for use in

quantitative branched DNA hybridization assays. Anal Biochem. 1995 20; 226:120-9.
14. Lelie PN, Van Drimmelen AAJ. Calibration of native and inactivated HBV, HCV and HIV-1 standards. VR4060, Technical documentation BioQControl
15. Heermann KH, Gerlich WH, Chudy M, Schaefer S, Thomssen R.J. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. Clin Microbiol. 1999;37:68-73.
16. Saldanha J, Gerlich W, Lelie N, Dawson P, Heermann K, Heath A; WHO Collaborative Study Group. An international collaborative study to establish a World Health Organization international standard for hepatitis B virus DNA nucleic acid amplification techniques. Vox Sang. 2001;80:63-71.
17. Van Drimmelen AAJ, Lelie PN. Performance evaluation of ViraQ run controls for HBV, HCV and HIV-1 detection in different NAT assays. VR4061. Technical documentation BioQControl
18. Van Drimmelen AAJ, Lelie PN. Stability of ViraQ run controls for HBV/HVC/HIV-NAT. VR4058. Technical documentation BioQControl
19. Centers for Disease Control (CDC). Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. MMWR 1988; 37:377-388.
20. Centers for Disease Control (CDC). Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989; 38(S-6): 1-36.
21. Nelson LS, "The Shewhart Control Chart—Tests for Special Causes". Journal of Quality Technology 1984;16, no. 4: 238-239 https://doi.org/10.1080/00224065.1984.11978921

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