

NAT for HCV Screening in Blood Banking

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Blood Safety-Challenges

- High prevalence of , HCV, and HBV
- Low percentage of volunteer donors
- Lack of Standardization of screening procedures
- Fragmented blood transfusion services

Pre-requisites for blood safety

- Safe blood donors - through repeat voluntary donations and thorough donor screening
- Safe blood transfusion practices - better screening
- Appropriate / Rational use

Potential Impact of Single Unit of Blood Collected from Infected Donor

- A single unit of whole blood collected from a donor in the window period of infection may be transfused into up to four recipients or may be added to pools of more than 1,000 units to manufacture blood-derived products

Abstract

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Background & objectives: Pakistan has a high prevalence of, hepatitis C and B virus (HCV and HBV) in the blood donors but yet to implement nucleic acid testing (NAT) in blood screening. We undertook a pilot study to evaluate blood donor testing by NAT for HCV to determine the feasibility of NAT implementation in Pakistan's blood bank setting.

Methods: A total of 1000 samples seronegative for Anti HCV were tested manually in small pools of 16 samples by the HCV Real time QualtRG Sacace Italy assay using **SmartCycler** for detection of HCV RNA. In another study we tested 100 Anti HCV positive samples for presence of HCV RNA

Results: Of the 1000 seronegative samples tested non was found reactive for HCV RNA . In another study we found those cases with S/CO values more than 1 and less than 5 are mostly negative for HCV RNA

Interpretation & conclusions: Our observed NAT yield for HCV RNA was 0 in 1000 samples tested. If a sensitive assay is utilized for serological screening of HCV NAT testing for HCV RNA may be deferred till it becomes economically viable in our setting. Moreover release of seropositive and RNA negative units of blood also cast doubt on implementation of NAT in our setting .

What Tests are Available to Detect Viral Infectious Diseases?

- Tests that look for antibody produced by the body against viral antigens
 - Window phase for current antibody tests is 22 days for HIV, 59 days for HBV, and 70 days for HCV

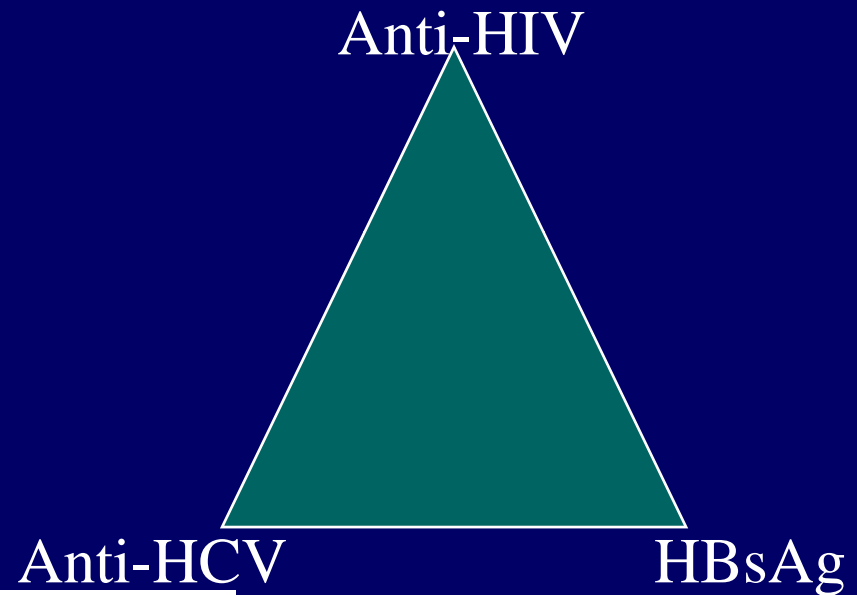
What Tests are Available to Detect Viral Infectious Diseases?

- Tests that look for virus specific antigens (HIV p24Ag, HBsAg)
- Tests that look for viral DNA or RNA (NAT)
 - Become positive more quickly
 - Remain positive as long as virus is present
 - Window period for HIV is 11 days, HBV 20-30 days, and HCV 10-12 days
- Virus can be transmitted in the window period

Tests used for TTI

Front-line screening

- Rapid tests
- ELISA tests
- Chemiluminescence methods



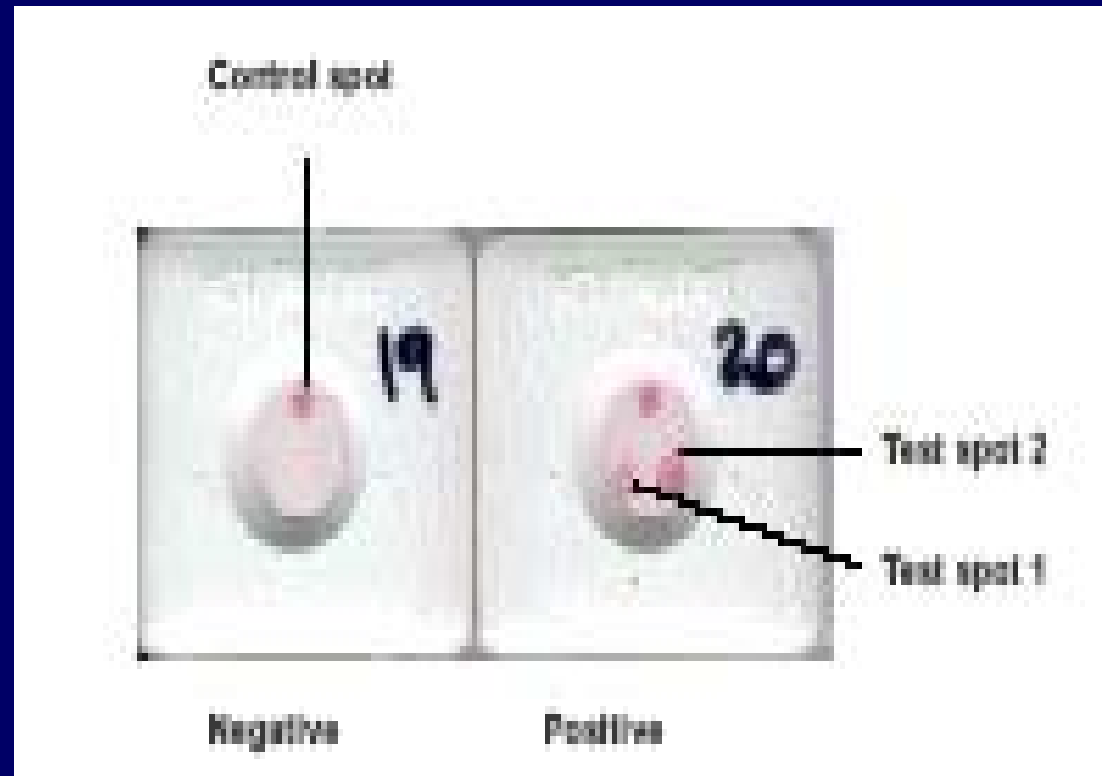
Tests for residual risks:

- NAT test methods
- Supplemental marker tests



Rapid tests

- Agglutinating tests
 - Latex tests
- Color forming tests
 - IC tests



Rapid tests

- Advantages

- Ease of use
- No capital equipment
- Use in Remote areas
- Quick TAT
- Bed-side tests
- Random test

- Disadvantages

- Sensitivity and specificity is a concern
- Limited area of coating
- Limited washing efficiency
- Subjective visual results

ELISA methods

- Advantages

- 96 tests format
- Objective results
- Automatable
- Appreciable sensitivity & Specificity
- Narrower detection window

- Disadvantages

- Demands skill sets
- Decade old method
- Detection capability surpassed by newer methods
 - Sero-conversion detection panels

Chemiluminescence methods

- **Advantages**

- Lasting luminescence
- Increased sensitivity
- High precision when automated
- Wider detection limits

- **Limitations**

- Limited suppliers
- Capital equipment

TESTS FOR RESIDUAL RISK for TTI

- NAT methods
- Supplemental marker tests

NAT Testing

- ❑ NAT is a recently developed technology that allows detection of very small amounts of genetic material (DNA or RNA) by a process of massive copying (amplification) of a gene fragment.
- ❑ Currently, donors of blood and plasma are tested for Hepatitis B surface antigen, antibodies to HCV, antibodies to HIV and sometimes HIV-1 antigens, which are the virus' own proteins. However, there is still a "window period" during which a donor can be infected, but have negative screening tests.
- ❑ With the use of Individual Donor Testing (IDT) NAT for HCV, the "window period" for detection of HCV is reduced by 67 days (from an average of 72 days to 5 days). For HIV-1, the average window period with antibody tests is 22 days. Antigen testing cuts the window period to approximately 16 days and NAT further reduces this period to 5.6 days
- ❑ Given the high rate of sero-positivity of HIV, HCV and HBV in Pakistan and keeping in mind the high percentage of first time and replacement donors, it is likely that adding NAT to the current screening tests will have a very significant reduction in Transfusion Transmitted Infections making our blood safer.

NAT testing

- How is NAT Testing Performed in Blood Screening?
 - In most countries NAT testing for blood screening is performed on pooled samples (pool size varies between 1 to 96 across countries) by one of the following methods
 - PCR - Polymerase Chain Reaction
 - Roche Cobas Ampliscreen
 - TMA – Transcription Mediated Amplification
 - Chiron Geneprobe
- Others
 - Nucleic acid sequence-based amplification (NASBA), ligase chain reaction (LCR), branched DNA signal amplification (bDNA)

Nucleic Acid Tests (NAT)

General Characteristics

- Sample preparation, including viral concentration and extraction of DNA or RNA
- Amplification of the target viral DNA or RNA
- Detection of the amplified product

NAT methods

- Advantages

- Direct detection of viruses
- Higher sensitivity than ELISA
- Closure of window period of detection

- Limitations

- High skill sets
- High TAT
- Infra-structure
- Sample processing step yet to be automated
- Room for error
- Cost of single NAT: 10X ELISA

What NAT means to the Window Period (Blood)

EIA Window

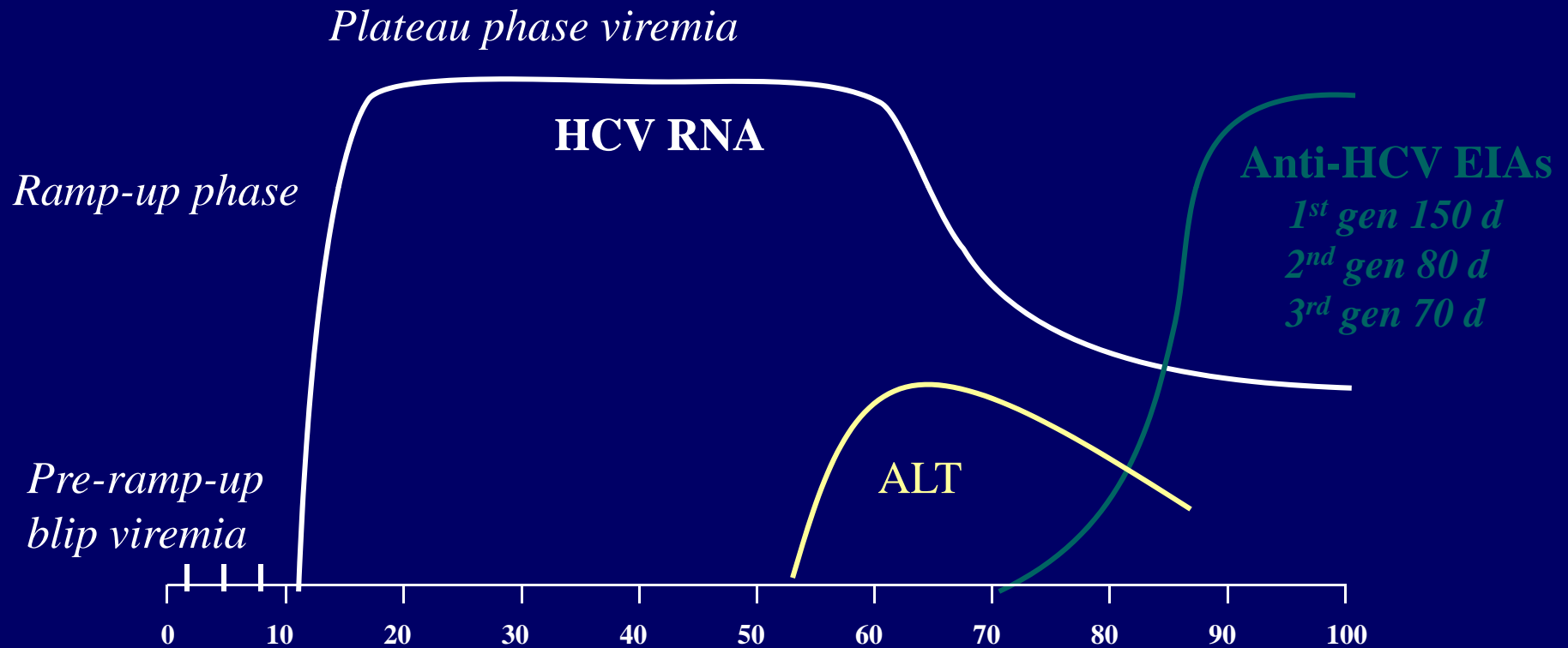
Post-NAT Window

- | | | |
|-------|------------|------------|
| • HCV | 70-80 days | 10 days |
| • HIV | 16 days | 10 days |
| • HBV | 56 days | 20-30 days |

Viral Characteristics

- HBV-DNA harder to detect by virtue of the slower reproductive cycle of the virus
- Virus Doubling Times
 - HCV--17.7 hrs.
 - HIV-- 21.5 hrs.
 - **HBV--2.8 days (67.2 hrs.)**

HCV Markers During Early Infection



NAT experience of various countries in the SE Asia

- In Singapore, among the 466,779 samples tested by NAT since October 2007 they were able to pick 9 HCV and 10 HBV NAT yield samples (1 in 24,567).
- Similarly in Thailand, Hong Kong and in Korea the NAT yield rate is 1 in 11, 676, 1 in 202,500 and 1 in 1, 46,628 respectively. Despite these countries having a very stringent donor counseling and screening process, a high rate of regular repeat voluntary donation, and use of the most sensitive serological tests, they were able to identify a significant number of samples which were NAT reactive but sero-negative.
- In India, Indraprastha Apollo Hospitals, Delhi has taken the initiative for NAT implementation for the first time in the country. In the first nine months of implementing NAT, they were able to pick five (3 HBV and 2 HCV) NAT yield samples among 13,331 samples tested (1 in 2,666).

NAT Testing

- Will NAT Testing replace existing Immunoassay screening tests in Blood Screening?
 - **NO**,
 - Small percentage of Antibody positive donors have been tested negative by NAT tests.
 - It is possible that an antibody positive and NAT Negative donation might transmit infection to the recipient.
 - Therefore NAT Testing will not replace current serology tests in blood screening ^{1,2}
 - So far no country has discontinued the serology screening for HBsAg, Anti HIV and Anti HCV after implementation of NAT screening