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# Stability of rapid Human Immunodeficiency Virus (HIV) tests beyond their recommended storage temperature

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## Abstract

Blood of blood donors is screened for Human Immunodeficiency Virus (HIV) type I & II and other viral markers at blood banks by using suitable rapid tests / Enzyme Linked Immuno-Sorbent Assay (ELISA) or molecular assays. The rapid HIV tests are low cost, robust, provides quick results, useful for laboratory without having sufficient infrastructure, on-site to test, does not require highly skilled manpower and used in resource-poor settings at primary health centres. Thesensitivity as well as specificity of rapid HIV test is reported to be similar to those of the standard ELISA. The aim of the present study was to check the stability of rapid HIV tests within and particularly beyond their recommended storage temperature. Five commercially available rapid tests were exposed at 25±2°C and higher than their recommended storage temperature like 37±2°C, 45±2°C up to 80 days and 60±2°C up to 2 days. Though all the five HIV RTs retained 100% sensitivity when stored at 37±2°C up to 30 days but only two out of five rapid HIV tests showed 100% stability even after exposure at higher temperature viz 60±2°C up to 2 days. The study suggests that rapid HIV tests are worth using for screening of HIV at blood banks and primary health centres situated particularly in remote areas of tropical countries including India but does not intend to encourage people to use rapid HIV tests exposed to beyond their recommended storage temperature.

**Keywords:**Human Immunodeficiency Virus (HIV), Rapid Tests,Stability beyond storage temperature.

## 1.Introduction

Nearly 33 million people are infected with the HIV worldwide. Sub-Saharan Africa continues to bear the brunt of the global epidemic. Two thirds (68%) of all adults and children with HIV globally live in Sub Saharan Africa, with its epicentre in Southern Africa. One third (32%) of all people with HIV globally live in southern Africa and 34% of all deaths due to AIDS in year 2006 occurred there [1]. India has the third highest number of estimated people living with HIV in the world. According to the HIV estimations 2012, the estimated number of people living with HIV/AIDS in India was 20.89 lakh, with an estimated adult (15-49 age group) HIV prevalence of 0.27% in year 2011[2]. For early and accurate testing of HIV, fast development has been seen for diagnosis of this disease since first HIV antibody tests became commercially available in year 1985 and development of rapid tests in year 1990[3].

Rapid HIV tests are widely used in resource-poor settings, especially in developing countries [4]. Rapid screening for HIV infection can be performed on-site which does not require sophisticated laboratory infrastructure or highly skilled man power, and can help identify those who may be infected with the virus and can facilitate immediate counselling to help prevent the individual from spreading the virus to others by introducing them to risk-reducing behaviour [5]. Rapid HIV tests offer additional advantages of low cost, providing results rapidly and are likely to gain increasing acceptance for HIV screening and diagnosis in both developed and developing countries[6]. To meet the continuously increasing need for testing in resource-limited countries, the primary method has shifted from Enzyme Linked Immunosorbent Assays (ELISAs) to Rapid Tests (RTs). Many studies showed that the laboratories

having limited infrastructure usesHIV rapid testing algorithms as effective as ELISA / Western blot algorithms[3,4,7,8,9]. There is substantial evidence from studies in many countries that the sensitivity and specificity of rapid tests are similar to those of the standard ELISA [10].

According to ASSURED criteria, the ideal rapid test is A= Affordable, S= Sensitive, S= Specific, U= User friendly (simple to perform in a few steps with minimal training), R= Robust and rapid, E= Equipment free or minimal equipment and D= Deliverable to those who need them [11,12]. Most of the manufacturers recommend to store rapid HIV tests at 2-30$^{o}$C / room temperature. But some time temperature may be higher than manufacturers claim during transportation or storage particularly in rural and remote areas of the country because of electricity problem and higher environmental temperature. Keeping this view in mind present study was carried out to see the impact of higher than recommended storage temperature on stability of some of rapid HIV tests.

## 2. Methods

Well characterized confirmed HIV positive samples and five commercially available rapid HIV tests namely 1. Determine™ HIV-1/2 of M/s Alere Medical Co., Ltd., Japan; 2. Meriscreen HIV 1-2 of M/s Meril Diagnostics Pvt. Ltd., Gujrat, India; 3. SD Bio line HIV 1 / 2 3.0 of M/s Standard Diagnostics inc., Korea, 4. Instachk HIV 1+2 of M/s In Tech Products Inc, China and 5. Reliable one step HIV 1 & 2 rapid test of M/s Reliable Pro-detect Biomedical Pvt. Ltd., Shimla were used in this study. All these Rapid Tests (RTs) of HIV were randomly coded prior to study with coding like RT-A, RT-B, RT-C, RT-D, and RT-E. As per kit insert each manufacturer of rapid HIV tests claimed 100% sensitivity. In the present study all five type of rapid HIV test were exposed to temperature viz. 25±2°C, 37±2°C, 45±2°C up to 80 days and 60°±2°C up to 2 days. The sensitivity was calculated after performing the test as per instruction given by each manufacturer.

## 3. Results

All the five HIV RTs were found to retain 100% sensitivity when stored at 25±2°C up to 80 days. The sensitivity of RT-A at 37±2°C up to 30 days was 100% which decreased to 96.66% when incubated at 37±2°C from 60$^{th}$ day to 80$^{th}$days. The sensitivity of RT-Aat 45±2°C up to 15 days was 100% but after 30$^{th}$, 60$^{th}$and 80$^{th}$ days sensitivity decreased to 96.66%, 90.00% and 86.66% respectively. The sensitivity of RT-A at 60°C up to2 days was also decreased to 86.66%.The sensitivity of RT-B at 25±2°C, 37±2°C and 45±2°C up to 80 days was 100% but at 60±2°C up to2 days the sensitivity was decreased to 90.00%.

The results of the study revealed 100% sensitivity of RT-C when stored at 25±2°C, 37±2°C and 45±2°C up to 80 days as well as at 60±2°C up to2 days. In case of RT-D the 100% sensitivity was maintained at 25±2°Cand 37±2°C up to 80 days and at 45°C up to 30 days but up to 60 days sensitivity decreased to 96.66% at 45$^{o}$C and even retained sensitivity of 96.66% up to 80 days. However, the sensitivity of RT–D decreased to 86.66% at 60°Cup to 2 days. Study also revealed 100% sensitivity of RT-E when stored at 25±2°C, 37±2°C and 45±2°C up to 80 days as well as at 60±2°C up to2 days.

**Table: Percentage (%) sensitivity of five HIV Rapid Test (RT) stored at 25±2°C, 37±2$^{o}$C, 45±2$^{o}$C and 60±2$^{o}$C for various days**

| Exposure days | Stored Temperature | Percentage (%) sensitivity of Coded HIV Rapid Tests (RTs) | | | | |
|---|---|---|---|---|---|---|
| | | RT-A | RT-B | RT-C | RT-D | RT-E |
| 15 days | 25±2°C | 100% | 100% | 100% | 100% | 100% |
| | 37±2°C | 100% | 100% | 100% | 100% | 100% |
| | 45±2°C | 100% | 100% | 100% | 100% | 100% |
| 30 days | 25±2°C | 100% | 100% | 100% | 100% | 100% |
| | 37±2°C | 100% | 100% | 100% | 100% | 100% |
| | 45±2°C | 96.66% | 100% | 100% | 100% | 100% |
| 60 days | 25±2°C | 100% | 100% | 100% | 100% | 100% |
| | 37±2°C | 96.66% | 100% | 100% | 100% | 100% |
| | 45±2°C | 90.00% | 100% | 100% | 96.66% | 100% |
| 80 day | 25±2°C | 100% | 100% | 100% | 100% | 100% |
| | 37±2°C | 96.66% | 100% | 100% | 100% | 100% |
| | 45±2°C | 86.66% | 100% | 100% | 96.66% | 100% |
| 02 days | 60±2°C | 86.66% | 90.00% | 100% | 86.66% | 100% |

95% Confidence interval for 100% sensitivity (96.44-100%), 96.66% sensitivity (90.24-100%), 90.0% sensitivity (79.27-100%) and 86.66% sensitivity (74.5-98.8%).

## 4. Discussion

Rapid HIV tests are not only used for screening of blood donors at blood banks but also used in public health clinics, occupational exposures, testing pregnant women, alternative testing strategies and in developing countries with financial restraints and electricity limitations etc. The rapid tests as in present study are usually used for screening. They can produce results in as little as 1-2 minutes and all provides results within 30 minutes [13].

Most rapid assay incorporate an internal procedural control to verify that all reagents are performing adequately used that the sample has been added (an important attributes that EIAs lack). This internal procedural control, usually an antibody to human IgG, detect any immunoglobulin in a sample and are primarily incorporated to eliminate false negative results that could occur because of failure to add a sample. However, it also acts to verify that conjugate system is working.

A number of rapid HIV tests are considered to be robust particularly as compared with ELISA screening tests. Some rapid assays can be stored at ambient temperature (room temperature) or allow a wide range of storage temperature 2 – 30$^{o}$C that allows them to be transported easily, even carried with in a pocket. This eliminates for a cold chain (refrigerated transport boxes) and the use of smaller transport containers. The allowances for higher temperature storage (30$^{o}$C) is particularly valuable in laboratories where there is poor temperature control and outside temperatures are high. Also many rapid tests allow an extended time to read results (e.g. 15 to 60 minutes) after the last reagent are added. This not only gives flexibilities in their use but also the stability of results for extended times allows verifications of results in real time by other personnel. Because many rapid tests are designed to be stable at ambient temperature, their reagents are usually in a dried form, sometimes within device itself. This allows a longer shelf life as compared to some ELISA methods, where liquid conjugate for example have a short duration for use. Besides all these, many rapid tests are packaged in sealed packages, allowing ease of transportation and resistance to spillage of liquid reagents [13].

In the present study all the five HIV RTs retained 100% sensitivity even after their exposure at 37±2°C and 45±2°C up to 30 days except RT-A at 45±2°C up to 30 days wherein sensitivity was declined to 96.66%. Over all study results indicate better quality of RT-C and RT-E than the other 3 RTs. Learmonth *et al* [14] also found that six of seven HIV RTs those were exposed to ambient (22 or 30°C), warm (35 or 37°C) or hot (45°C), temperature for up to 90 days and 60$^{o}$C for 72 hours were relatively robust despite exposure to higher than recommended temperatures.

The reasons of the variability in HIV RT-A, RT-B and RT-D performance beyond their recommended storage temperature could be due to the differences in HIV protein sequence, structure and impurities used in the rapid tests. The denaturation of proteins binding (antigen and / or antibody, conjugate) on the solid support like nitrocellulose membrane and different stabilizer(s) may also be the reasons. However, the results of this study are not intended to encourage people to use rapid tests after they are exposed to temperature beyond the recommendations of manufacturers but it suggests that if such exposure is unavoidable they may be used rarely but only if reagents and controls of RTs are working satisfactory.

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