Bio QControl

P0069
ViraQ HBV Trend 25

CE 0088

IVD

REF P0069

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

P0069 ViraQ HBV Trend 25 is intended to be used as external trend control for hepatitis B virus (HBV)-DNA detection by the multiplex transcription mediated amplification (TMA) assays on the automated nucleic acid amplification technology (NAT) plat-forms defined in Table 1. The trend control helps laboratories to ensure that HBV is detected with sufficient analytical sensitivity by consecutive reagent batches of the Procleix Ultrio assay versions and by each of the Tigris or Panther instruments in use. 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 batches
- installation qualification of instruments
- training of technicians.

Table 1. Test kits and platforms covered by P0069 ViraQ HBV Trend control

Platform	Test kits	Test environment
Grifols Procleix Tigris®	Procleix Ultrio Plus®	Blood screening
Grifols Procleix Panther®	Procleix Ultrio Elite®	

The run control should not be used to replace the internal controls or calibrators in the test kits.

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

Store below -30°C

CE mark with Notified Body number

Caution

In vitro diagnostic medical device

Expiry date

Contents

Biological substance Category B

Read instructions for use

Principle of method

P0069 ViraQ HBV Trend 25 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 platforms. The trend control contains 25 copies/mL of HBV-DNA (equivalent to 4.7 International Units (IU)/mL) and has been designed to ensure sufficient analytical sensitivity of transcription mediated amplification (TMA) tests in blood screening laboratories. The HBV-DNA concentration in the run control has been set near the 95% lower limit of detection (LOD) of the Ultrio Plus and Elite assays (table 2)[1-5]. P0069 ViraQ HBV Trend 25 Control enables laboratories to be alerted in case of a reduction of analytical sensitivity of NAT instruments or reagent batches and to identify changes in TMA performance over time. The run control is a dilution of the S0043 HBV-RNA genotype A2 standard, prepared by heat-inactivation of a pool of HBsAg plasma units from the same

donor[6-8]. 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 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 run 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 TMA assays)[4,9]. 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 run 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 of time allows for comparison of analytical performance of NAT reagent batches and laboratory instruments. The trend control can also be used in multiple replicates in the same test run to ensure that TMA reagents or instruments fulfil the minimum requirements for analytical sensitivity before they are accepted for routine blood screening.

Table 2. Detection limits on native and inactivated HBV standard dilution panels in Procleix Ultrio assay versions

standard	panel	NAT method	n	50% LOD (CI) cp/mL	95% LOD (CI) cp/mL
S0043 BioQ HBV-DNA genotype A inact.	P0031	Ultrio Plus	24	6.6 (2.7-17.4)	64 .2 (22.4-1099)
	P0031	Ultrio Elite	25	5.7 (4.0-8.2)	40.8 (24.3-91.7)
	P0031	Ultrio Plus/Elite	49	7.6 (5.9-9.5)^	33.3 (23.8-56.4)^
S0011 VQC-Sanquin HBV-DNA genotype A	P0007	Ultrio Plus	48	4.8 (3.7-6.2)	38.8 (25.6-68.5)
	P0007	Ultrio Elite	74	3.4 (2.3-4.8)	43.2 (24.8-98.0)
	P0007	Ultrio Plus/Elite	122	4.3 (2,9-6,1)^	35.4 (20,6-87,8)^
S0010 Eurohep HBV-DNA genotype A	P0001	Ultrio Plus	96	3.6 (2.9-4.4)	40.4 (29.2-60.2)
	P0001	Ultrio Elite	24	7.9 (5.5-11.2)	49.1 (29.4-116)
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)

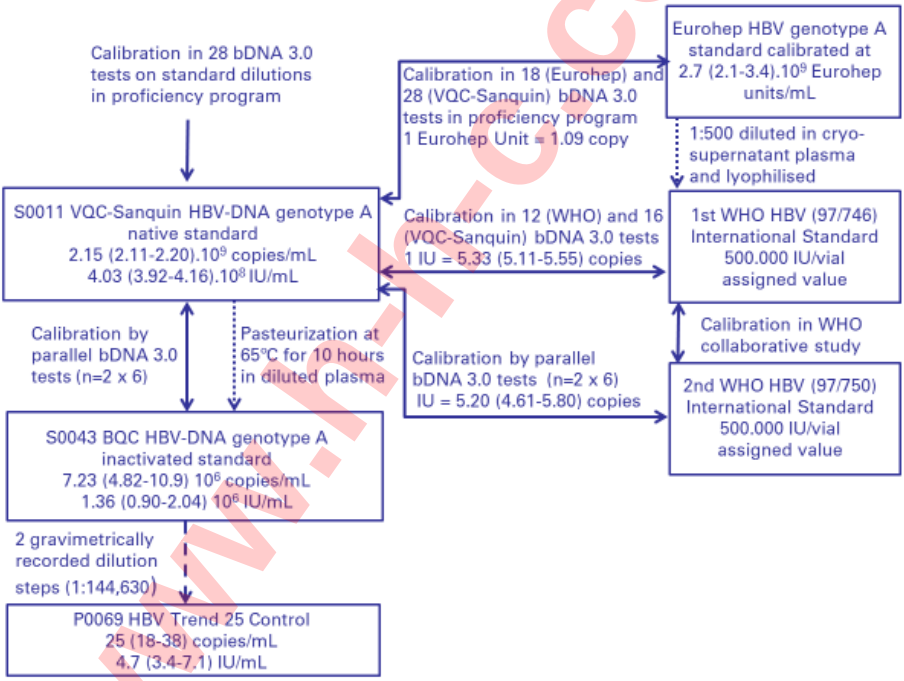
^ probit analysis without two lowest concentrations in panel P0031 # 1 IU = 5.33 copies

Traceability to HBV-DNA copies and International Units

Figure 1 shows the traceability chain between the ViraQ run control, the Bio Quality Control (BQC) standard, 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 P0069 ViraQ trend control) has been calibrated in copies/mL by replicate testing in the Siemens Versant bDNA 3.0 assay[10] against the historically established S0011 VQC-Sanquin HBV-DNA genotype A standard[11]. 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[12]. 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[13] used for preparation of the WHO standards[14]. The accurate calibration of the VQC-Sanquin and the inactivated BQC standard 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[4,12]. The BQC manufacturing and quality control procedures guarantee consistent virus concentrations in consecutive ViraQ HBV Trend 25 batches[15]. The inactivated BQC HBV genotype A standard is available in sufficient supply to ensure batch to batch consistency of ViraQ trend controls for a prolonged period of time.

Figure 1. Traceability chain between trend control, BQC and VQC-Sanquin standards and WHO International Standards



Stability of HBV standards and run control

The long term stability of the liquid frozen S0043 HBV standard stored at ≤65°C has been firmly established[16]; hence the stock solution from which the trend control is prepared has shown to be stable in the BQC storage facilities. Real time stability experiments using quantitative NAT assays showed no degradation of HBV-DNA in P0065 ViraQ HBV Check 125 control when stored at -30°C[16]. Hence, it can be guaranteed that also the P0069 trend control is still functional and should generate a reactivity rate near 95% when stored at -30°C and used before the expiration date (two years after preparation of the run control batch)[15,16].

Kit contents (materials provided)

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

P0069/01 and P0069/02 are intended to accommodate both blood screening and diagnostic 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 barcode identifying the product, sequential batch number and marker HBV. The barcode can be read by the automated NAT systems.

Table 3. Description of kit formats and contents

Cat. Code	Description of contents	Primary packing	Secondary packing
P0065/01	60 x 1.5 mL run control	10 mL vial	60 vial rack in box
P0065/02	10 x 1.5 mL run control	10 mL vial	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 trend controls should be stored at or below -30°C for a maximum of two years[16]. 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[17]. 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 P0069 ViraQ HBV Trend 25 contains inactivated HBV particles[6-8] 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-HBs, 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. Observe the universal precautions for prevention of transmission of infectious agents when handling these materials[17,18].

- 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 and calculations

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.

The following sections in this package insert provide guidance on interpretation and analysis of test results on P0069 ViraQ HBV Trend 25. The statistical evaluation methods were developed by BioQ Control and not reviewed nor approved by the manufacturer of the Ultrio assay versions

The results of the Procleix Ultrio Plus and Ultrio Elite assays are expressed as a sample to cut-off ratio (S/CO). P0069 ViraQ HBV Trend 25 Control should react positive in approximately 90% to 95% of TMA test runs. Approximately 90% of test results on the trend control are expected in the saturated range of the TMA assay with S/CO values equal to or above 12.0 (range 11.0-13.0). Approximately 2% to 10% of test results are expected in the dynamic range of the TMA assay with S/CO rations below 12.0 (see interpretation of test results below)[15].

The S/CO responses on ViraQ HBV Trend 25 in the Ultrio Plus and Elite assay versions are not normally distributed (figure 2). A Gumbel distribution is 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 $\Delta(S/CO_{M-A})$ becomes higher with lower analytical sensitivity of the NAT system and can be used for trend analysis or comparison of experimental conditions (see interpretation of test results below)[15].

Interpretation of test results on trend control in Procleix Ultrio assay versions

The expected frequency of S/CO values on P0069 ViraQ HBV Trend 25 control in the dynamic and saturated range of the TMA assay as well as the interpretation of three categories of test result are shown in table 4. The majority of S/CO values on the run control reach maximum TMA response levels and are found between 12.0 and 15.0 (figure 2). A fifth to a quarter of TMA reactions on the trend control are not yet complete and have S/CO values in the dynamic range of the assay (between 1.0 and 12.0). The threshold S/CO value between dynamic and saturated response levels varies over time (between 11.0 and 13.0) and is dependent on the Ultrio (Plus and Elite) reagent batch[15]. This affects the frequency of S/CO response values above and below the arbitrarily chosen threshold value of 12.0. In a one month observation period of 190 Ultrio Elite test runs the overall proportion of reactive results on P0069 HBV Trend 25 control was 92.6% as compared to 98.3% on P0154 HBV Trend 50 control tested in parallel (table 5)[16]. The latter trend control containing 50 (instead of 25) copies/mL was tested during 4 years with reactivity rates varying between 97.2% and 100% between TMA and trend control reagent batch combinations)[15].

Table 4. Interpretation of a single TMA test result on P0069 ViraQ HBV Trend 25 in Procleix Ultrio assay versions and expected frequency of S/CO values in three ranges

Result	S/CO	Expected frequency per 1000[#]	Interpretation
Reactive saturated	>12.0	736 – 1000	The test signal on the trend control reaches maximum values in the saturated range of the TMA assay. This is an expected result.
Reactive dynamic	1.0–12.0	24 –92	The test signal on the run control is in the dynamic range of the assay because the TMA reaction is not yet complete. This is an expected result.
Non-reactive	<1.0	35-112	The test signal on the run control is below the cut-off. This is an expected result

[#]95% confidence limits found in 190 Ultrio Elite test runs

Table 5. Comparison of reactivity rate on HBV Trend Control (TC) batches of 50 and 25 copies/mL in two Ultrio Elite (UE) reagent batches.

Ultrio Elite batch	P0154 ViraQ HBV 50			P0069 ViraQ HBV 25		
	Trend control batch	reactive/n	%	Trend control batch	reactive/n	%
UE1	TC4	427/434	98.5%	TC1	54/58	93.1%
UE2	TC4	138/141	97.9%	TC1	122/132	92.4%
UE all	TC4	565/575	98.3%	TC1	176/190	92.6%

Monitoring performance of Procleix Ultrio assay versions on trend control

The difference between the median and the average of S/CO values can be used as an indicator of the analytical sensitivity of the NAT system (table 6). To illustrate this the mean and median at each time point of testing of the run control was calculated for 50 earlier and 50 later S/CO measurements and the same was done for the proportion reactive and proportion of saturated reactive responses (figure 3). From the sliding values it can be seen that the highest values of Δ (median S/CO – average S/CO) coincided with the lowest proportions of saturated responses. Based on the available results one may conclude that $\Delta(S/CO_{M-A})$ should be below 1.60 when the system is properly functioning[15]. The presence of non-reactive results also coincides with high values of $\Delta(S/CO_{M-A})$, thereby confirming its ability to be a trend indicator for analytical performance of the TMA assay. An alert threshold value for this parameter that is indicative for poor NAT performance cannot be given with the available data.

Table 6. Reproducibility of Ultrio (Plus and Elite) S/CO values on P0069 ViraQ HBV Trend 25 control

n test runs	Median S/CO	Average S/CO	$\Delta(S/CO_{M-A})$	S/CO Percentile	
				95%	99%
190	13.00	12.02	0.98	0.09 – 14.0	0.07 – 14.4

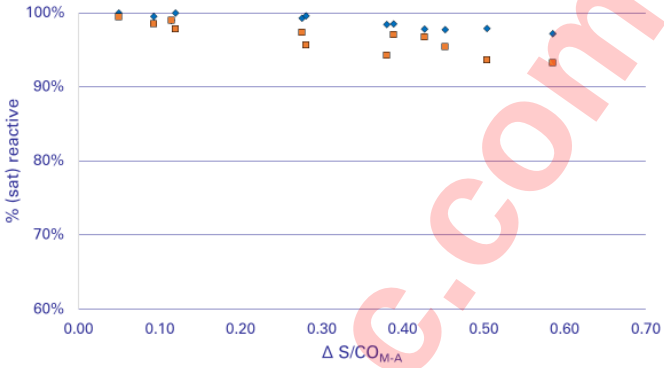
The parameter $\Delta(S/CO_{M-A})$ can also be applied to compare other experimental conditions such as the TMA reagent batch, the ViraQ trend control batch or the testing robot (Tigris or Panther). An example using $\Delta(S/CO_{M-A})$ as performance indicator is shown in figure 4 for

another trend control containing 50 (instead 25) copies/mL comparing different TMA reagent batches[15]. [Note that in this case, all data per experimental condition are used without 'sliding']. The result shows that the values of $\Delta(S/CO_{M-A})$ for TMA/trend control reagent batch combinations correlate with the reactivity rates. Hence, if the reagent batch performance indicator $\Delta(S/CO_{M-A})$ has an outlier value it could be used as an alert signal for checking technical performance of that particular TMA reagent (or trend control) batch.

Figure 2 Distribution of S/CO values in Ultrio Elite test runs on P0069 ViraQ HBV Trend 25 ontrol

Figure 3. Sliding course of $\Delta(S/CO_{M-A})$ over time in relation to proportions reactive and saturated reactive on P0069 ViraQ HBV Trend 25 Control. [Each data point represents a value derived from 50 S/CO measurements before and 50 S/CO measurements after the retrospective monitoring date].

Figure 4. Correlation between $\Delta(S/CO_{M-A})$ and proportion reactive (diamonds, S/CO≥1.0) and saturated (squares, S/CO≥12.0) response levels observed with different Ultrio Plus and Ultrio Elite reagent batches on P0154 ViraQ HCV Trend 50 control. Each point represents a TMA/trend control batch combination.



Acceptance testing of NAT system component using trend control

P0069 ViraQ HBV Trend 25 can also be used for acceptance testing of a new TMA reagent batch, a new trend control batch, a reagent transport integrity check, a (re)-installation qualification of a Panther or Tigris instrument or training of an operator. For these applications it is recommended to test 20 vials of the trend control in one Ultrio Plus or Elite test run. The reagent batch, instrument or operator performance is approved when at least 17/20 (85%) of tests are reactive and the median S/CO value is above 12.7. If either one of these criteria is not fulfilled it is recommended to repeat the acceptance test procedure in another test run. If in the repeat test either one of these criteria is again not fulfilled further investigation of the performance of the reagent batch or instrument is recommended. These acceptance criteria were established by a simulation study with sliding sets of 20 sequential results out of a data base of only 190 Elite test runs[15]. A preliminary decision algorithm for accepting the NAT system component is summarized in table 7.

Table 4. Preliminary decision algorithm and criteria for acceptance of reagents, instruments or operators by replicate testing of 20 vials of P0067 ViraQ HCV Trend 25 control in one Procleix Ultrio (Plus or Elite) assay run

Acceptance criteria		Expected frequency	Decision
reactivity rate ≥17/20 (85%)	Median S/CO ≥12.7		
OK	OK	>95%	Accept
either one of criteria not fulfilled on initial test		<5%	Repeat acceptance test protocol
either one of criteria not fulfilled on repeat test		<0.25%	Initiate root cause analysis

Limitations

- P0069 ViraQ HBV Trend 25 Control cannot be used to determine the analytical or diagnostic sensitivity of NAT blood screening assays (although changes in analytical sensitivity of the NAT system can become apparent with the trend control).
- P0069 ViraQ HBV 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.
- A single nonreactive test result on P0069 ViraQ HBV Trend 25 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 distributions of assay response values on P0069 ViraQ HBV Trend 25 Control that are presented in this package insert were based on evaluation studies involving a limited number of tests and NAT reagent batches. Therefore it cannot be guaranteed that different results will be found on other assay versions or NAT reagent batches.
- The parameter $\Delta(S/CO_{M-A})$ as performance indicator of Ultrio Plus and Elite assays and the proposed threshold value of 1.60 above which a deterioration of the test system is possible needs to be further evaluated and confirmed in post-market surveillance studies.
- The preliminary decision algorithm for acceptance testing of NAT system components was based on testing only two Ultrio Elite reagent batches. The acceptance criteria need to be confirmed and re-established after evaluating a larger data base of Ultrio Plus and Elite test results on the P0069 HBV trend control.

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. Lelie PN, Van Drimmelen AAJ. Positioning of ViraQ Check and Trend Controls compatible with analytical sensitivity of NAT assays. VR4059. www.bioqcontrol.com

6. Lelie PN. Van Drimmelen AAJ. Preparation of inactivated secondary viral standards: Safety assessment of quality control samples for viral serology and NAT assays in blood screening laboratories.CE4006. www.bioqcontrol.com
7. 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 Nov;23(3):289-95
8. 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.
9. 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
10. 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.
11. Lelie PN, Van Drimmelen AAJ, Cuypers HTM, Best SJ, Stramer Hyland SL C, J.-Allain P, Moncharmont P, Defer C, Nubling CM, Glauser A, da Silva Cardoso M, -F. Viret J, Lankinen M, Grillner L, Wirthmuller U, Coste J, Schottstedt V, Masecar B. and E.M. Dax. Sensitivity of HCV-RNA and HIV-RNA blood screening assays. Transfusion. 2002,42:527-36.
12. Lelie PN, Van Drimmelen AAJ. Calibration of native and inactivated viral standards and traceability to viral nucleic acid copies and International Units. VR4060, www.bioqcontrol.com
13. 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.
14. 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.
15. Van Drimmelen AAJ, Lelie PN. Performance evaluation of ViraQ run controls for HBV, HCV and HIV-1 detection in different NAT assays. VR4061. www.bioqcontrol.com
16. Van Drimmelen AAJ, Lelie PN. Stability of ViraQ run controls for NAT. VR4058. www.bioqcontrol.com
17. 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.
18. 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.

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