Stability of Human Immunodeficiency Virus serological markers in samples collected as HemaSpot and Whatman 903 Dried Blood Spots

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Key Words: Dried Blood Spot, HemaSpot, 903 cards, HIV diagnostics, stability,

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Abstract

Dried blood spots (DBS) are frequently used in clinical testing for biosurveillance, infectious disease and confirmatory testing, and clinical trials, particularly for populations in remote areas. The HemaSpot™-HF Blood Collection Device (HS) provides an alternative format to the Whatman 903 cards (903) to simplify sample collection and processing. In this study, the performance of the HS device was compared to that of the 903 using previously characterized clinical specimens and HIV seroconversion panels known to exhibit markers of early Human Immunodeficiency Virus (HIV) infection. HS and 903 samples were prepared and tested by Bio-Rad GS HIV Combo Ag/Ab EIA, GS HIV-1/-2 Plus O EIA, GS HIV-1 Western Blot, and HIV-1 Genius assays. Both HS and 903 performed well for up to six months at room temperature but a marked loss of Western Blot and low titer antibody signal from early infection samples was observed in samples stored for 180 days at elevated (37-45°C) temperature and high humidity (95%). HemaSpot samples placed in sealed bags with additional desiccant were protected from degradation and showed improved signal recovery relative to 903. HS was easier to use than the 903 and showed higher sensitivity and reproducibility for early infection samples and improved stability.
Background

The ability to easily and efficiently collect, preserve, and transport high quality biospecimens is critical for the success of clinical screening and biosurveillance programs for infectious pathogens detection, particularly among populations living in remote and/or low resourced areas. Dried blood spots (DBS) offer a simple and practical alternative to acquire specimens in cases where traditional blood collection methods involving venipuncture by a trained phlebotomist or on site centrifugation of fresh blood samples may not be feasible [1-4]. Collection of DBS by fingerstick is minimally invasive, and can be performed by individuals with minimal technical skills. DBS are stable for several weeks at ambient temperature, and are considered non-regulated, exempt materials that can be easily shipped to laboratories by regular mail without requirement for cold-chain transportation [5-7].

Dried Blood spots have been successfully used for testing of populations for prevalence of HIV-1/2, hepatitis C virus (HCV), hepatitis B virus (HBV) and herpes simplex virus type (HSV), with test results comparable to those from venous blood [8-11]. HIV-1 enzyme immunoassay (EIA) and Western blot (WB) testing of DBS have enabled cost-effective early infant diagnosis [12], and culturally appropriate HIV serosurveillance [1,13]. Surveillance of HIV infection among various high risk groups based on HIV antibody, antigen and nucleic acid test results of DBS demonstrated a more accurate estimation of HIV prevalence than those based on data from prenatal clinics, STI clinics, and programs monitoring female sex workers [15,16]. Test results of HIV-1 EIA and HIV-1 WB of matched dried blood spots samples proved to be a practical and efficient approach for monitoring of the accuracy of field performance of HIV Rapid Tests at peripheral diagnostic centers in Niger, prenatal clinics in rural Cameroon, or testing of men who have sex with men (MSM) in South Africa [17-19]. Dried blood specimens provided a convenient and reliable format for confirmation of positive HIV rapid tests of Guinean women by Geenius™ HIV 1/2 in a central lab [20]. Testing of DBS with fourth generation HIV Ag/Ab Combo may enable more sensitive detection of early infections, and had been shown to identify more (34/39) HIV early infections than the Determine HIV1/2 rapid test (24/39) [21]. With improvement in technology, DBS are increasingly being used for clinical monitoring of viral load in HIV infected individuals on antiviral treatment or treatment naïve patients [22,23], and drug resistance genotyping analysis, with results similar to those of plasma [24-26].

As DBS devices are more widely employed, issues attendant to variability of test results due to inappropriate handling of specimens, contamination, or inadequate laboratory quality control [27,28] need to be addressed. A study of infants born to HIV-1-infected women in Equatorial Guinea found discordant results with use of DBS in both serological and molecular assays, with frequent (17/68) false-positive results on viral load testing [29]. A DBS HIV surveillance study in India found poor concordance between HIV rapid test and DBS laboratory test results, with many of the initially reactive samples not confirmed on repeat testing [14]. DBS and centralized testing revealed higher rates of HIV positivity with the Calypso BED Incidence enzyme
immunoassay (EIA) than expected from the local prevalence data [30]. Higher unconfirmed positivity rates were also observed in monitoring mother-to-child transmissions by testing of DBS samples in a central lab than those obtained by use of Rapid Diagnostic tests. [31]. In addition to concerns about higher false positive rates, issues of analyte sensitivity for detection of early infection [14], and stability of analytes such as HBsAg and anti-HBc upon prolonged storage [32] must be considered when using DBS for clinical applications.

The extensive handling of 903 cards during collection and processing can lead to variability of results or cross-contamination when specimens are applied too closely together or from accidental splatter or smearing during application. Prior to packaging, 903 DBS samples must be dried for 2-18 hours in open air [6], which can allow contamination from air and surfaces. Recovery of antibody, antigen, nucleic acid and other biological analytes from incompletely dried DBS can be quite variable, particularly for markers near the clinical end point of detection [6,33,34]. Additional opportunities for contamination can also occur during the laboratory processing steps, including excision of DBS by filter punch. In contrast, the HemaSpot™-HF Blood Collection Device (SpotOn Sciences, Inc., Austin, TX) is a self-contained unit consisting of a pre-cut segmented 8-wedge HemaForm (HF) membrane positioned in a crush resistant, moisture resistant, hard plastic cartridge that can be decontaminated as general biohazard waste and disposed in regular trash with no additional safety precautions. The device is simpler to use, less prone to contamination from other samples, and does not require extended drying times. A desiccant within the cartridge ensures efficient drying of the sample, with no refrigeration or cold chain requirement. Individual wedges can be readily removed by a slight twisting motion with forceps, for simplified processing in the laboratory. The HemaSpot is CE marked and is registered as a Class I with FDA. In this study, we compared the performance of the HemaSpot™-HF Blood Collection Device to the Whatman 903 card for testing of HIV positive specimens and seroconversion panels containing early HIV infection panel members.

Materials and Methods

Specimens used for testing of DBS were prepared from previously tested, HIV-1 positive or negative serum samples submitted for clinical testing, and were supplemented with well-characterized plasma specimens from the PRB204 panel that included weakly reactive HIV EIA members, or from PRB910 and PRB947 seroconversion panels containing early HIV-1 infection members (SeraCare, Inc., Milford, MA). Serum or plasma specimens were mixed with an equal volume of whole blood (O Negative) from uninfected individuals (Biological Specialty Corporation, Colmar, PA) prior to spotting on the filter membranes.

Samples were applied as 20 µl, 80 µl, or one drop to the center of the spot on Whatman 903 Protein Saver Cards (903) (Whatman #10534612). The cards were placed into a card drying rack, dried overnight in a Biological Safety Cabinet, and placed into Ziploc sandwich bags with 2-3 Desiccant Packs (Fisher #09923360). A duplicate set of samples was prepared on the
HemaSpot™-HF Blood Collection Device (SpotOn Sciences, Inc., Austin, TX) by applying two drops, or 100 μl of blood to the middle of the central disk and allowing the sample to absorb for at least five min before the Device was snapped shut. Samples were stored at room temperature for 2-5 days prior to testing. During preliminary testing, DBS and HS were eluted in PBS-T buffer, and were found to work well in the Bio-Rad 1/2/O EIA (1/2/O), but a high background was observed in the Bio-Rad GS HIV Combo Ag/Ab EIA (Combo) test (Bio-Rad, Redmond, WA). The thermo/humidity stability study, in which samples were incubated for up to 180 days at room temperature (20-23°C) and ambient humidity (<40%), or in a 37°C or 45°C incubator at high (95%) humidity, was therefore performed using the 1/2/O assay. A duplicate set of spots (in both DBS and HS) were prepared for the Geenius testing and for each time point in the stability study. Once removed from its original bag or cartridge, the samples were tested that same day and not returned to storage. Subsequently, an alternative elution procedure using Western Blot Buffer was obtained from the CDC [35] which worked well for the Ag/Ab Combo assay, allowing us to complete the performance evaluation HS/903 DBS for sensitivity, specificity and reproducibility.

For Bio-Rad HIV-1 Western Blot testing, a 6.4 mm (1/4 inch) disc was cut-out from a 903 card or two HemaSpot wedges were removed by slight twisting motion with clean forceps, placed directly into the Western Blot sample tray, and processed as per manufacturer’s instructions for the assay. For the stability studies, a 12.9 mm (1/2 inch) disc cut-out from the 903 cards or the remainder of the HS (6 wedges plus the center) (Fig 1) was eluted overnight into 300 μl of PBS-T at 2-8°C, and 75 μl of eluted material was tested by HIV-1/2/O. For HIV Ag/Ab Combo testing, two HS wedges or a 1/4 inch cut-out from 903 were eluted into 150 μl Western Blot Specimen Diluent/Wash buffer (Bio-Rad catalog # 32574) at room temperature for 30-60 minutes and 75 μl of eluted material were tested in the Combo, or 40 μl in the Bio-Rad HIV-1/2 Geenius test. The Ag/Ab Combo Cutoff Value was set as the mean absorbance for the cutoff calibrators (CC) + 0.15 [35].

Ethical considerations

The results reported represent an analysis of a quality assurance activity on clinical discard samples or commercial panel materials within an existing clinical regulated activity. The WRAIR Human Subjects Protection Branch has determined this study (WRAIR #2188) involves analysis of anonymized samples with no link to individual patients. As such, the study does not meet the definition of research involving Human Subject, and no IRB Review was required.

Results

The recovery of HIV Ag/Ab Combo EIA signal from HS and 903 DBS was evaluated on serial dilutions of an HIV reactive clinical sample diluted in normal human blood up to a 1:10,000 dilution (Fig. 2). Values for signal to cut-off (S/CO) greater than 1.0 are considered reactive.
The original plasma sample yielded the highest S/CO signal; and was reactive at up to a 1:1,000 dilution while the HS and 903 samples were reactive at a 1:100 dilution. Samples which yielded S/CO values in the linear range (3-12) of 3rd or 4th Gen EIA assays, yielded consistently higher S/CO values (3.05 +/- 0.88) when eluted from two HS wedges than those from the 1/4 inch cut-out from 903 cards.

The reproducibility of HIV Ag/Ab Combo signal from 903 and HS DBS was evaluated by replicate testing of spots prepared from the Bio-Rad Virotrol-2 Low Copy Antibody Control. Spots prepared from a 1:1 dilution of Virotrol-2 in negative whole blood were tested in seven (7) replicates per day over three (3) days (21 replicates total). The results are shown in Fig 3 and Table 1. The S/CO of HS samples was consistently reactive with a mean of 4.19, while that from 903 was 1.23, with 4 of the 21 replicates below the S/CO value of 1.0. The reproducibility of signal was also tighter for the HS with intra-assay CV at 10.4% and inter-assay CV at 9.7 %, respectively, while that for the 903 card was 20.8% and 21.1%.

Recovery of the HIV Ag/Ab Combo signal from HS or 903 membranes was evaluated in 51 previously tested clinical samples (30 Reactive plus 21 Non- Reactive samples). All 30 reactive samples were reactive in the HS and 903 DBS, while all 21 non-reactive samples were non-reactive. The recovery of HIV Combo was also evaluated in more challenging samples, prepared from each of the 18 members of the PRA204 low titer HIV panel. The results are plotted in descending order of HS S/CO, with the last two members being the negative panel members (Fig 4). Again, the original plasma samples gave the highest signals. The S/CO signal of the low titer samples showed consistently better recovery than those from the 903 card, except for member #12. Two HS and three 903 samples prepared from the very low plasma reactive members of PRA204 were non-reactive (Fig. 4). The two negative PRA204 samples were also negative on both HS and 903.

Based on the results of the 51 clinical and 18 PRA204 panel members, we calculated the sensitivity of the 903 at 93.5%, and of HS at 95.6%, with a Specificity and Positive Predictive Value of 100% for both (Table 2). The Negative Predictive value was 88.5% for 903 and 92.0% HS, with accuracy at 95.7% and 97.1%, respectively.

The recovery of low titer HIV-1 antibody signal from early infection was also evaluated by the third Generation HIV-1/2/O EIA on both 903 and HS using the SeraCare PRB914 and PRB947 seroconversion panels (Fig. 5). The signal from the HS DBS was consistently higher than from 903, with all positive members being detected except for one member (PRB914-03) that was just below the cut-off value for the 903 sample. The first member of PRB947 was HIV 1/2/O non-reactive on the original plasma and was also non-reactive on both the HS and 903.

The recovery of HIV-1 Western Blot signal from samples collected on HS was evaluated for three plasma samples from individuals with established HIV infection. The signal recovered...
from one or two HS wedges was indistinguishable from that of the original plasma (Fig 6). An additional ten positive samples were also tested by HIV-1/2 Geenius assay following storage of HS or 903 spots at room temperature. All samples were reactive for HIV-1, with 10/10 still reactive on HS and 9/10 reactive on 903 spot. The remaining sample was Geenius Indeterminate (IND) after 300 days of storage at room temperature.

Since DBS are frequently used for evaluation of populations in tropical environments, we evaluated the impact of high temperature (37°C and 45°C) and high humidity (95%) on antibody stability as detected by the Bio-Rad HIV-1/2/O assay. Controls were stored at room temperature (20-23°C) and ambient humidity (<40%). All 10 of 10 (100%) clinical samples from confirmed HIV infection remained reactive for up to 180 days when stored on 903 cards sealed in Ziploc bags with additional desiccant (903-S) at room temperature (RT) or 37°C in high humidity (Fig 7). However, 4/10 (40%) 903 samples stored at 45°C in high humidity became non-reactive by 180 days. The corresponding HS samples snapped shut in their cartridges but stored in open bags with no additional desiccant (HS-O) at room temperature remained reactive for 180 days, but 2/5 (40%) HS-O samples stored at 37°C for 180 days, 2/5 (40%) stored at 45°C for 30 days, and 3/5 (60%) stored at 45°C for 180 days became non-reactive. When the HS cartridges were sealed in Ziploc bags without additional desiccant (HS-S), all (100%) remained reactive except for those at 45°C for 180 days. An HS sample stored in a Ziploc bag with additional desiccant remained reactive even at 45°C for 180 days (not shown).

All 903-S samples stored at room temperature for up to 180 days were reactive on HIV-1 WB, but 1/10 (10%) became WB (IND) after 30 days at 45°C or 180 days at 37°C, and 9/10 (90%) became IND or non-reactive by 180 when stored at 45°C. HS samples stored in open bags (HS-O) remained reactive for 180 days at room temperature, but 1/5 (20%) became WB IND after 30 days storage at 37°C, and 4/5 (80%) were IND or Neg when stored at 45°C. By 180 days, 4/5 (80%) samples stored at 37°C, and all 5/5 (100%) stored at 45°C became WB IND or Neg. All 5/5 (100%) HS samples sealed in Ziploc bags without additional desiccant (HS-S) remained HIV-1 WB positive at 180 days when stored at room temperature or 37°C and high humidity, but all became IND or Neg by 180 days at 45°C. HS samples sealed in Ziploc bags with additional desiccant remained 1/2/O and WB reactive even after 180 days at 45°C (not shown).

Stability of antibody signal (HIV-1/2/O) from early HIV infection was also conducted on initial reactive members from SeraCare HIV-1 seroconversion panels (Fig 8). Seroconversion member PRB947-04 stored as 903 cards in a sealed Ziploc bag with desiccant (903-S) showed lower reactivity (S/CO), but remained reactive when stored for 30 days at RT, 37°C, or 45°C. Samples stored at 37°C or 45°C for 180 days were non-reactive. The corresponding HS sample (HS-O) was reactive at 180 days if stored at room temperature, but non-reactive by 30 days when stored at 37°C or 45°C at high humidity. The stability of the 1/2/O signal was also compared in another seroconversion panel member PRB910-03, in which the HS sample was stored in a Ziploc bag with additional desiccant (HS-S), while the 903 was left in an open bag with no desiccant (903-S).
O). The HS-S samples were stable up to 180 days at room temperature or 37°C. These samples were also stable for 30 days at 45°C, but became borderline-reactive by 180 days at 45°C at high humidity. The 903 samples stored in unsealed bags with no additional desiccant (903-O) at room temperature, 37°C or 45°C at high humidity became non-reactive by 30 days.

Discussion

In this study, we evaluated the suitability of Whatman 903 and HemaSpot DBS for serological testing for HIV infection and their stability under conditions mimicking those in tropical climates. Both the 903 and HS showed good recovery of HIV antibody and antigen signal as detected by the Fourth Generation HIV Ag/Ab Combo, the Third Generation HIV 1/2/O EIA, as well as the supplementary HIV-1 Western Blot and HIV-1/-2 Geenius assays. The Western blot patterns obtained from elution of sample from one or two wedges of HS was virtually indistinguishable from that of the original plasma sample. However, the HS and 903 dried blood spots were somewhat less sensitive for detection of very early infection as shown by decreased sensitivity on commercial panels containing low levels of antibody (Low titer and Seroconversion Panels). The HS showed a higher recovery (4.43 vs 1.30 S/CO) and greater consistency (10.4% vs 20.8% CV) for the HS as compared to 903 when tested on Bio-Rad Virotrol-2 low titer serological control. Similarly higher HIV Ag/Ab Combo signals were obtained from HS than 903 in low titer samples of PRA204 panel and HIV 1/2/O signal from early seroconversion members. The lower recovery from 903 cards can translate to decreased sensitivity, particularly for detection of early infection as one additional low titer member (PRA204-22) and at least one seroconversion member (PRB914-03) were detected from the sample stored as HS, but not the corresponding 903 card.

The decreased sensitivity in the Combo and EIA assays from both HS and 903 relative to the original plasma can be in part accounted for by a smaller sample size. The HIV Combo assay, for example, uses a volume of 75 μl of plasma for testing. The amount of blood eluted from 2 HS wedges would be about one fifth of the blood applied to HS (approx. 100 μl), with half of that (75 μl of the 150 μl eluted), or an equivalent of 10 μl used for testing. In the case of the 903 card, the 1/4 inch cut-out represents approx. one third of the spot created by one drop of blood (50 μl) and half of that (75 μl of 150 μl elution), or about 8 μl used for testing.

Our results are in agreement with those of Luo et al which shows good recovery of HIV signal from 903 cards using modified FDA protocols for GS Combo HIV Ag/Ab EIA and Geenius HIV-1/2 supplemental assay [35]. Although the dried blood spots in their studies performed well on samples from established infection, they were less sensitive at detecting acute HIV infections than the corresponding plasma or serum, consistent with lower signal observed for seroconversion members in our study. The decreased sensitivity could result in part, from use of lower volume, although more than one punch did not improve sensitivity. Their study also showed that Geenius testing of DBS was comparable to that of plasma, with very few HIV-
untypable or HIV-Indeterminate results [35]. Equivalent signal recovery of HIV-1 p24 antigen was also reported in samples collected as dried plasma spots (DPS) or venous plasma in another study [36]. Comparable results were also reported between matched plasma and DBS samples from HIV-1 infected individuals as tested on the BioPlex assay, which measures direct antibody binding and avidity to multiple HIV-1 analytes [37].

Previous studies had shown that 903 DBS are quite stable when stored at room temperature and low humidity conditions, and retain activity for up to several months. HIV-1/2 antibody detection from whole blood spots samples stored 4°C, -20°C and -70°C showed comparable performance to the gold standard of fresh serum and remained stable for several weeks at room temperature and low humidity as tested by EIA, immunofluorescence, or HIV-1 Western Blot [2,5,8,38]. However, antibody stability started to deteriorate in high humidity after one month storage at room temperature or one week at 37°C [5,33]. Our studies extend these observations to show that both HS and 903 DBS are stable for up to 6 months at room temperature, but may lose reactivity on prolonged storage of 30 days at high humidity at 37-45°C as may be encountered in many tropical climates. Sealing the samples in Ziploc bags with additional desiccant extended stability, suggesting that humidity rather than temperature alone is the significant factor in loss of reactivity. The HS sample showed superior recovery and stability to 903 cards under these conditions if protected from high humidity and temperature in a Ziploc bag with additional desiccant.

Our studies had several limitations. The use of simulated samples prepared from frozen plasma or serum diluted in whole blood may not perform identically to those collected in the field. Dilutions of sample in whole blood as used in Fig 2, is not equivalent to low reactive samples present in early infection. The volumes of serum used in the laboratory testing (75 µl for HIV-1/2/O and HIV Ag/Ab Combo, 5 µl for Geenius) are higher than the equivalent volume recovered from HS and 903, which can account at least in part for the lower sensitivity of DBS.

We have shown that samples collected as DBS including Whatman 903 cards and HemaSpot provide reliable test results in HIV screening (HIV 1/2/O, HIV-1/2 Ag/Ab Combo) and supplemental/confirmatory (HIV-1/2 Geenius and HIV-1 Western Blot) assays even under harsh environmental conditions. The HemaSpot device (about $6.00 per unit in the US, or $2.53-$3.00 outside US global public health clients) is easier to handle, requires shorter drying times, and provides more convenient processing in the laboratory with decreased opportunity for potential for cross-contamination than the standard 903 card ($2.30 per unit). The HS device demonstrated higher yields and more consistent recovery of analyte for detection of early infection, with improved stability at high temperature and humidity when stored in Ziploc bags with additional desiccant. Since this study was conducted, SpotOn has reformatted the HemaSpot-HF product packaging to include a Ziploc with additional desiccant, which should further improve preservation of samples under conditions of high humidity. Use of HemaSpot for molecular
testing including HIV drug resistance studies [39], and other nucleic acids testing applications [40], is the subject of ongoing studies.

References


40 SpotOn Sciences Publications: https://www.spotonsciences.com/knowledge-center/

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Figure Legends

Fig. 1. A. HemaSpot with two wedges torn off. B. 903 cards with 80 µl of blood before and after a 13.0 mm (1/2 in.) cut-out or 20 µl before and after a 6.5 mm (1/4 in.) cut-out.

Fig. 2. Recovery of HIV-1 Ag/Ab Combo signal from serial dilutions of a reactive plasma sample eluted from a 1/4 inch cut-out of a 903 card or two wedges of a HemaSpot. Seventy-five (75 µl) of the 150 µl eluate were used for testing. An S/CO >1.0 designates a reactive sample.

Fig. 3. The reproducibility of signal recovery from HS and 903 cards was evaluated for the low titer Virotrol-2 Control (Bio-Rad) in 7 replicates per day over 3 days. The HS gave consistently higher signal, with tighter CVs for both Intra-assay and Inter-assay reproducibility.

Fig. 4. Relative signal intensity for Bio-Rad HIV Ag/Ab Combo obtained by testing the original plasma samples from the PRA204 Low Titer Panel compared to that recovered from a 1/4 inch cut-out of 903 cards or two wedges of HS. Samples are arranged in decreasing order of HS reactivity, with the members at the far right representing the negative panel members.

Fig. 5. Recovery of HIV-1/2/O signal in 903 and HS membranes prepared from early seroconversion samples. The HS signal is consistently higher. Member -01 from PRB947 was also negative in the original plasma sample.

Fig. 6. Comparison of Western Blot signal obtained from three patient plasma samples to that of one or two wedges from the corresponding HemaSpot samples.

Fig. 7. Percent of positive serum samples stored on 903 and HS dried blood spots that remain HIV-1/2/O reactive. Ten replicate aliquots of serum samples from established HIV infection were spiked into whole blood, spotted on HS or 903 DBS, and stored at room temperature/ambient humidity (<40%), or at 37°C or 45°C at 95% humidity for up to 180 days. 903-S samples were stored in sealed Ziploc bags with additional desiccant. HS-O HemaSpot
samples snapped shut in their cartridge were stored in an open bag allowing moisture to come in contact with the cartridge. HS-S HemaSpot samples were stored sealed in Ziploc without additional desiccant.

Fig 8. Comparison of 903 DBS to HS for HIV 1/2/O stability from samples acquired at early HIV infection. 903 DBS and HS from early HIV-1 infected seroconversion Panel members PRB947-04 or PRB910-03 stored in sealed Ziploc bags with additional desiccant for 903-S or without additional desiccant for HS-S. Control samples were stored in an open bag with no added desiccant (903-O or HS-O). Samples stored without additional desiccant at high temperature and high humidity rapidly lost reactivity.
Serial Dilutions of HIV Pos

903

HS

Serum
cut-off

1.0      0.1       0.01     0.001    0.0001

Dilution

s/co

on July 25, 2018 by guest http://jcm.asm.org/ Downloaded from
PRA204 Low Titer Panel

- Plasma
- HS
- 903

s/co cut-off

Reactive Panel Member

Neg
Table 1. Precision of recovery of HIV Ag/Ab Combo signal from 903 and HS Dried Blood Spots.

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*The Bio-Rad Virotrol-2 Low titer HIV control was run in 7 replicates over 3 days (21 total)
Table 2. Sensitivity and Specificity of HS and 903 dried blood

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*HS and 903 spots were prepared from 30 Reactive plus 20 Non-Reactive clinical samples, plus 16 Reactive and 2 Non-Reactive members from SeraCare PRA204 low titer panel.