P0068
ViraQ HIV-1 Trend 25

CE
0483

IVD

REF P0068

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

P0068 ViraQ HIV-1 Trend 25 is intended to be used as external trend control for detection of human immunodeficiency virus type 1 (HIV-1) RNA by the nucleic acid amplification technology (NAT) assays on the platform defined in Table 1. P0068 ViraQ HIV-1 Trend 25 is a heat-inactivated HIV-1-RNA standard in plasma at a concentration of 25 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.

P0068 ViraQ HIV-1 Trend 25 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 P0068 ViraQ HIV-1 Trend 25 control.

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

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

In vitro diagnostic medical device

Country of origin

Contains human blood derivatives

Store below -65°C

Expiry date

Number of controls

Caution

CE mark with Notified Body number

Unique device identification

Read instructions for use

Biological substance Category B

Principle of method

P0068 ViraQ HIV-1 Trend 25 control has been formulated to mimic natural plasma specimens with a low HIV-1-RNA 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 25 copies/mL of heat-inactivated HIV-1 RNA (equivalent to 43 IU/mL). The trend control has been designed to ensure sufficient analytical sensitivity of TMA tests in blood screening laboratories. The HIV-1-RNA 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 S0041 inactivated HIV-1-RNA subtype B standard, prepared by heat-inactivation of tissue culture derived virus spiked in plasma[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 S0041 HIV-1 standard has been calibrated in copies/mL and IU/mL against the Viral Quality Control (VQC)-Sanquin and World Health Organization (WHO) International Standards (Figure 1). The low concentration of HIV-1 subtype B in the trend control is meant to be representative for HIV-1 subtypes A-K and circulating recombinant forms that are prevalent in different geographical regions of the world (although in previous studies not all genetic variants were detected with comparable analytical sensitivity by commercial NAT assays)[1,4,10-12]. 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 the analytical performance of NAT reagent lots and laboratory instruments. For this purpose, users of the Procleix assays in combination with the P0068 ViraQ HIV-1 Trend 25 Control can generate a statistical report for post-market performance follow-up[13] 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 P0068 ViraQ HIV-1 Trend 25 control will be made available at the EUDAMED website of the European Union, and is currently available upon request[14].

Table 2. Detection limits on native and inactivated HIV-1 standard dilution panels in Procleix Ultrio assay versions calculated by probit analysis.

standard	BQC panel	NAT method	n	50% LOD (CI) copies/mL	95% LOD (CI) copies/mL
S0041 BioQ HIV-1 RNA subype B inactivated	P0026	Ultrio	52	3.1 (2.4-3.9)	20.2 (13.9-33.3)
S0012 VQC-Sanquin HIV-1 RNA subtype B native	P0025	Ultrio	60	1.5 (1.0-2.2)	11.2 (6.3-29.8)
	P0025	Ultrio Plus	48	1.7 (1.3-2.2)	15.1 (9.9-26.9)
	P0025	Ultrio Elite	24	2.1 (1.5-2.9)	9.0 (5.8-19.5)
	P0290	Ultrio Elite	48	3.3 (2.7-4.2)	13.8 (10.0-22.2)
	P0290	UltrioPlex E	104	2.0 (1.5-2.7)	15.4 (9.7-28.8)
WHO HIV-1 RNA 97/650#	P0022	Ultrio	40	2.6 (2.1-3.3)	11.8 (8.2-20.7)
	P0022	Ultrio Plus	288	2.4 (2.2-2.6)	13.4 (11.4-16.3)
	P0022	Ultrio Elite	229	2.2 (1.4-3.2)	17.2 (10.3-40.1)

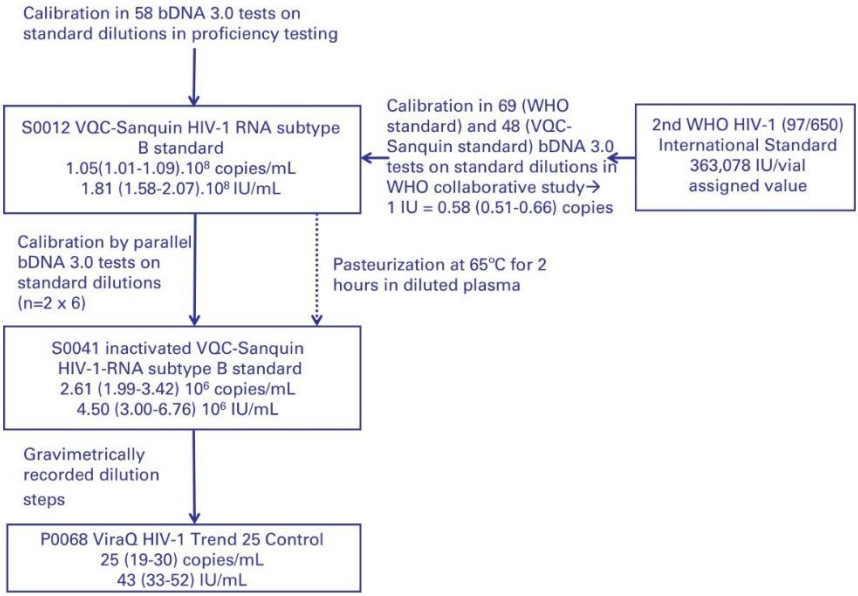
1 IU = 0.58 copies

Traceability to HIV-1 RNA copies and International Units

Figure 1 shows the traceability chain between the ViraQ trend control, the heat-inactivated and native VQC-Sanquin standard and the 2nd WHO 97/650 International Standard for HIV-1 RNA. The inactivated S0041 HIV-1 RNA standard (used for preparation of the P0068 ViraQ trend control) is derived from the S0012 VQC-Sanquin HIV-1 RNA subtype B

standard and has been calibrated in copies/mL by replicate testing in the Siemens Versant bDNA 3.0 assay[15] against this primary standard[16-18]. The VQC-Sanquin HIV-1 RNA subtype B standard has been calibrated at 0.58 (0.51-0.66) copies per IU against the second WHO HIV-1 RNA 97/650 standard in multiple replicate bDNA 3.0 assays[17-20]. It must be emphasized that this conversion factor from copies to IU values was dependent on the quantitative NAT method used and differed for the 1st 97/650, the 2nd 97/656 WHO HIV-1 standards and the later heat-inactivated 3rd 10/152 and 4th 16/194 WHO replacement standards[17,18]. The accurate calibration of the native and inactivated VQC-Sanquin HIV-1 subtype B standards in copies/mL and IU/mL has been confirmed in analytical sensitivity studies of the Grifols Procleix TMA and Roche cobas MPX assays[1-5,16-18]. The manufacturing and quality control procedures of BioQControl guarantee consistent virus concentrations in consecutive ViraQ HIV-1 Trend 25 batches[13,21]. The inactivated S0041 HIV-1 subtype B 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 ViraQ trend control and inactivated and native VQC-Sanquin standards and WHO International Standard.



Stability of HIV-1 standards and trend control

P0068 ViraQ HIV-1 Trend 25 control can be stored at ≤–65°C for five years and if stored at –30 °C for no longer than 6 months. The long-term stability of the liquid frozen S0041 HIV-1 standard stored below -65°C has been firmly established[22]; 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 less than 10% degradation of HIV-1 RNA per year in standard dilutions of higher concentration than in P0068 HIV-1 Trend 25 control when stored at -30°C[22]. Since the relative degradation is independent of concentration the degradation estimate can be extrapolated to P0068 HIV-1

Trend 25 control. Hence, it can be guaranteed that the trend control is still functional and should generate a reactivity rate of 88-98% when stored at ≤–65°C or less than 6 months at ≤–30°C[6,13,21].

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 P0068 ViraQ HIV-1 Trend 25# kit formats and contents.

Cat. Code	GTIN/UDI-DI code^	Quantity	Tube size	Sample volume	Secondary packing
P0068/01	8718719832813	60 tubes	10 mL	1.4 mL (+overfill)	Rack in box
P0068/02	8718719832059	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 P0068 is 871871983P0068EV

P0068/01 and P0068/02 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: HIV-1. 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 –65°C for a maximum of five years after preparation of the trend control batch and not be used for more than six months after storage at –30°C[22]. 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. 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 P0068 ViraQ HIV-1 Trend 25 contains inactivated HIV-1 particles[7-9] 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, 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[23,24].

- 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 control 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). P0068 ViraQ HIV-1 Trend 25 Control is expected to be reactive in approximately 95% to 99% of TMA test runs.
The positioning of P0068 ViraQ HIV-1 Trend 25 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 S0012 HIV-1 subtype B standard (Figure 2) and of the inactivated S0041 HIV-1 subype B standard used for preparation of the trend control (Figure 3). At the concentration of 25 copies/mL S/CO ratios follow a non-parametric distribution.

Figure 2. Distributions of S/CO ratios on dilutions of the native S0012 HIV-1 standard dilutions in reference panel P0290 tested in 55 replicates by the UltrioPlex E assay with a 95% LOD (CI) of 12.5 (9.5-18.2) copies/mL.

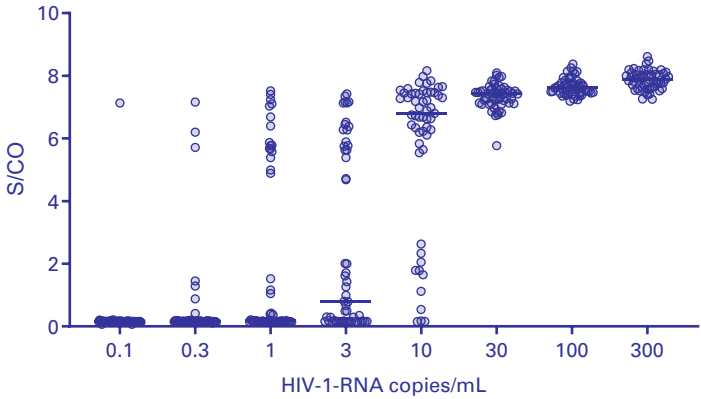
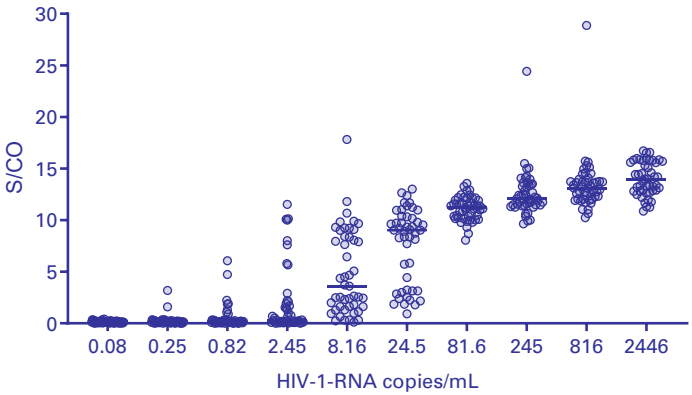


Figure 3. Distributions of S/CO ratios on dilutions of the inactivated S0041 HIV-1 standard dilutions in the P0026 reference panel tested in 47 replicates by the Ultrio assay with a 95% LOD (CI) of 20.2 (13.9-33.3) copies/mL being near the trend control concentration of 25 (19-30) copies/mL.



Expected assay response values on trend control

The expected results for the P0068 ViraQ HIV-1 Trend 25 Control are as follows:

Procleix Ultrio Elite assay
HIV-1-RNA S/CO ratios range: 0.0 – 12.1

Procleix UltrioPlex E assay
HIV-1-RNA S/CO ratios range: 0.0 – 8.8

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 HIV-1 standard dilutions and on P0068 ViraQ HIV-1 Trend 25 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[25] to identify deviations in the Levey-Jennings trend analysis.

The Procleix Ultrio Elite and UltrioPlex E test results on P0068 ViraQ HIV-1 Trend 25 control are expected to be reactive in 95-99% of cases according to probit analysis on the inactivated S0041 HIV-1 subtype B standard dilutions in the P0026 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 HIV-1-RNA 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 three P0068 ViraQ HIV-1 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 three batches of P0068 ViraQ HIV-1 Trend 25 Control in 1298 Ultrio Elite test runs in the period from 11-11-2022 to 9-02-2024 (data in Figure 5).

Assay	n	Median S/CO	95% range S/CO	99% range S/CO
Ultrio Elite	1298	8.54	0.58 – 10.53	0.07 – 11.91

Table 9 summarizes the Levey-Jennings chart parameters in Figure 6, which were calculated from the UltrioPlex E S/CO ratios reported on one P0068 ViraQ HIV-1 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 P0068 ViraQ HIV-1 Trend 25 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	8.26	5.98 – 8.79	1.80 – 9.20

Figure 5. Levey-Jennings QC chart of Ultrio Elite assay S/CO ratios for HIV-1-RNA on eleven batches of P0068 ViraQ HIV-1 Trend Control.

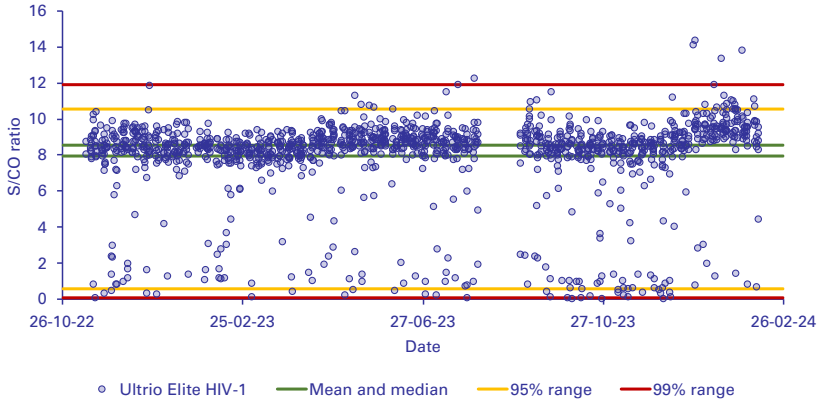
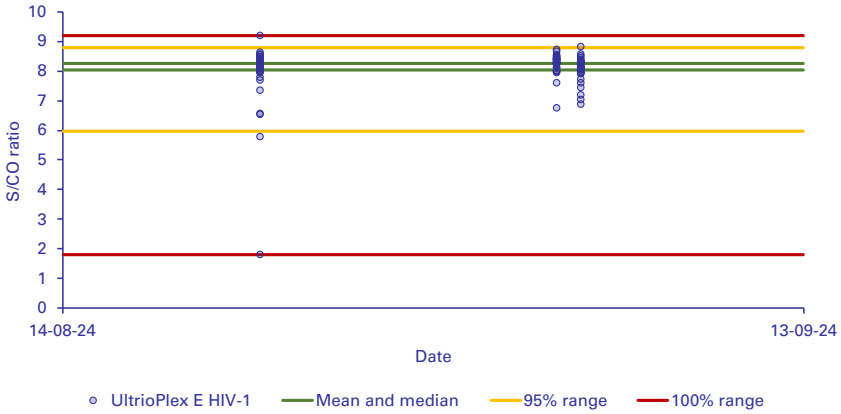


Figure 6. Levey-Jennings QC chart of UltrioPlex E assay S/CO ratios for HIV-1-RNA on one batch of P0068 ViraQ HIV-1 Trend Control.



Post-market performance follow-up of trend control batches

One can use P0068 ViraQ HIV-1 Trend 25 in combination with the DataQ Analytics system on www.bioqcontrol.com to compare the S/CO ratios on different trend control batches, Procleix Ultrio reagent lots or Procleix Panther instruments. Table 10 and Figure 7 compare the median S/CO ratios in the Ultrio Elite assay on P0068 ViraQ HIV-1 batches and Procleix Ultrio reagent lots for HIV-1-RNA, showing moderate variability in analytical sensitivity. However, all ViraQ batch-reagent lot combinations had a reactivity rate of >85%, and 36 out of 43 combinations (84%) had a reactivity rate of >95%. The preliminary data of the UltrioPlex E assay is based on test runs with only one P0068 ViraQ batch (B4063-017) and one test reagent lot (707270). Therefore, no comparisons can be made for the UltrioPlex E assay. However, the reactivity rate of 100% in this limited data set indicates sufficient analytical sensitivity in the UltrioPlex E assay for HIV-1-RNA.

Table 10. Comparison of Ultrio Elite S/CO on P0068 ViraQ HIV-1 Trend 25 Control batches and between consecutive Procleix Ultrio Elite reagent lots.

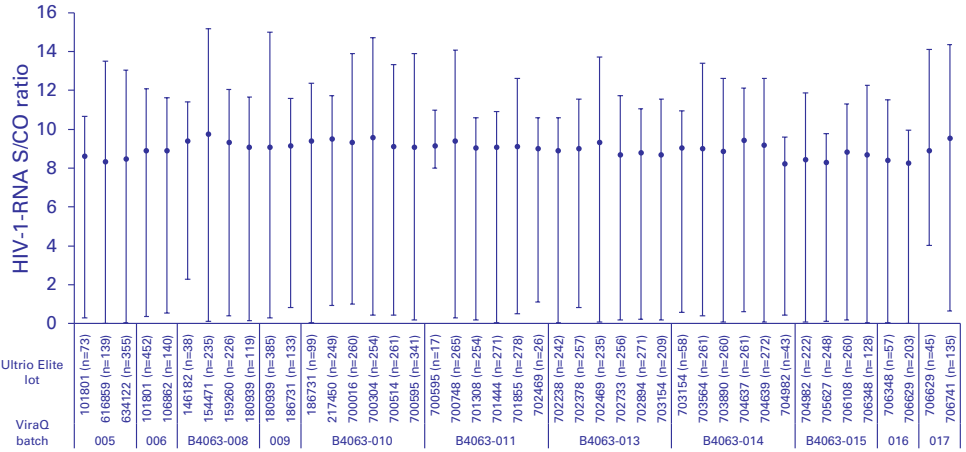
P0068 ViraQ batch	Ultrio Elite reagent lot	n	Median S/CO (range)	Avg. S/CO	$\Delta S/CO_{M-A}$	S/CO values ≥ 1
B4063-005	101801	73	8.62 (0.30 - 10.67)	7.10	1.52	67 (91.78%)
	616859	139	8.32 (0.03 - 13.51)	6.84	1.48	120 (86.33%)
	634122	355	8.48 (0.06 - 13.03)	7.58	0.90	338 (95.21%)
	All	**567**	**8.45 (0.03 - 13.51)**	**7.34**	**1.11**	**525 (92.59%)**
B4063-006	101801	452	8.91 (0.37 - 12.08)	8.65	0.25	445 (98.45%)
	106862	140	8.89 (0.55 - 11.61)	8.76	0.13	139 (99.29%)
	All	**592**	**8.90 (0.37 - 12.08)**	**8.68**	**0.22**	**584 (98.65%)**
B4063-008	146182	38	9.40 (2.28 - 11.40)	9.09	0.31	38 (100.00%)
	154471	235	9.73 (0.14 - 15.16)	9.31	0.42	229 (97.45%)
	159260	226	9.33 (0.40 - 12.06)	8.71	0.62	225 (99.56%)
	180939	119	9.08 (0.15 - 11.64)	8.60	0.48	117 (98.32%)
	All	**618**	**9.39 (0.14 - 15.16)**	**8.94**	**0.45**	**609 (98.54%)**
B4063-009	180939	385	9.07 (0.31 - 15.01)	8.85	0.22	380 (98.70%)
	186731	133	9.14 (0.83 - 11.57)	8.87	0.27	131 (98.50%)
	All	**518**	**9.10 (0.31 - 15.01)**	**8.86**	**0.24**	**511 (98.65%)**
B4063-010	186731	99	9.37 (0.06 - 12.38)	9.02	0.35	97 (97.98%)
	217450	249	9.51 (0.94 - 11.74)	9.27	0.24	248 (99.60%)
	700016	260	9.31 (1.00 - 13.88)	8.92	0.38	260 (100.00%)
	700304	254	9.55 (0.46 - 14.72)	8.97	0.58	250 (98.43%)
	700514	261	9.09 (0.45 - 13.33)	8.77	0.32	258 (98.85%)
	700595	341	9.07 (0.19 - 13.91)	8.77	0.30	339 (99.41%)
	All	**1464**	**9.27 (0.06 - 14.72)**	**8.93**	**0.34**	**1452 (99.18%)**
B4063-011	700595	17	9.14 (7.99 - 11.00)	9.29	-0.15	17 (100.00%)
	700748	265	9.38 (0.30 - 14.08)	9.06	0.32	262 (98.87%)
	701308	254	9.03 (0.19 - 10.58)	8.80	0.23	251 (98.82%)
	701444	271	9.05 (0.04 - 10.91)	8.51	0.54	266 (98.15%)
	701855	278	9.10 (0.51 - 12.61)	8.42	0.67	272 (97.84%)
	702469	26	8.98 (1.10 - 10.61)	8.67	0.31	26 (100.00%)
	All	**1111**	**9.13 (0.04 - 14.08)**	**8.70**	**0.43**	**1094 (98.47%)**
B4063-013	702238	242	8.89 (0.04 - 10.60)	8.56	0.33	235 (97.11%)
	702378	257	9.01 (0.83 - 11.55)	8.64	0.37	254 (98.83%)
	702469	235	9.31 (0.08 - 13.73)	8.86	0.45	231 (98.30%)
	702733	256	8.68 (0.18 - 11.74)	7.97	0.71	239 (93.36%)
	702894	271	8.80 (0.24 - 11.06)	8.24	0.56	262 (96.68%)
	703154	209	8.69 (0.18 - 11.54)	7.99	0.70	197 (94.26%)
	All	**1470**	**8.88 (0.04 - 13.73)**	**8.38**	**0.50**	**1418 (96.46%)**
B4063-014	703154	58	9.05 (0.60 - 10.94)	8.84	0.21	57 (98.28%)
	703564	261	9.00 (0.39 - 13.39)	8.62	0.38	258 (98.85%)
	703890	260	8.85 (0.10 - 12.63)	8.03	0.82	249 (95.77%)
	704637	261	9.41 (0.63 - 12.12)	9.30	0.11	258 (98.85%)
	704639	272	9.16 (0.08 - 12.61)	8.79	0.37	265 (97.43%)
	704982	43	8.23 (0.45 - 9.61)	7.33	0.90	39 (90.70%)
	All	**1155**	**9.08 (0.08 - 13.39)**	**8.64**	**0.44**	**1126 (97.49%)**

(continued)

Table 10 *(continued)*

P0068 ViraQ batch	Ultrio Elite reagent lot	n	Median S/CO (range)	Avg. S/CO	$\Delta S/CO_{M-A}$	S/CO values ≥ 1
B4063-015	704982	222	8.43 (0.08 - 11.86)	7.90	0.53	213 (95.95%)
	705627	248	8.27 (0.13 - 9.76)	7.76	0.51	245 (98.79%)
	706108	260	8.82 (0.20 - 11.32)	8.34	0.48	252 (96.92%)
	706348	128	8.68 (0.07 - 12.25)	7.91	0.77	122 (95.31%)
	All	**858**	**8.52 (0.07 - 12.25)**	**7.99**	**0.52**	**832 (96.97%)**
B4063-016	706348	57	8.39 (0.05 - 11.50)	7.67	0.72	54 (94.74%)
	706629	203	8.24 (0.03 - 9.97)	6.94	1.30	177 (87.19%)
	All	**260**	**8.29 (0.03 - 11.50)**	**7.10**	**1.19**	**231 (88.85%)**
B4063-017	706629	45	8.88 (4.02 - 14.11)	8.90	-0.02	45 (100.00%)
	706741	135	9.53 (0.66 - 14.35)	9.19	0.34	132 (97.78%)
	All	**180**	**9.35 (0.66 - 14.35)**	**9.12**	**0.23**	**177 (98.33%)**
All	**All**	**8793**	**8.97 (0.03 - 15.16)**	**8.51**	**0.46**	**8559 (97.34%)**

Figure 7. Median S/CO (and min – max range) ratios for HIV-1-RNA in Procleix Ultrio Elite assay reagent lots on P0068 ViraQ HIV-1 Trend 25 control batches (data in Table 10).



The S/CO responses on P0068 ViraQ HIV-1 Trend 25 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[13,21].

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 P0068 ViraQ HIV-1 Trend 25 control in the Ultrio Elite assay only (97%, see Table 10), since the UltrioPlex E assay data is too limited.

Table 11. Decision algorithm and criteria for acceptance of reagent lots by replicate testing of 20 vials of P0068 ViraQ HIV-1 Trend 25 Control in one Procleix Ultrio Elite or UltrioPlex E assay test run.

Phase	Number of reactives	Expected frequency	Decision
Initial test	≥19	>88%	Accept
	≤18	<12%	Repeat acceptance testing procedure
Repeat test	≤17	<2.1%	Initiate root cause analysis

Limitations

- P0068 ViraQ HIV-1 Trend 25 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).
- P0068 ViraQ HIV-1 Trend 25 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 P0068 ViraQ HIV-1 Trend 25 Control cannot be used to invalidate a test run. The Poisson distribution in samples with low HIV-1 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 P0068 ViraQ HIV-1 Trend 25 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 $\Delta(S/CO_{M\text{-}A})$ 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 $\Delta(S/CO_{M\text{-}A})$ 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 P0068 ViraQ HIV-1 Trend 25 control due to matrix effects or other manufacturing variables.

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