PREPARATION OF INACTIVATED VIRAL STANDARDS

SAFETY ASSESSMENT OF QUALITY CONTROL SAMPLES FOR VIRAL SEROLOGY AND NAT ASSAYS IN BLOOD SCREENING LABORATORIES

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Approved by:	A.A.J. van Drimmelen (General Manager)	Signature:	Date:
	P.N. Lelie (Scientific Director and CEO)	Signature:	Date:

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1. INTRODUCTION

The run controls manufactured by Biologicals Quality Control (bioQControl or BQC) for serologic or nucleic acid amplification testing (NAT) are prepared from infectious plasma or tissue culture supernatant. The implicated viruses are Human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human T-cell Leukemia virus type 1 (HTLV-1), West-Nile Virus (WNV) and the bacterium Treponema pallidum. These agents pose a serious health risk when transmitted sexually or via parenteral routes but the infection risk in laboratory setting is considered to be negligible. Manufacturers of run control samples for daily quality control of blood screening assays usually inactivate the source material to eliminate potential infectivity in the products. For example for HIV one hour heating at 56 °C is often used and HBV in plasma is mostly pasteurized for 10 hours at 60°C. However, are these heat treatments enough to inactivate the virus? The choice of the pathogen reduction method is made by finding a balance between the capacity in reducing infectivity and the yield of the analyte after inactivation. For serological methods the virus particles can be completely destroyed as long as the antibodies or antigens remain present for detection by the immunoassays. By contrast for detection by NAT methods the viral particles should remain intact to avoid RNA or DNA being released as free nucleic acid in plasma. For the inactivated BQC standards pathogen reduction methods were used that have been extensively validated for safety of commercial plasma products. The reduction capacity of these inactivation methods has been established in animal or tissue culture infectivity experiments. In this report we describe the preparation of the inactivated BQC standards. In addition a risk analysis is performed to establish the maximum residual infectivity in the run control samples expressed in 50% human, tissue culture (TC) or chimpanzee (C) infectious doses (ID50) per mL.

For this safety assessment the viral reduction capacity of the inactivation methods is combined with the dilution factors for preparation of the ViraQ and SeraQ run control samples. Moreover for some of the evaluated viruses the 50% chimpanzee minimum infectious dose (CID_{50}) has been expressed in nucleic acid copies (cps) by direct comparison of the viral RNA and DNA concentrations in the native BQC standards before inactivation and in Japanese infectivity standards (kindly provided by Prof. Yoshizawa and Prof. Tanaka, Hiroshima University).

For nonenveloped viruses (HEV, HAV, parvo B19V) BioQControl also manufactures ViraQ run controls, which are prepared from non-inactivated viral plasma standards. The risk-benefit analysis in this report explains why inactivation of these viruses is considered to be not necessary.

2. METHODS AND RESULTS

2.1. **Infectivity of HBV**

The minimum infectious dose of HBV is estimated at approximately 3.16 (1-10) virions of HBV-DNA copies for genotypes A and C[1]. This estimate comes from chimpanzee experiments using challenge plasmas taken from the ramp-up phase of viremia and calibration of the HBV concentration against the BQC standard (see results described below). HBV is known to be approximately 100-fold less infectious in the declining phase of viremia when anti-HBc (and anti-HBe) antibodies are present and HBsAg and HBV is present in immune complexes with anti-HBs[2]. The plasma standards of HBV used for preparation of the inactivated BioQControl standards for HBsAg and for HBV-DNA were composed of a pool of plasma units from the same HBeAg and HBsAg positive donor with an HBV-DNA concentration of approximately 2.10^9 cps/mL. The infectivity of HBV in chronic HBeAg positive HBsAg carriers may be somewhat

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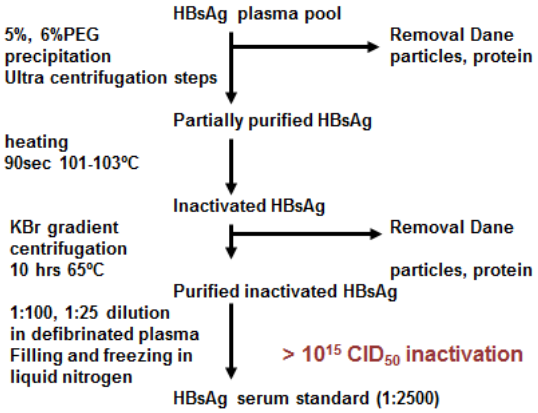
lower than in the ramp up phase of viremia as reported by Hsia et al[3] . However, more in depth analysis of the latter chimpanzee infectivity studies indicates that HBV infectivity in HBeAg positive chronic infection is probably comparable to that in the pre-acute phase[4]. We therefore assume a worst case scenario of a minimum infectious dose of 3.16 (1-10) HBV-DNA copies in the native plasma standards used for preparation of the inactivated BQC reagents for detection in serologic and NAT assays.

2.1.1. *Preparation of heat-inactivated S0001 Sanquin-VQC HBsAg (adw2) standard*

For preparation of the inactivated HBsAg standard a similar process was followed as was used for production of a plasma derived heat inactivated hepatitis B vaccine. The purified and heat-inactivated standard has been used for preparation of the World Health Organization (WHO) HBsAg International Standard and is also used for preparation the SeraQ Multi-Marker run controls.

In the 1980s the Central Laboratory of the Blood transfusion Service prepared an experimental batch of aqueous vaccine from a 25 liter pool of HBeAg positive plasma units (negative for anti-HIV) with a high HBV-DNA concentration (>10^9 cps/mL). The preparation scheme is shown in figure 1.

Figure 1. Preparation scheme of the S0001 heat-inactivated VQC HBsAg serum standard



After extraction of lipoid material with freon 113 the clarified plasma was diluted in PBS for overnight precipitation at 4 °C with 5% PEG to remove the majority of the Dane particles. The supernatant was adjusted to a 6% PEG concentration at pH 3.5 for overnight precipitation at 4 °C. The obtained precipitate was collected by centrifugation and the PEG pellet was resuspended in PBS and adjusted to a pH of 7.4. HBsAg in the PEG resuspendate was ultracentrifuged for 3.5 hour at 200,000 g. The ultracentrifuge pellet was dissolved in PBS and the partially puried HBsAg plasma protein solution with a concentration of approximately 1 mg/ml (Kjeldahl) was heated for 90 seconds at 103 °C. Unsoluble proteins were removed by centrifugation at 48,000g for 1 hour. Typically ca 50% of detectable HBsAg was lost by this heat inactivation cycle. The heat inactivated material was then subjected to overnight potassium bromide gradient centrifugation and HBsAg containing fractions were collected, pooled and dialyzed against PBS. The HBsAg concentration in this aqueous vaccine solution was measured against the first standard of the Paul Ehrlich Institute (PEI) by testing the vaccine and standard dilutions in duplicate in a radio-immunoassay (Ausria, Abbott). The concentration in the aqueous vaccine solution was determined at 86 ug/ml or 86,000 Paul Ehrlich Institute (PEI) units/ml against the first PEI standard by parallel line analysis on dose response curves.

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Normally the aqueous vaccine solution would have been sterile filtered and absorbed to aluminum phosphate after which ampoules of heat inactivated vaccine containing 3 ug HBsAg were pasteurized for 10 hours at 65 °C. In this case the aqueous vaccine batch (No 30) was not further processed to pasteurized vaccine ampoules but the solution was kept at 4 °C for several years. In order to claim the same 15 logs reduction of HBV infectivity for preparation of the VQC HBsAg standard as was established for the hepatitis B vaccine in a chimpanzee infectivity study[5], the aqueous vaccine solution was pasteurized for 10 hours at 65°C. Insoluble proteins were removed by centrifugation at 2,000g and the standard was snap frozen in liquid nitrogen before storage at –30°C. A 50 ml volume of the purified and heat inactivated VQC standard was diluted 101 fold in 5 liter defibrinated plasma prepared according to standard manufacturing procedures. This material and a further (gravimetrically recorded) 1:10,201 dilution were stored frozen at -30°C. The 1:101 VQC standard dilution was used for preparation of the WHO 00/588 HBsAg replacement standard (see below). The HBsAg concentration in the 1:10,000 standard dilution was determined in the Ausria (Abbott) by comparison with both the first WHO standard (80/549) and the first PEI standard. At three days two-fold dilutions of the three standards were prepared in negative serum and the dose response curves of duplicate assays at each day were compared by parallel line analysis. The average HBsAg concentration in the undiluted purified heat inactivated VQC standard was established at 66,000 PEI units/mL and 98,000 IU/mL. These data were in good agreement with the quantitative values obtained in a WHO collaborative study[6] and were confirmed by Schüttler et al[7] who also have investigated the biochemical and immunological properties of the purified VQC standard. The infectivity of the original plasma pool from which vaccine batch 30 was prepared is maximally $10^{9}CID_{50}$/mL According to the 15 logs inactivation demonstrated in the chimpanzee studies the infectivity titer in the purified aqueous vaccine solution was estimated below $10^{-6}CID_{50}$/mL.

2.1.2. *Preparation of 2nd lyophilised WHO International Standard for HBsAg (00/588)*

A volume of 160 ml (163.0 g) of the 1:100 VQC standard dilution was diluted 25-fold in defibrinated plasma to 4 L (4112.8 g), which is a 1:2,500 dilution of the purified VQC standard. This material was sterile filtered and filled off in bottles that were shipped to the National Institute for Biological Standards and Control (NIBSC, Pottersbar, UK). One mL volumes were filled off in approximately 4000 ampoules, which were freeze dried according to the standard lyophilisation procedure of NIBSC. This material was accepted by the Expert Committe of Biological Standardization (ECBS) as the second WHO international HBsAg standard (00/588) and contains 33 IU per vial. This unitage was found as the overall mean of potencies of all laboratories. The geometric coefficient of variation (%gcv) of all potency results found by parallel line assays on datasets provided by the laboratories was 30%. For the individual methods the potencies assigned to the standard varied from 22 IU/mL to 47 IU/mL.

2.1.3. *Preparation of WHO HBsAg reference panel and EQAS panels*

A four-fold WHO standard dilution panel was prepared of 1:10,000, 40,000, 64,000 and 256.000 dilutions of the purified inactivated VQC standard. These standard dilutions were also sterile filtered and lyophilized at NIBSC for preparation of a WHO HBsAg standard dilution panel. The lyophilized ampoules of the VQC standard dilutions were labeled as panel members A, B, C and D and contain 8.25, 2.06, 0.52 and 0.13 IU/vial. Similar 2-fold dilutions in defibrinated plasma are used for preparation of lyophilised samples distributed internationally in an external quality assessment scheme (EQAS) organized by Dr C. Weycamp (Beatrix Hospital, Winterswijk) on behalf of the Dutch Foundation for Quality Assessment in Hospital Laboratories (SKZL). Table 1 gives an impression of the average S/CO values in four different HBsAg assays that were found during the EQAS in the late1990s on the lyophilised HBsAg standard dilution panels.

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Table 1: Mean S/CO values in four HBsAg assays found during the late 1990s in the Dutch SKZL EQAS on standard dilutions of the purified and inactivated VQC HBsAg standard expressed in IU/mL.

Dilution x10,000	HBsAg concentration IU/ml	Abbott PRISM n~200	Abbott AxSYM n~400	Behring Enzygnost 5.0 n~40	Ortho HBsAg n~200
1:1	8.25				
1:2	4.12				
1:4	2.06				
1:8	1.03	35.90	11.66	22.36	16.96
1:16	0.52	18.17	5.94	12.87	8.16
1:32	0.26	9.05	3.58	6.75	4.30
1:64	0.13	4.65	2.14	3.49	2.17
1:128	0.064	2.62	1.52	1.98	1.16
1:256	0.032	1.48	1.14	1.36	0.67
1:512	0.016	0.86	0.99	0.78	0.41

2.1.4. *Preparation of SeraQ run controls for HBsAg testing*

For preparation of the SeraQ Multi-Marker Controls typically 1:50 to 1:130 dilutions of the HBsAg serum standard (1:10,201 dilution of the purified inactivated standard) are used (Table 1). Since the HBV infectivity titer in the purified and inactivated HBsAg preparation (the aqueous CLB HB vaccine solution batch 30) was established at 10^{-6} CID_{50}/mL the infectivity in the SeraQ control samples has been reduced to $<10^{-10}$ CID_{50}/mL.

2.1.5. *Preparation of S0043 pasteurized HBV-DNA standard*

Figure 2 shows the preparation process of the inactivated S0043 HBV-DNA standard out of the native S0011 HBV-DNA standard. To be able to claim 6 logs inactivation by 10 hours pasteurization at 65°C as was found in chimpanzee infectivity experiments of the Dutch semi-purified HB vaccine[5] the native Sanquin-VQC HBV-DNA standard was first diluted 100-fold in PBS to a protein concentration of approximately 0.6 mg/mL. In this protein concentration (comparable to the semi-purified HB vaccine) HBV was inactivated by 10 hours pasteurization at 65°C. After removal of the insoluble denatured proteins by centrifugation at 2000g, the heat treated material was diluted 2.5 fold in normal human plasma negative for all viral markers. The HBV-DNA concentration in the pasteurized BQC HBV-DNA plasma standard was compared with the native Sanquin-VQC HBV-DNA standard by parallel testing of 1:66667 and 1:235 dilutions of the standards respectively in 6 replicate bDNA 3.0 assays. The geometric mean HBV-DNA concentrations in the test samples were 40 625 and 38 777 cps/mL respectively. These values became 32 250 and 30 783 cps/mL respectively when the HBV-DNA concentration in the native VQC standard was adjusted for the geometric mean concentration measured in a total of 28 bDNA 3.0 assays[8]. After this 1.26 fold adjustment of the measured bDNA test results the HBV-DNA concentration in the pasteurized BQC HBV-DNA standard was established at $7.23.10^6$ cps/mL as compared to $2.15.10^9$ cps/mL in the native Sanquin-VQC standard. The yield of HBV-DNA after the preparation process of the inactivated standard was estimated at 84% (Figure 2).

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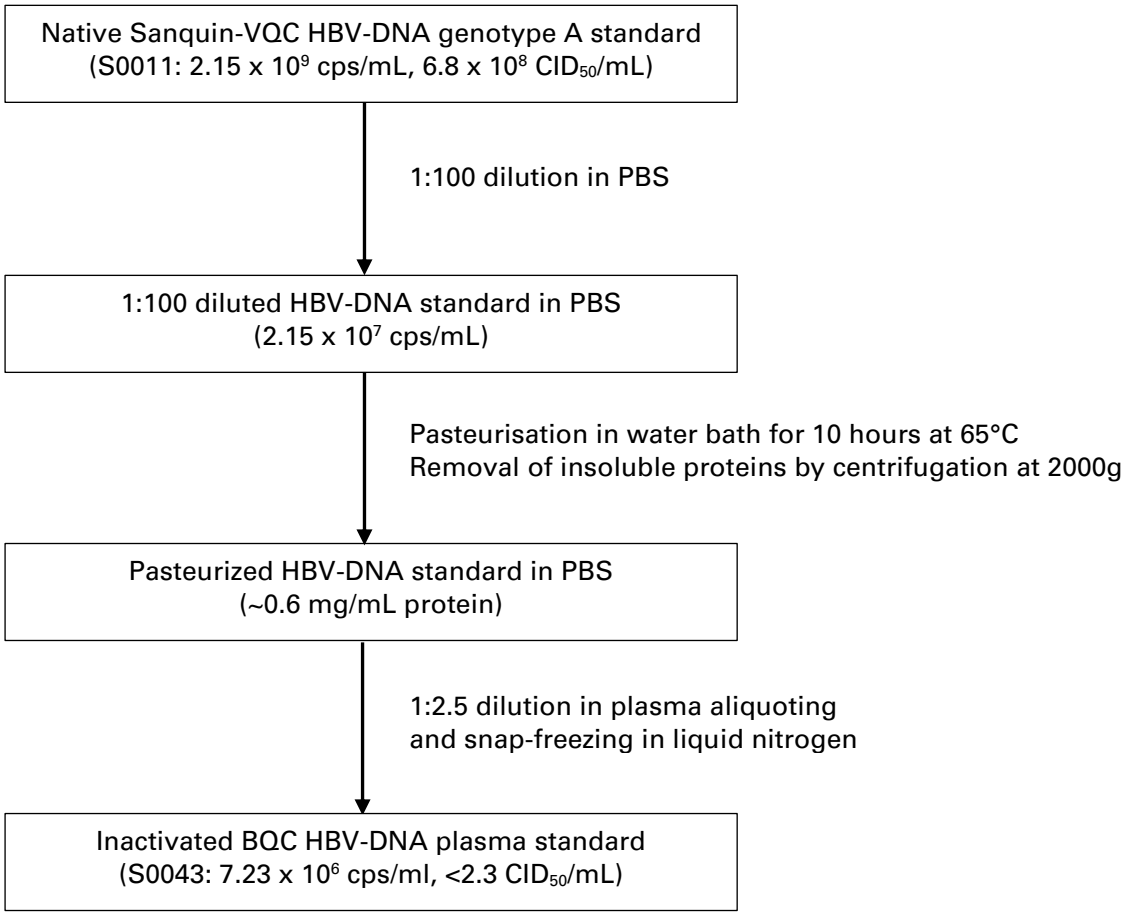
Figure 2. Preparation of pasteurized BQC HBV-DNA plasma standard



In the same calibration experiment a 1:100 dilution of sample P57 of chimpanzee C246 (kindly provided by Prof Yoshizawa and Prof Tanaka, Hiroshima University, Japan) was also tested in 6 bDNA 3.0 assays. The HBV-DNA concentration in this challenge plasma taken from the ramp up phase of viremia was established at $1.26.10^6$ cps/mL when compared to the concentration of $2.15.\ 10^9$ cps/mL in the native Sanquin-VQC HBV-DNA standard. According to interpretation of the chimpanzee infectivity data reported by Komiya et al[1] the 50% chimpanzee minimum infectious dose or CID_{50} (and 0% to 100% infectivity range) lies at 8.2 (2.6-26) copies for this HBV genotype A plasma. These estimates were based on quantification in the Roche TaqMan assay. The recalibration of the 1:100 dilution of the P57 ramp up phase plasma against the Sanquin-VQC standard showed a conversion factor of 2.06 TaqMan copies to one bDNA copy. As a consequence one CID_{50} (0% to 100% infectivity range) of the Japanese HBV genotype A strain contains 4.0 (1.3-12.6) copies when calibrated against the Sanquin-VQC standard. This value does not differ significantly from a 50% minimum infectious dose of HBV estimated at the geometric mean value between 1 and 10 virions or 3.16 HBV-DNA copies. With this worst case infectivity level of HBV the infectivity in the native VQC HBV-DNA standard is estimated at $6.8.10^8$ CID_{50}/mL. After >6 logs reduction of infectivity[5] the pasteurized BQC HBV-DNA standard contains < 2.3 CID_{50}/mL.

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2.1.6. *Preparation of ViraQ run controls for HBV-DNA testing*

The inactivated BQC HBV-DNA genotype A standard is diluted to 125 cps/mL or lower in Controls for blood screening NAT methods. The further dilution of the pasteurized standard reduces the maximum infectivity in these run controls samples to $<4.10^{-5}$.

2.1.7. *Preparation of SeraQ run controls for anti-HBc testing*

For preparation of the first S0072 anti-HBc standard plasma units were kindly obtained from Dr. C Niederhauser that tested anti-HBc reactive in multiple anti-HBc assays[27] and only units with anti-HBs titers below 50 mIU/mL were used. These plasma units were tested non-reactive in 12 replicate Ultrio Plus assays (Grabarczyk, Institute of Haematology and Transfusion Medicine, Warsaw, personal communication). Therefore the HBV-DNA level in the plasma pool was estimated to be below 0.1 cps/mL. Since the 50% minimum infectious dose of HBV in plasma of anti-HBs nonreactive occult HBV carriers is estimated at 316-1000 virions[28] the HBV infectivity in the plasma pool was estimated to be below $10^{-3.5}$ CID_{50}/mL. The dilution factor for preparation of the SeraQ Multi-Marker run controls is approximately 30-fold so that the HBV infectivity titer in the quality control samples is below $10^{-4.5}$ CID_{50}/mL. Therefore it was not necessary to treat this S0072 anti-HBc standard by solvent detergents. However presence of a low titer of anti-HBs (2-3 mIU/mL) un the run controls interfered with HBsAg stability and therefore this standard was no longer used for multi-marker controls. More recently this S0072 anti-HBc standard was replaced by the S0219 anti-HBc standard prepared from an anti-HBs negative (< 1 mIU/mL) SANBS OBI plasma unit (23754638) with a viral load of 62 IU/mL or approximately 330 copies/mL. This corresponds with an infectivity titer (range) of 1 (0.1-10) ID_{50}/mL. Typically this anti-HBc standard is diluted >100-fold to obtain the SeraQ run control. Hence it cannot be excluded that the SeraQ run control is borderline infectious (<0.1 ID_{50}/mL). We therefore decided to inactivate the S0219 anti-HBc standard by solvent detergent treatment. The plasma unit was treated with 0.3% TNBP and 1% Tween 80 and incubated for 4 hours at 37 °C in a water bath and overnight at room temperature. Thereafter the inactivated serum standard was stored frozen at -30 °C. This inactivation is known to inactivate HBV with at least 5 logs[37, 38] and hence run controls contain less than 10^{-6} CID_{50}/mL. Also if in the future the anti-HBs negative anti-HBc replacement standard would contain a 10-fold higher viral load (e.g. 3000 copies/mL or 10-100 ID_{50}/mL) the SeraQ run controls will not be infectious for HBV.

2.2. **Infectivity of HIV**

Kleinman et al[4] reviewed the infectivity of HIV in blood and plasma products and estimated the 50% minimum infectious dose in the early negative window period between 100 and 1000 virions. This estimate came from an analysis of the infused amounts of HIV in a number of transmission and non-transmission cases by blood products drawn in the early anti-HIV negative window period. From the viral load analysis of the implicated minipool NAT nonreactive window period donations a 50% minimum infectious dose (ID_{50}) of 424 virions (with a wide range of uncertainty) was estimated by probit analysis[9]. However, the infectivity of HIV in stored cellular blood products at room temperature or at 4°C is known to reduce with a half-life of 5 to 14 days[10]. Therefore it may be that the infectivity of HIV in fresh frozen plasma may be ~10-fold higher. For risk estimation we assume a worst case of an ID_{50} of 31.6 virions or 63.2 HIV-RNA copies in plasma derived HIV in the seronegative window phase. This level is 10-fold higher than that found by Ma et al for SIV in a Macaque infectivity model[11]. Bruhn et al[12] used a ID_{50} of 3.16 virions as worst case for transmission risk analysis of window period donations in an international survey. This difference may be caused by the higher proportion of defective particles in HIV infection than in SIV infection. The infectivity of HIV from window period

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plasma in tissue culture experiments is found to be less than in the transfusion setting, and this may be explained by the lower sensitivity of the cell culture systems than in human infection. One 50% Tissue Culture Infectious Dose ($TCID_{50}$) in window period plasma was found to be equivalent to 1.000 to 10.000 HIV-RNA copies[13] and Prince et al[14] estimated 1 $TCID_{50}$ of cultured HIV to be equivalent to 10 CID_{50}. Interpreting these data an ID_{50} of 31.6 virions seems to be a reasonable infectivity estimate for humans in window period plasmas or tissue culture supernatants

2.2.1. *Preparation of solvent detergent treated S0071 anti-HIV standard*

The anti-HIV standard is composed of South-African plasma units that tested positive for both HIV-RNA and anti-HIV. The plasma units were pooled and converted to serum using the standard BQC defibrination protocol. To each bottle of the serum pool 4.5 mL of 6.4 % tri(n-butyl)phosphate TNBP/24 % polyoxyethylensorbitan mono-oleate (Tween-80) solution was added per 100 mL serum. The bottles with serum containing 0.3% TNBP and 1% Tween 80 were incubated for 4 hours at 37 °C in a waterbath and overnight on a shaker at room temperature. Thereafter the inactivated serum standard was stored frozen at -30 °C. Unfortunately, the viral load has not been determined in the inactivated plasma pool, but the average HIV-RNA concentration can be predicted. When the viral load of 203 anti-HIV positive units in the US were tested the geometric mean HIV-RNA concentration (range) was 9960 (44-2,700,000) cps/mL. (Prof. M Busch, personal communication). We therefore assumed that the average viral load in the South African plasma units was <100,000 cps/mL. The solvent detergent method using TNBP and Tween-80 reduces infectivity of HIV at least $10^{4.2}$ fold[15,16]. Therefore the infectivity of the inactivated anti-HIV serum standard is estimated at $<10^{0.8}$ $TCID_{50}$ or $<10^{1.2}$ CID_{50}. In addition, presence of anti-HIV antibodies are known to reduce the infectivity of HIV approximately 100-fold[11]. The infectivity titer in the inactivated anti-HIV serum standard is estimated at $<10^{-1}$ ID_{50}/mL. The anti-HIV antibody titer in the inactivated serum standard is >1:10,000.

2.2.2. *Preparation of SeraQ run controls for anti-HIV testing*

For preparation of the SeraQ Multi-marker run controls the solvent detergent treated anti-HIV standard with an estimated infectivity titer of $<10^{-1}$ ID_{50}/mL is typically diluted 1000-fold or more. We claim that in the final product less than 10^{-4} ID_{50}/ml is present.

2.2.3. *Preparation of native S0012 Sanquin-VQC HIV-RNA standard.*

An HIV clade B (env V3) field isolate was propagated on MT2 cells for 8 weeks until an infectivity titer of $10^{4.5}$ $TCID_{50}$ /mL was reached. The culture supernatant was diluted 1:10 in pooled plasma negative for all serologic and molecular markers to obtain the Sanquin-VQC HIV-RNA standard. Over time the HIV-RNA concentration in this plasma standard has been calculated at $1.05.10^9$ cps/mL as was established from the geometric mean of 58 bDNA 3.0 assays[8]. Since the infectivity titer in the plasma standard is estimated at $10^{3.5}$ $TCID_{50}$ /mL one can calculate that one $TCID_{50}$ is equivalent to 33,200 HIV-RNA copies or 16,600 virions, approximately 10-fold higher than found by Piatak et al[13] in window period plasma. This may indicate that the amount of defective particles (e.g. p24 capsids without envelope) in the cultured Sanquin-VQC standard is higher than in window period plasma derived HIV. Assuming that the human minimum infectious dose is 10-fold lower[14] the ID_{50} would be 1660 virions, 5-50-fold higher than the estimated ID_{50} of 31.6-316 virions in window period plasma. In the worst case situation of an ID_{50} of 31.6 virions the infectivity titer in the native Sanquin-VQC standard (containing $10.5.10^8$ cps/mL or $5.25.10^8$ virions/mL) would be $1.66.10^6$ ID_{50}/mL.

2.2.4. *Preparation of pasteurized S0041 HIV-RNA standard*

Figure 3 shows the preparation of the heat-inactivated S0041 HIV-RNA standard from the native S0012 VQC-Sanquin HIV-RNA standard. The native S0012 Sanquin-VQC HIV-RNA standard

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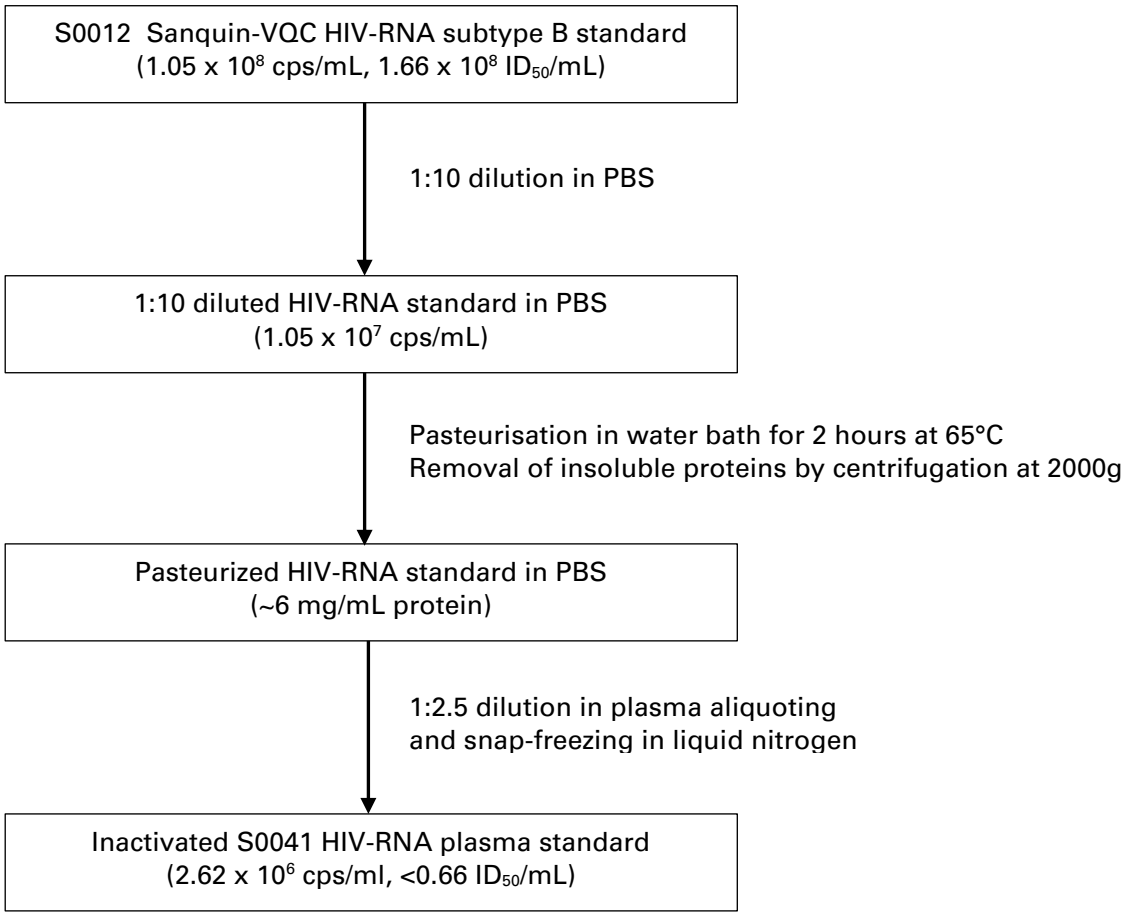
containing $1.05.10^9$ cps/mL had an estimated infectivity titer of $1.66.10^6$ ID_{50}/mL (see above). This material was diluted 10-fold in PBS to a protein concentration of ~6 mg/mL and was heat inactivated for 2 hours at 65°C. After removal of insoluble denatured proteins by centrifugation at 2000g the pasteurized material was diluted 2.5 fold in plasma to obtain the heat inactivated HIV-RNA standard. The HIV-RNA concentration in this standard was established at $2.62.\ 10^6$ cps/mL by parallel testing of 1:8333 and $1:10^5$ dilutions of the native and heat inactivated plasma standards respectively in 6 replicate bDNA 3.0 assays. The HIV-RNA recovery after heat inactivation was calculated to be 62%. A similar concentration of $2.28.10^6$ cps/mL or a recovery of 54% was measured at time point T=0 in a stability study (data not shown). During virus validation experiments of a heat inactivated HB vaccine[18] it was demonstrated that 4 logs of HIV infectivity were already completely destroyed during the 15 minutes warming up phase to 65°C. In lyophilized plasma products 4 logs of HIV was inactivated after pasteurization for 8 hours and 5 logs after 72 hours[19]. Pasteurization of a factor VIII preparation (Behring) for one hour at 60°C in the liquid phase completely inactivated 5 logs of HIV (CLB-Sanquin Virus Validation Studies, J. Over, personal communication). We therefore are confident to claim at least 5 logs of HIV inactivation during the pasteurization step of the S0041 HIV standard which contains $1.31.10^6$ heat treated virions/mL or <0.66 ID_{50}/mL.

Figure 3. Preparation of pasteurized S0041 HIV-RNA plasma standard



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2.2.5. *Preparation of ViraQ run controls for HIV-RNA testing*

The heat-inactivated S0041 HIV-RNA plasma standard containing $2.62.10^6$ cps/mL and <0.66 ID_{50}/mL is diluted to concentrations of 125 cps/mL or lower in blood screening controls. These further 1:20,960 fold dilution of the inactivated S0041 HIV-RNA standard reduces the maximum infectivity to $<3.10^{-5}$ ID_{50}/mL.

2.3. **Infectivity of HCV**

Katayama et al[20] determined the infectivity titer of a chimpanzee HCV RNA genotype 1b window period sample taken 7 weeks after inoculation. The 50% chimpanzee minimum infectious dose or CID_{50} (and 0% to 100% infectivity range) was established at 7.0 (2.2-22) copies based on quantification in the Roche TaqMan assay. The plasma used for preparation of dilutions for the chimpanzee infectivity experiment has been kindly provided by Prof H. Yoshizawa and Prof. J Tanaka (Hiroshima University, Japan) for standardization research. The HCV-RNA concentration of a 1:100 dilution of this chimpanzee C-210 window phase plasma was below the quantification limit of the bDNA 3.0 assay. Therefore dilution panels prepared from this plasma and the Sanquin-VQC S0009 standard were tested in 24 replicates in the Ultrio assay and probit analysis in parallel line model showed a conversion factor of 1.16 from bDNA to TaqMan copies. As a consequence one CID_{50} (0% to 100% infectivity range) of the HCV genotype 1b infectivity standard contains 8.1 (2.6-25.6) copies when calibrated against the S0009 Sanquin-VQC standard. Busch et al[21] inoculated a 5 x 50 ml of five pre-ramp up donations (different donors) from HCV seroconversion panels in chimpanzees. Phylogenetic sequencing identified one donor causing the infection. The pre-ramp donation was reactive in 2/27 TMA replicates. They estimated a concentration of 1.2 copy/mL and reported that 60 HCV-RNA copies in 50 mL were infectious and caused transient viremia in a chimpanzee. However reassessment of the viral load by probit analysis on S0009 HCV standard dilutions shows a lower amount of HCV in the inoculum. According to probit analysis on the Japanese HCV genotype 1 chimp plasma in the study of Grabarczyk et al[8] the 50% and 95% LODs in TMA (Ultrio) were 2.0 and 18.2 cps/mL. Using probit analysis the concentration at a rate of 2/27 TMA replicates is estimated as 0.29 cps/mL (with wide confidence limits). Hence, probably ~14 virions were present instead of ~60 copies in the inoculum of pre-ramp up phase plasma. In a second chimp previous 4 donations from the same donor (the closest three days before the infectious donation) were infused and caused no infection.. These data are in line with our estimate of the 50% infectious dose between 1 and 10 virions from the Japanese infectivity study[20].

Anti-HCV is known to have a neutralizing effect on infectivity of HCV in immune complexes. Hijikata et al[22] showed that HCV window period plasma (H strain) had a 100-fold higher infectivity than HCV present in immune complexes (F strain) originating from a chronic carrier. One CID_{50} of the H strain and F strain contained approximately 1-10 and 100-1000 HCV-RNA copies respectively, whereas the two strains banded at densities of 1.06 and 1.17 g/mL respectively in equilibrium sucrose gradient centrifugation[22]. From these data Kleinman et al[4] assumed that one CID_{50} of HCV in the window period plasma contains 3.16 virions (between 1 and 10 virions or HCV-RNA copies) and that the minimum infectious dose of HCV in anti-HCV positive plasma from chronic carriers is 100-fold higher or 316 virions (between 100 and 1000 HCV-RNA copies). *[A lower infectivity of immune complexed HCV is confirmed when interpreting the non-infectivity of so called blip viremia in the chimpanzee study of Busch et al[21]. They showed that inoculation of ~585 HCV-RNA copies of five blip viremia samples (as could be estimated from the proportion of TMA replicates on the S0009 HCV standard probit curve) did not cause infection. Most probably the 'blips' in the seroconverting plasma donor panels*

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represent passive transfer of virus from chronic carriers through shared needles. Therefore this latter chimpanzee study[21] provides another indication that the infectivity of HCV in anti-HCV positive plasma has been 100-fold reduced].

2.3.1. *Preparation of inactivated S0070 anti-HCV standard*

Anti-HCV and HCV-RNA positive plasma donations from Polish blood donors were used for preparation of a solvent detergent inactivated anti-HCV serum standard. The plasma units were pooled and converted to serum that was inactivated by the same solvent detergent inactivation method as described in detail for the anti-HIV standard above. Unfortunately the viral load has not been measured in the original plasma pool, but the average viral load can be predicted. In Poland genotypes 1 and 4 are prevalent. Dr. El Ekiaby et al[39] tested the HCV-RNA concentration in 50 HCV-RNA and anti-HCV positive genotype 1 and 4 plasma samples from Egyptian blood donors (unpublished results) and the geometric mean HCV-RNA concentration was $1.05.10^5$ IU/mL which is equivalent to $2.85.10^5$ cps/mL using a conversion factor of 2.73 copies/IU[8]. Since the pool was composed of a relatively large number of plasma units we assumed that the infectivity titer before inactivation was ~10^5 CID_{50}/mL. Prince et al[15] demonstrated that 10^4 CID_{50} of HCV (H strain) were inactivated by solvent detergent treatment on the basis of histological examination of chimpanzee liver biopsies for non-A non-B hepatitis. More recently inactivation studies are carried out in HCV cell culture systems and treatment with detergents like 0.2% Triton X-100 completely eliminated an infectivity titer of $6.0.10^4$ focus forming units (FFU)/mL[23]. The solvent detergent method also inactivated >10^5 $TCID_{50}$ of West Nile Virus (WNV), another flavivirus, like HCV[24]. We are confident inactivation with prolonged treatment with TNBP and Tween-80 of the anti-HCV standard has reduced the estimated HCV infectivity titer of 10^5 CID_{50}/mL with at least 5 logs to <1 CID_{50}/mL. As the standard is anti-HCV positive the average HCV infectivity in plasma units before inactivation is estimated at 10^3 CID_{50}/mL, 100-fold lower[22] than the estimated infectivity level of 10^5 CID_{50}/mL in case of free HCV in window period plasma. Therefore the infectivity titer in the solvent detergent treated anti-HCV standard is probably reduced to an infectivity titer below 10^{-2} CID_{50}/mL.

2.3.2. *Preparation of SeraQ run controls for anti-HCV testing*

The dilution factors of the inactivated anti-HCV standard to obtain the SeraQ Multi-Marker run controls are between 100 and 10,000-fold. Therefore the final quality control products contain less than 10^{-4} CID_{50}/mL.

2.3.3. *Preparation of S0109 HCV-RNA standard inactivated by betapropiolactone*

Initial inactivation of an anti-HCV and HCV-RNA positive plasma genotype 1 standard by pasteurization at 65 °C (following the same process in 1:10 diluted plasma as used for HIV, see Figure 2) destroyed >90% of HCV-RNA indicating that the particle integrity is affected by heat treatment. Therefore it was decided to inactivate the HCV-RNA standard by betapropiolactone. This is an aldehyde (like glutaraldehyde or formaldehyde) that cross links (membrane) proteins, does not interact with the lipid envelope and so inactivates HCV without disrupting the particles.

The inactivated S0109 HCV-RNA plasma standard was prepared from a highly viremic HCV-RNA genotype 3a window period donation from a Lithuanian blood donor. This plasma unit was tested against the S0009 Sanquin-VQC HCV-RNA genotype 1 standard in six parallel bDNA 3.0 assays on dilutions. The geometric mean concentrations in the undiluted plasmas were determined at $3.83.10^7$ cps/mL and $5.49.10^6$ cps/mL in the genotype 3a and genotype 1 samples respectively. However, over time the Sanquin-VQC standard has been tested in 27 bDNA assays and the geometric mean titer has been established at $6.30.10^6$ cps/mL[8]. Therefore we adjusted the HCV-RNA concentration in the HCV genotype 3a plasma 1.15 fold to $4.39.10^7$ cps/mL. This plasma unit was treated with 0.14% beta-propiolactone for 5 hours at 23°C followed by 18 hours

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incubation at 4°C[26]. The beta-propiolactone treated plasma was diluted 1000 fold and tested in parallel with the genotype 3a plasma before inactivation and the Sanquin VQC standard in six bDNA assays. The geometric mean concentration in the undiluted beta-propiolactone treated material was determined at $3.58.10^7$ cps/mL which was adjusted 1.15 fold to $4.11.10^7$ cps/mL when calibrated on the Sanquin-VQC genotype 1 standard. The HCV recovery after treatment with beta-propiolactone was calculated at 94%. In another calibration experiment a 1:295.6 dilution of the Sanquin-VQC standard and a 1:1000 dilution of the beta-propiolactone inactivated BQC standard were tested in 6 replicate bDNA assays respectively. The geometric mean concentrations in the undiluted standards were measured at $4.99.10^6$ and $4.80.10^7$ cps/mL respectively. After 1.26 fold adjustment to the Sanquin-VQC standard concentration of $6.30.10^6$ cps/mL we established the concentration in the beta-propiolactone treated standard at $6.06.10^7$ cps/mL. We have no explanation why this value is 1.47 fold higher than in the previous calibration experiment. This latter value was first assigned to the inactivated S0109 HCV standard. However later we calibrated this inactivated S0109 HCV genotype 3 standard against the S0009 HCV genotype 1 standard in different quantitative assays and re-established the concentration at $3.64.10^7$ copies/mL (see table 2 taken copied from our calibration report VR4060).

Table 2. Overview of potency of inactivated S0109 HCV genotype 3a standard against native S0009 HCV genotype 1 standard

Assay	n	S0009/S0109 potency	Potency of S1431 HCV genotype 3a In copies/mL	Undiluted S0109 HCV genotype 3a inactivated copies/mL
original bDNA 3.0 tests	6	1.00	1000	60600000
bDNA 3.0 repeat tests	3	1.47	680	41224490
Probit analysis Ultrio versions	48-3393	2,08	481	29134615
Probit analysis Cobas MPX	10-80	1.47	680	41224490
Hologic Aptima	16	1.92	520	31512000
Cobas MPX Ct value	16	2.47	405	24543000
Overall geomean		**1.67**	**600**	**36385863**

What is the safety of the beta-propiolactone treated standard? We exactly followed the protocol for chemical inactivation as described by Stephan et al[25] who demonstrated that this beta-propiolactone treatment completely inactivated 3000 CID_{50} of the Hutchinson strain of non-A non-B virus (later known to be HCV). After inoculation of the same immunoglobulin preparation containing $10^{3.5}$ CID_{50} before inactivation the liver biopsies showed ultra-structural changes typical for non A non B hepatitis and aminotransferase (ALT) levels were elevated because of hepatitis C. Since these experiments were done before HCV was identified it cannot be excluded that a self-limited HCV infection would have occurred. We therefore also compared the 3.5 log reduction in the chimpanzee experiment with recent HCV inactivation studies with two other aldehydes (glutaraldehyde and formaldehyde) in a cell culture system[23] (Table 3). At 5-10 fold lower concentrations as used for beta-propiolactone treatment in the chimpanzee experiment 2.6 logs reduction of HCV infectivity could be established. The reduction might be higher but the inocula of 104.6 FFU/mL needed to be tested in 1:100 dilution to avoid damage of the cells in culture by the aldehydes. Scheidler et al[26] used Bovine Diarrhea Virus (BVDV) as a

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model virus for HCV and showed that treatment of cryo-poor plasma and immunoglobulin with 0.1 to 0.25% beta-propiolactone already inactivated 3.8 to 4.1 logs infectivity after 45 to 60 minutes incubation. So we are confident that prolonged treatment of the S0109 HCV standard with beta-propriolactone reduces at least 3.5 logs of HCV infectivity. The infectivity titer in the S0109 standard before inactivation is 0.5 log lower than the concentration in cps/mL since one ID_{50} is equivalent to 3.16 copies in window period plasma (see above). Therefore the inactivated S0109 HCV standard containing $3.64.10^7$ cps/mL has an infectivity titer of less than 3.6×10^3 ID_{50}/mL.

Table 3. Overview of inactivation by treatment of HCV and BVDV model virus by treatment with betapropiolactone and other aldehydes in chimpanzee and tissue culture infectivity experiments.

Reference	aldehyde inactivation method	Matrix	model virus (mL inoculum)	Cell culture animals	Log infectivity titer before inactivation	Log reduction infectivity titer	infecivity units
Song et al	formaldehyde: 0.037%, 4 hours	serum	HCVcc, JHF-1 (1 mL)	Huh7-25-CD81 cells	4.6	>2.6*	FFU/mL
Song et al	glutaaraldehyde: 0.01%, 40 min	serum	HCVcc, JHF-1 (1 mL)	Huh7-25-CD81 cells	4.6	>2.6*	FFU/mL
Scheidler et al	betapropiolactone : 0.25%, 60 min	cryopoor plasma	BVDV (1 mL)	Fetal Bovine lung cells	5.6	3.8 ± 0.3	TCID50/mL
Scheidler et al	betapropiolactone : 0.1%, 45 min	IV immuno globulin	BVDV (1 mL)	Fetal Bovine lung cells	5.9	4.1 ± 0.3	TCID50/mL
Stephan et al	betapropiolactone : 0.14%, 5 hours 23°C, 18h 4°C	IV immuno globulin	non-A non-B Hutchinson (10 mL)	Two chimpanzees	2.5	3.5§	CID50/mL

culture of 1:100 dilution of aldehyde, §two chimps infected by 10 mL of untreated inoculum

2.3.4. *Preparation of ViraQ run controls for HCV-RNA testing*

The inactivated S0109 HCV standard containing $3.64.10^7$ cps/mL or <3600 CID_{50}/mL is diluted to 125 cps/mL or lower in blood screening controls. Hence the infectivity titers in ViraQ controls is $<1.2 \times 10^{-2}$ ID_{50}/mL. If 100 uL of the control would be infused directly in the blood stream the probability of transmission of one infectious HCV virion is estimated at approximately 1:1000. Since such an event is highly unlikely we consider that the HCV infection risk by ViraQ HCV Controls is negligible.

2.4. **Infectivity of West-Nile virus (WNV).**

Since WNV, like HCV is a flavivirus and beta-propiolactone inactivation has been used for development of vaccines[40] we assumed that the claims for this chemical inactivation for HCV also hold for WNV.

2.4.1. *Preparation of S0169 WNV Lineage 2 standard inactivated by beta-propiolactone*

VR4030 describes the preparation of the inactivated S0169 WNV Lineage 2 standard and the calibration in copies/mL.

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2.4.2. *Preparation of ViraQ run control for WNV-RNA testing*

VR4030 describes the preparation and characterization of the ViraQ WNV run control of 125 copies/mL. When assuming similar virucidal capacity by treatment with beta-propiolactone for WNV as for HCV the residual infectivity titer in the WNV run control is estimated below 10^{-2} ID_{50}/mL. Therefore we consider the risk of infection of laboratory workers handling this control negligible.

2.5. Preparation of inactivated anti-HTLV standard

Transmission of Human T cell leukemia virus type I and II occurs after transfusion of RBCs and platelet concentrates but not by Fresh Frozen Plasma or plasma products before introduction of inactivation methods[29]. The infectivity of HTLV-1 by blood transfusion or in maternal infant transmission is related to the proviral load in lymphocytes[30,31]. The anti-HTLV titers in plasma are high and typically >1:1000 dilutions are required for preparation of the SeraQ Multi-Marker run controls. Even if the infectivity of the plasma pools because of cell debris would be borderline infectious (~1 ID_{50}/mL) the infectivity titer in the final SeraQ run control products is estimated to be approximately 10^{-3} ID_{50}/mL. Hence it is in fact not necessary to inactivate anti-HTLV-1 standard batches. Nevertheless we have inactivated the S0127 anti-HTLV standard with the same solvent detergent method as applied for the S0071 anti-HIV standard. Therefore the SeraQ run controls containing the diluted S0071 anti-HTLV standard are safe for laboratory workers.

2.6. Preparation of anti-Treponema pallidum standard.

Treponema pallidum is only transmitted by fresh blood products and is not resistant to cooling or freezing. The anti-Treponema plasma pool is typically diluted 16-100 fold to obtain the SeraQ run controls which are supplied in a frozen state and therefore are considered to be non-infectious.

2.7. Preparation of plasma or serum matrix

The matrix for the ViraQ NAT controls is prepared by pooling of approximately 120 to 150 FFP units into ~30 L batches of citrate plasma to which EDTA is added to mimic the matrix in clinical samples. For preparation of the matrix in the SeraQ serology controls the citrate plasma pools are converted to serum by a defibrination process. The FFP units are derived from whole blood donations in European blood banks. All units tested negative for the serological markers (HBsAg, anti-HCV and anti-HIV 1 and 2) and for HBV-DNA, HCV-RNA and HIV RNA either by the Ultrio Elite assay (Grifols) in individual donation (ID) NAT format or by cobas MPX testing (Roche Molecular Systems) in minipools (MPs) of 6 donations. The plasma units are further tested for anti-HBc and ant-HBs to exclude presence of HBV from occult carriers and to avoid forming of immune complexes with anti-HBs in HBsAg or HBV-DNA containing run control products. The final pools are tested again for all serum markers and in 4 replicate cobas MPX tests in ID-NAT format to exclude contamination of the plasma pool in the rare event that an infectious unit had passed the NAT screening and FFP release process undetected in the blood banks.

The cobas MPX ID-NAT assays can detect 1-2 copies/ml of HBV, HCV and HIV with 50 % probability, whereas the 95% LODs are 10-20 cps/mL respectively[8]. For the donations screened by MP6 the ~50% and 95% LODs are approximately 6-12 and 60-120 cps/mL for the three viruses[32]. If one window period donation would have been missed by NAT screening due to technical or administrative failure the quadruplicate ID-NAT screening of the pools have a high probability to detect the 100-fold lower concentration in the pool, even if the concentration in the plasma unit was near the 95% LOD of the MP6 assay . However, this may not be the case for unrecognized low viral load window period units not detected by ID-NAT screening. In such a

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worst case situation 0.6-1.2 cps/mL of HBV-DNA, HCV-RNA or HIV-RNA could still be present in the pool. For HBV-DNA and HCV-RNA the ID_{50} is equivalent to 3.1 (1-10) copies and for HIV-RNA to 6.3 (2-20) copies. Therefore in the rare event a contamination of the plasma pool would not be detected in the quadruplicate ID-NAT release test procedure the infectivity in the run control products would not be higher than 0.2 - 0.4 CID_{50}/mL. However, the risk that an undetected window period donation would slip through the NAT screening is very low. In Poland the window period NAT yield rates are 1:109 401 for HCV, 1:240 266 for HBV and 1:1 501 341 for HIV[8]. When using the risk day equivalent method[33] and window period ratio modeling[34] it can be estimated from these yield rates that the risk of unrecognized window period donations in Poland with ID-NAT screening is in the order of 0.24 per million for HCV, 2.6 per million for HBV and 0.18 per million for HIV. Hence the risk of a low viral load pre-ID NAT WP contamination is highest for HBV and translates to a risk of approximately 1:3000 to 1:4000 plasma pools. If contaminated and not detected by the quadruplicate release test procedure such a contaminated pool would have an infectivity titer below the minimum infectious dose in 2.5 mL of plasma.

3. DISCUSSION

The production methods of quality control samples by Biologicals Quality Control (and in the past by Sanquin-VQC) differ from those of other manufacturers or providers of EQAS programs. Stock solutions containing high concentrations of viral standards calibrated in cps/mL by multiple bDNA 3.0 assays and in IU/mL by several WHO collaborative studies[8] are kept frozen at -80°C. At this temperature the standards have shown to be stable for more than two decades (VR4060). These plasma standards are diluted in gravimetrically recorded steps of less than 1:100 until the appropriate concentrations are reached that ensure meaningful external quality control of blood screening assays[8,32,35] . In the past Sanquin-VQC provided the PeliSpy run controls that were prepared from the native Sanquin-VQC plasma standards that were calibrated against the WHO standards[8] so that the commutability to the native virus and the traceability to copies and IUs was guaranteed. Later this activity was taken over by another company (Acrometrix/Life Technologies, Benicia, CA, USA) that replaced the PeliSpy standards by a new production process starting from heat inactivated raw materials that were no longer traceable to the old Sanquin-VQC standards. The inactivation methods of the raw materials used for the new so called 'PeliSpy Pro' run control products (Acrometrix/Life Technologies) were heating cycles, i.e one hour at 56°C for HIV and 10 hours pasteurization at 60°C for HBV. There are no data that guarantee that these methods are enough to inactivate the virus in the starting materials. Shikata et al[36] demonstrated in old chimpanzee studies (1978) that inactivation of 10^5 CID50 of HBV in crude plasma by this pasteurization process was incomplete. Therefore BioQControl decided to inactivate HBV at a 1:100 dilution in buffer solution by 10 hours pasteurization at 65°C, so that the conditions of inactivation of HBV during preparation of a plasma derived hepatitis B vaccine were copied and 6 logs reduction of infectivity could be claimed. The starting material of the heat inactivated S0043 HBV-DNA standard was the S0011 Sanquin-VQC standard and both standards have been cross calibrated in multiple bDNA assays showing that after removal of denatured heated protein the recovery of HBV-DNA was 84%. Sucrose gradient studies of the WHO and BioQControl HBV standards before and after pasteurization showed that the Dane particles in the inactivated standard were still intact (kindly performed by Prof. W. Gerlich, University of Giessen, Germany). A similar approach was followed for the preparation of the other BioQControl NAT standards, which were inactivated by methods of known virucidal capacity as validated by manufacturers of plasma products. The recovery of HIV-RNA after pasteurization of the 1:10 diluted Sanquin-VQC standard for 2 hours was calculated at 62% (and 55% in according to another calibration experiment). Interestingly

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the recovery after lyophilisation and reconstitution of the Sanquin-VQC HIV standard was found to be lower at 25% (data not shown) indicating that freeze drying is a harsher treatment than heating for the particle integrity of HIV. The damaging effect of heat inactivation is even higher for HCV. A similar pasteurization process of the Sanquin-VQC HCV-RNA standard destroyed >90% of the virions. This standard is antibody positive. The HCV particles are likely to be present in immune complexes and the physical properties are known to be different from free HCV in window period plasma[22]. The target for NAT assays primarily are the highly infectious donations drawn before anti-HCV seroconversion. Therefore a new standard was prepared from an HCV-RNA genotype 3a window period donation. We decided to inactivate HCV in this plasma standard by beta-propiolactone. Like other aldehydes beta-propiolactone reduces infectivity by cross linking of membrane proteins and leaves the viral particles intact. Indeed the recovery after this chemical inactivation process was 94% for HCV but variable (5-80%) depending on the method for WNV. Despite the rather modest 3-4 log reduction of HCV infectivity that can be claimed from experiments in chimpanzees and tissue culture[23,25,26] the dilution factor for preparation of the run controls is high enough to ensure almost absence of infectious HCV in the end products (Table 4). The traceability of the cps/mL and IU/mL values of the VQC-Sanquin HCV-RNA standard could be maintained by cross calibration of standards in multiple parallel bDNA assays (see methods and results). Further studies of the inactivated BioQControl NAT standards for HCV, HBV and HIV demonstrate particle integrity and stability when testing dilutions of the standards before and after heat inactivation.

The commutability of the purified and heat inactivated HBsAg standard that is used for preparation of the SeraQ run controls and the WHO International Standard has been studied in a calibration study of HBsAg assays[6] and the physico-chemical and immunological properties of the standard were examined by Schüttler et al.[7] They showed that the HBsAg particles in the purified heat inactivated standard were still intact as were the major p24 and gp27 S proteins to which the anti-HBs monoclonal antibodies in HBsAg assays are directed[7]. The purification and inactivation process guarantees at least 15 logs of HBV reduction[6] and therefore more than 10 logs overkill of HBV is claimed in the SeraQ HBsAg run controls. For the anti-HIV and anti-HCV run controls the plasma standards are inactivated by the solvent detergent method that disrupts the viral envelopes but does not affect the antibody reactivity of diluted standards. From the chimpanzee and tissue culture experiments that have been published the claim for the reduction of viral infectivity in the inactivated standards is limited by the height of the infectivity titers in the challenge materials. The solvent detergent method has proven to be a very robust inactivation method for envelope viruses in the plasma industry[37,38] and probably the reduction of viral infectivity is higher than we have claimed (Table 4).

The highest risk of infectivity of run controls for the NAT and serologic blood screening assays comes from the plasma and serum matrices in which the inactivated viral standards are diluted. We estimated that 1:3000 batches of 30L plasma could be contaminated by an HBV or HCV window period donation that is not detected by the NAT screening of plasma units and the quadruplicate release testing of the plasma pools. However if this happens the contamination level is still below the infectivity threshold even if 1-10 mL of run control plasma would be injected intravenously. Laboratory incidents with the run controls or donor samples in a screening laboratory are restricted to splashing of plasma to skin near cutting wounds or eyes, which can be prevented by wearing gloves or protective glasses. In fact, the highest infection risk of the run control and donor screening samples comes from contamination by viruses that are not screened for. Therefore BioQControls considers to test future plasma matrices also for

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HAV, HEV or B19V to avoid any chance of transmission in the unlikely event of spread by aerosols caused by highly viremic donations present in the starting pools.

Clear guidelines on acceptable risk levels similar to the classification of chemicals are lacking for quality control reagents. Recommendations made by the CDC and WHO use the principle: 'as low as reasonable achievable' (ALARA principle). In the warning and precaution sections of the BioQControl product inserts it is recommended to follow CDC guidelines for handling and disposal of the materials in order to reduce the risk of any adverse events. Complete absence of pathogens in quality control samples cannot be guaranteed. This risk analysis report proves that the evaluated run control products are very unlikely to contain infectious pathogens endangering laboratory workers or the environment during transport. Therefore the inactivated ViraQ and SeraQ products do not to need to be classified as UN3373 for transport of dangerous goods.

Table 4. Viral concentration and estimated infectivity titers in BioQControl standards before and after inactivation and in run control products.

BioQ standard	Inactivation method	before inactivation		in standard after inactivation			in run controls	
		cps/mL	ID50/mL	cps/mL	ID50/mL	yield	cps/mL	ID50/mL
HBV-DNA	10 hours pasteurization at 65°C, 1:100 diluted in PBS	2,15E+09	6,80E+08	7,23E+06	<2,30E+00	84%	<125	<0.4E-04
HCV-RNA	0.14% betapropiolactone for 5 hours at 23°C, 18h 4°C	4,39E+07	1,38E+07	4,11E+07	<4,11E+03	94%	<125	<1.2E-02
HIV-RNA	2 hours pasteurization at 65°C, 1:10 diluted in PBS	1,05E+08	1,66E+06	2,62E+06	<6,60E-01	62%	<125	<1E-05
HBsAg	90 sec at 103°C, 10 hours at 65°C of purified HBsAg	3,00E+09	1,00E+09	?*	<1,00E-06	~10%	?#	<1,00E-10
anti-HBc	no inactivation	<1,00E-01	<3,16E-04					<3,16E-05
anti-HCV	0.3% TNBP, 1% Tween 80 for 4 hours at 37 °C, 16h RT	~2,58E+05	~8,16E+02	?	~8,16E-03	~100%	?	<1,00E-03
anti-HIV	0.3% TNBP, 1% Tween 80 for 4 hours at 37 °C, 16h RT	<1,00E+05	<3,16E+03	?	<1,00E-01	~100%	?	<1,00E-04
anti-HTLV	no inactivation¥		<1,00E+00					<1,00E-03

**98 000 IU HBsAg/mL*
#<0.26 IU HBsAg/mL
¥ inactivation not necessary, but BQC anti-HTLV standard has been solvent detergent treated

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4. SUMMARY AND CONCLUSION

Table 4 summarizes the viral concentrations and residual HBV, HCV or HIV infectivity in the native Sanquin-VQC standards and in the BioQControl standards that are inactivated by validated methods used in the plasma industry. The log reduction data that can be claimed from infectivity experiments in chimpanzees and tissue culture systems guarantee absence of infectivity in the inactivated viral standards, except for the undiluted HCV-RNA and WNV-RNA standards in which some rest infectivity cannot be excluded. However our risk analysis demonstrates that the virus concentration in the final run control products is far below the infectivity threshold, also for the HCV-RNA and WNV-RNA run controls. The test and release process of the (defibrinated) plasma matrix guarantees less than 1 CID_{50} of HBV or HCV per mL plasma in case of contamination. This could occur once in 3000 plasma batches according to the estimated frequency of pre-NAT window period donations in Poland.

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