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Internal quality control for HIV testing of blood donors - Dried tube specimen as a cost-effective alternative

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Abstract:

BACKGROUND: An important aspect of ensuring blood safety is the performance of mandatory serological testing for transfusion transmissible infections. The practice of internal quality control (IQC) in blood banks in India is nonuniform, especially the use of third-party materials. Cited reasons are cost, lack of access to control materials, and need for deep-freezers for storage, if prepared in-house.

OBJECTIVE: Validation of dried tube specimen (DTS) from HIV-positive plasma as a low-cost, stable material for use as IQC material in blood banks.

METHODS: Fresh-frozen plasma (FFP) prepared from four HIV-positive blood-donors were pooled. Equal numbers of seronegative FFPs were pooled. Twenty microlitre aliquots of plasma were made in micro-centrifuge tubes and air-dried overnight at room-temperature. These were stored in 2–8°C refrigerators and tested once weekly for 6 months on multiple platforms with different detection principles: Rapid tests, second-generation enzyme-linked immunosorbent assay (ELISA), fourth-generation ELISA, and fourth-generation Chemiluminescence immunoassay. The protocol was sustained over the next 6 months with decreased testing frequency to study the extended stability of DTS.

RESULTS: A total of 139 positive-DTS and 139 negative-DTS were tested with 100% samples showing consistent results on all platforms over 1 year. There was mild deterioration in reaction strengths, which did not interfere in result interpretations.

CONCLUSION: Plasma in form of DTS maintained stability when stored at 2–8°C for 1 year. This provides evidence that DTS can be a modality for the production of cost-effective, stable, in-house control material for resource-restricted countries.

Keywords:

Blood bank, dried tube specimen, HIV, internal quality control, transfusion transmissible infection screening

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Introduction

Blood transfusion remains a vital aspect of the modern medicine. Although life-saving, transfusion has its risks, including the transmission of blood-borne infections. This is because there are situations where donors remain asymptomatic although harboring infection. This is especially true of HIV where after initial mild morbidity the individual can remain apparently well till a decade, depending on immunological state.[1]

In India, donor selection begins with donor screening with questionnaires to exclude those with history of risk factors, followed by the clinical examination to ensure fitness. Thereafter, all donated blood are tested for HIV 1/2, Hepatitis B/C, syphilis, and malaria.[2,3]

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The practice of quality assurance is important to ensure reliability of laboratory results,[4] including internal quality control (IQC) for precision and external quality assessment schemes for accuracy. Good laboratory practice requires the inclusion of independent/third-party controls as routine. Practice of IQC in transfusion transmissible infections (TTI)-screening in blood-banks across India is highly variable and most are dependent on controls included within rapid-devices or manufacturer provided kit-controls.[5] Reasons cited are that IQC material is not easily accessible, lack long shelf-life and is expensive. Some blood-banks use frozen aliquots of seropositive plasma stored at –20°C for IQC. This requires a separate, dedicated freezer for storing seropositive samples leading to additional expenses for equipment/power. This is not a viable alternative in smaller blood-banks with poor infrastructure. Thus, there is a pressing need for an alternative material for IQC.

We propose an alternative method to utilize seropositive donor plasma in the form of dried tube specimen (DTS) that can be prepared and stored in blood-banks and used for IQC. It can be done with existing equipment and expertise in small-setting blood-banks. DTS has been assessed as external quality assessment (EQA) material for HIV testing laboratories and was found suitable for storage and transport at ambient temperatures, especially in warm Africa.[6,7] However, shelf-life required for this is very short, only for the period of survey cycle, which is about 4–6 weeks.[8-10] If stability of DTS can be validated over extended periods, it can be considered a cost-effective alternative to expensive third-party IQC material. The preparation and storage will then be a function of the frequency of usage and available space.

In this study, we have evaluated the stability of DTS prepared from seropositive donor plasma over a 12-month period to consider it as an alternative to commercial IQC for HIV testing in the blood-banks.

Methods

Whole blood was collected over 6 months from consenting blood donors who fulfilled all regulatory requirements. The components were prepared. Fresh–frozen plasmas (FFPs) of samples that tested sero-reactive for HIV on routine screening by Evolis™ Automated enzyme-linked immunosorbent assay (ELISA) (Biorad™, France) and positive on nucleic acid test (NAT) (ID-NAT, Griffols™, Switzerland) were quarantined over the 6-month period at –80°C for further processing. These were confirmed by serological and Western Blot testing. Plasma co-infected with other TTIs was excluded to prevent cross-reactivity.

Four HIV-positive FFPs collected during this 6-month period were thawed simultaneously. 10 ml plasma from each was pooled and mixed adequately. Twenty microlitre aliquots were transferred into sterile microcentrifuge tubes using sterile pipette tips. These were left in stopper-open position and air-dried at room temperature in biosafety-cabinet by the cabinet's inbuilt air-flow system for 16 h without preservative/antimicrobial agent. Loss of mobility of the dried-up material was considered as end-point of drying.

DTS from HIV-negative plasma samples was made since any IQC material will be incomplete without negative QC. These negative DTS should again be stable for long term and not produce false-positive reactions or generate interferences in any of the testing platforms on ageing. DTS for negative controls (NC) were prepared from pooling 10 ml aliquots from four units of FFP that were negative for HIV and other TTIs. The NC DTS were prepared following same procedure as above on a different day to avoid cross-contamination. Seropositive and NC DTS were labelled, stoppered, and stored in separate containers at 2–8°C. This was considered as day-zero for start of the stability period of DTS and it was tested for the next 1 year. Adequate number of samples was created to allow for contingency testing. Lipemic/icteric plasma was avoided. Donor identification was delinked from all samples to maintain confidentiality.

Samples were tested on a variety of platforms as different blood-banks use different platforms for TTI screening. Kits were chosen from the list notified by Central Drugs Standard Control Organization of India, the blood-bank regulatory authority, to be used for HIV screening in blood-banks.[11] The parallel testing used the following six methods, of which three were immunochromatography/immunoconcentration based rapid tests-CombAids-RS Advantage™ (ARKRAY Healthcare Private Limited™, Gujarat, India)(Dot immunoassay for qualitative detection of immunoglobulin G (IgG)/M anti-HIV antibodies), HIV TRI-DOT™ (J. Mitra and Company Private Limited™, Delhi, India)(Rapid immunochromatographic qualitative assay for IgG anti-HIV antibodies detection) and TRUELINE™ (Alere Medical Private Limited™, Haryana, India) (Rapid immunochromatographic qualitative assay for IgG/M/A anti-HIV antibodies detection). A second-generation manually performed ELISA, used by many small blood-banks, was included: Microlisa-HIV™ (J. Mitra and Company Private Limited™, Delhi, India) (Qualitative enzyme immunoassay for IgG anti-HIV antibodies detection). A fourth-generation automated ELISA (used by bigger blood-banks) was performed using Evolis™ (BioRad™, Marnes La Coquette, France) (Ag/Ab Combo qualitative detection). Tests were performed on Architect™ (Abbott™, Pennington,

USA), (fourth-generation Chemiluminescence Microparticle-based Immunosorbent Assay (CMIA)-Ag/Ab Combo qualitative detection). All tests were performed as per manufacturer's instructions.

Any platform that tests the titer of antigen/antibody/RNA concentration to show their change over time was not included as this was out of scope of the study, since blood-banks are not expected to perform any of these investigations.

A day prior to scheduled tests, required number of aliquots of positive/negative DTS was kept out in biosafety cabinet. Reconstitution was done with 200 µl of phosphate-buffered saline with Tween 20™.[6] The tubes were stoppered and gently tapped ten times at the tip to dislodge dried plasma. Then, they were left overnight at 2–8°C for complete sample dissolution. This resulted in ×10 dilution of plasma after compensating for dried-up plasma volume. No further sample dilution was done during testing. To ensure homogeneity, DTS tubes were inverted ten times and then mixed ten times using micropipettes immediately before testing. Alternately, vortex-mixer can be used for homogenization. Nonvortex mixing was validated as blood-banks usually do not have vortexes. Hence, for the purpose of preparing DTS, they do not need to spend for infrastructure addition.

Homogeneity test[12,13] was performed using 10 HIV-positive DTS samples chosen from different areas of the various storage containers and each tested in duplicate on Microlisa-HIV™ platform. The NC DTS was tested similarly. Appropriate statistics recommended for ensuring homogeneity of samples was performed as described in The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (IUPAC Technical Report).[13] Both the positive/NC samples were found to be homogeneous.

For initial 6 months, tests were performed in triplicates on the three types of rapid-tests and the micro-ELISA platforms and in singlet on CMIA, every week. For the last 6 months, tests were done on monthly frequency on all platforms. Samples were tested on ELISA in singlet for the entire period at a monthly frequency, except for the 1st month, when this platform was not used.

For rapid platforms, the results were recorded as positive/negative. For micro-ELISA and ELISA, optical density (OD), cutoff (CO), and OD/CO ratio (E-RATIO) were recorded. For CMIA, relative light units (S/CO) were recorded.

Due to sudden nonavailability of Trueline™ rapid-test kits during the study period, the schedule of testing for this kit was revised and performed only for initial 7 months of evaluation period. Thereafter, testing on this platform was discontinued.

Both positive/negative in-house QC were included with micro-ELISA while commercial IQC was used for automated ELISA and CMIA. Levey-Jennings charts were plotted and Westgard rules were applied for interpretation.[14]

Softwares Epidata Manager™ Vs.2.0.11.61 (EpiData Association, Denmark)[14], Epidata Entry Client™ Vs.2.0.9.25 (EpiData Association, Denmark)[15], MS Excel™ Vs. 2016 (Microsoft™, Washington, USA)[16] and SPSS™Vs. 16 (IBM™, New York, USA)[17] were used to tabulate and analyse data.[18] Agreement statistics were performed for each platform. Besides concordance of results, linear regression of results obtained on the two ELISA and CMIA was performed to demonstrate stability.

Results

All platforms showed 100% sensitivity and specificity for the positive and negative DTS [Table 1].

The HIV-positive and negative DTS did not show significant deterioration [Table 2] on Microlisa™ [Figure 1] and Evolis™ platforms [Figure 2] over the 1 year period. Although positive samples appeared to show mild deterioration when tested on the very sensitive CMIA platform-Architect™ [Figure 3], all samples remained clearly positive on it till the end of the study.

Discussion

HIV screening of donated blood has been a practice in Indian blood-banks since 1989.[19] The prevalence of HIV in India and other developing/under-developed countries is high. India has a huge load of people living with HIV with NACO's estimate as 2.14 million in 2017.[20] There are about 88,000 new HIV infections getting diagnosed every year in the country.[20,21] Globally, there are about 36.9 million PLHIV, making the global prevalence as 0.8%.[21]

The prevalence in the population implies that blood donors drawn from this population may have a higher

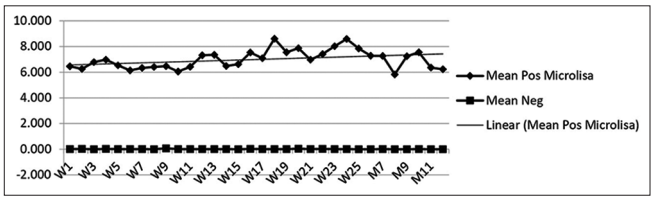

Figure 1: Trend of reactivity rations of dried tube specimen and controls on Microlisa™. In weeks for the first 26 weeks (6 calender months), thereafter in month for the next 6 months

Table 1: Quantity of samples tested with their concordances on the various platforms

Platform	DTS control status	Number of samples tested	Positive concordance (%)	Negative concordance (%)
Rapid-combaids™	Positive	96	100	100
	Negative	96	100	100
Rapid-HIV tridot™	Positive	96	100	100
	Negative	96	100	100
Rapid-trueline™	Positive	70	100	100
	Negative	48	100	100
Manual ELISA-microlisa™	Positive	96	100	100
	Negative	96	100	100
Automated ELISA-evolis™	Positive	11	100	100
	Negative	11	100	100
CMIA-architect™	Positive	32	100	100
	Negative	32	100	100

DTS=Dried tube specimen, CMIA=Chemiluminescence microparticle-based immunosorbent assay, ELISA=Enzyme-linked immunosorbent assay

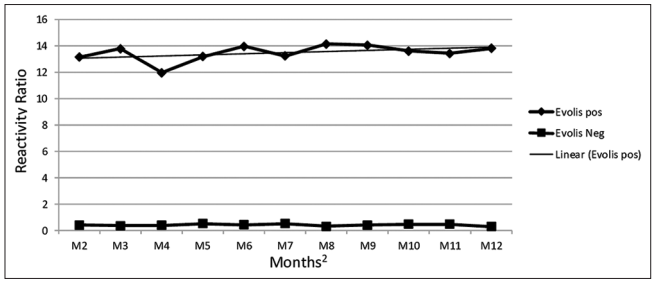

Figure 2: Trend of dried tube specimen and negative controls on Evolis™. This platform was not used for the testing of dried tube specimen in the 1st month

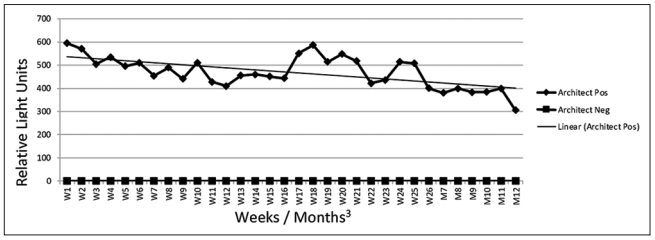

Figure 3: Trend of dried tube specimen and controls on Architect™. Week for the first 26 weeks (6 calender months), thereafter in month for the next 6 months

chance to harbor this pathogen. Since HIV has a prolonged asymptomatic incubation period, often lasting up to 10 years postinfection, it may be likely that such a donor may be missed on screening by conventional questionnaire/physical examination method.[1] As the fragmented Indian blood transfusion services struggle to get more repeat, voluntary donors and reduce dependency on replacement donors,[22] chances of getting a high-risk behavior donor in the system is not an unlikely scenario. 1% of HIV infections is attributable to transfusions as per the NACO.[23] This attribution number may be disputed since there is no reliable look back system with unique patient/donor identification systems for traceability. However, the residual risk still persists.

There has been a significant increase in availability of high-quality test kits/platforms for HIV screening, having high sensitivity/specificity for testing of blood-donors. However, there appears to be transfusion-transmitted HIV disproportionate to what could be attributed to just spread by blood donation in the window period.

Performance characteristics of ELISA are defined under highly standardized conditions. Lots of variables affect the quality of highly user-dependent ELISA. It has multiple steps where user has to add a number of reagents in sequential manner, especially in nonautomated ELISAs used in small-setting blood-banks. It is temperature dependent and has steps that are light-sensitive. Again, temperature fluctuations at places of storage by local dealers, transport and storage at the point of their use may invalidate the kits. Inadequately trained technicians further add to the challenge in the increasing number of small blood-banks, gradually mushrooming in developing/under-developed countries. India's hot, humid tropical climate provides ample opportunity for kits to become defective unless specifically cared to prevent it. Automation is one of the solutions by a reduction of manual intervention. However, even automation may not be an answer to all of the above-mentioned problems. Besides, automation increases the cost. Control reagents that come with ELISA kits are designed to calculate CO values and are of high strength reactivity. An IQC has high-positive, borderline-positive and negative samples to test the capacity of the kits optimally for their ability to detect similar strength patient samples, ensuring that weak positive ones are not missed. The rapid tests, although quite simple in approach, also to some extent depend on these variables and may not demonstrate stated sensitivity under field testing conditions.

IQC for TTI screening is practiced by only 52% of Indian blood-banks according to the baseline assessment of blood-banks conducted by National Blood Transfusion Council in 2016.[24] Even larger centers often restrict themselves to using kit-based controls and are averse to implementing third-party

Table 2: Linear regression of dried tube specimen with time on three immunoassay platforms

	Sample	Coefficient of regression for reactivity ratios	95% CI
Microlisa™	HIV positive DTS	0.028	0.0012-0.055
	Negative control DTS	0.00	−0.001-0.00
Evolis™	HIV positive DTS	0.0849	−0.073-0.242
	Negative control DTS	−0.0017	−0.019-0.016
Architect™	HIV positive DTS	−4.409	−6.572-−2.246
	Negative control DTS	−0.001	−0.002-0.00

DTS=Dried tube specimen, CI=Confidence interval

IQC material, which are definitely out of bounds to the small-setting blood-banks due to their high cost. Conventionally, freezing has been the storage method for QC materials. This requires dedicated −20°C freezers for storage of seropositive samples and is often not possible again due to cost and maintenance factors. Hence, the majority of centers suffer not because they do not have access to high-end diagnostics but because they do not have proper QC tools. Hence, there is a pressing need for an alternative cost-effective method for production and maintenance of in-house controls to overcome these issues.

Prior studies have demonstrated that HIV-DTS has higher endurance than liquid-plasma samples. They can be stored for long periods at higher temperatures. However, all these studies have looked at DTS as EQA material that is transported to other centers, many times under hot and humid conditions, and then tested within a very small finite time period of the proficiency testing cycle. A study by Parekh *et al*. showed for the first time that DTS is stable at 4°C and 25°C for 4 weeks and had marginal deterioration at 37°C and 45°C over the same time period.[6] In order to ensure efficiency and longer shelf life, we studied the stability and potency of HIV DTS for an extended period of 1 year at 2–8°C. The long-term stability is very important for DTS to qualify as IQC reagent for the following reasons:

1. The main ingredient, HIV-positive plasma with adequate strength of reactivity, may not be available frequently in small blood-banks with limited number of blood donations
2. The added work of preparing DTS should be limited and infrequent, otherwise, due to newly added work upon the already overloaded and limited workforce of such blood-banks, the exercise may end up being not done at all, especially when performing IQC does not bring any incentive for technicians.

It is now potentially possible that an IQC may be prepared in-house by a blood-bank once or twice a year only instead of at more frequent intervals. In addition, we validated DTS for second and fourth-generation ELISA, fourth-generation CMIA and three types of rapid tests as these all are used by different large and small-setting blood-banks in India, thus further enhancing the horizon of platforms where HIV-DTS can be used. All blood-banks have 2–8°C reagent refrigerators where these DTS can be stored.

Subsequently, another study by Chopra *et al.* showed that HIV antibodies in DTS are stable at 37°C for up to 30 days.[25] Ramos *et al.* evaluated DTS as a proficiency testing tool for HIV viral load determination on molecular platforms. Samples with viral RNA concentrations ranging from 10^2 to $10^{6.5}$ copies/ml examined up to 8 weeks showed no viral load reduction at 37°C while a little deterioration in DTS stored at 45°C.[7]

A good IQC reagent must be homogenous, stable, sensitive/responsive, retain potency, reproducible and be reasonably priced to be affordable. In our study, HIV-DTS has been stable for 1 year without losing potency. It has responded on all platforms it was tested upon. The DTS is very economical, costing about INR 12 (<US$ 0.25) for a pair of positive and NC DTS including cost of preparation, storage and consumables utilized in all procedures. Once suitable consent is taken from all donors, HIV-positive plasma bags which are not usable for transfusions can be utilized productively. The equipment required for production and its reconstitution are easily available in all blood-banks as their regular inventory. There is no requirement of any costly, high-energy consuming −20°C freezers for its storage. Lyophilization and vortex devices are also not required for their preparation/usage. DTS is homogeneous and meets all of the above-mentioned properties to be IQC reagent. DTS can be used to make negative, weak positive and strong positive controls by appropriate dilutions of the pooled plasma before drying, serving the purpose of IQC. Apart from small-setting blood-banks, DTS will find favor with developed blood-banks due to its low-cost, since all of the currently available commercial third-party IQC are many times costly than DTS. Moreover, they have a limited, short shelf-life too, making DTS a better alternative. Similarly, the diagnostic laboratories which perform qualitative tests for diagnosing HIV infection can use this form of IQC material.

Limitations

HIV-2 positive donor samples were not available and so not included in the validation process of HIV-DTS.

The rapid platform Trueline™ became unavailable midway in the market in between the study period and the number of tests performed on this platform had to be reduced midway so that the remaining kits last for at least 7 months.

Microlisa-HIV™ and EVOLIS™ platforms have shown a very slight inexplicable increase in the strength of reaction of HIV-positive DTS (note: The reactivity was well within the linear range of the two platforms) while the highly sensitive Architect™ gave an expected mild decrease in the strength of reactions of DTS at 1 year. The batch of reagents could not be maintained the same over the 1-year period for EVOLIS™ and Achitect™ platforms due to high blood donor/patient sample load.

The DTS was made from undiluted plasma with ×10 dilution happening during the reconstitution stage for testing. The goal of the study was to study the deterioration of the specimen reactivity over time and it was expected that the strength of reactions will fall down soon when stored at 2–8°C, becoming undetectable in some platforms. However, the strength of the reactions did not fall significantly and remained detectable in all of the platforms. For IQC material, apart from using high-positive samples, it is suggested to users to include DTS to be made from diluted plasma, titred as per the individual testing platform used, to have reactivity closer to the detection limits of the platform (borderline-positive).

Conclusion

Our study has demonstrated the application of HIV DTS as a stable, homogenous and low-cost IQC material for use in blood-bank setting for an extended period of 1 year when stored at 2–8°C. This material fulfils all the characteristics of an ideal IQC reagent. Access to source material and competency for preparation and validation exist in all blood-banks in the country. We have validated it on a variety of platforms commonly used in small and large blood-banks. Inclusion of independent IQC is an important, though often neglected aspect of ensuring validity of screening blood for HIV in blood-banks. Further studies are required to validate this method for other infectious markers in the blood-bank. We propose DTS as an alternative IQC material especially for resource-restricted countries to maintain quality and provide safe blood.

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Conflicts of interest

There are no conflicts of interest.

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