NAT reveals high risk of Hepatitis B and C transmission by Serologic screening practice in India

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India

Human Immunodeficiency virus
Hepatitis C virus
Hepatitis B virus
Status of Transfusion Transmitted Infections in India
Status of Blood Safety in India

Topics for Discussion

• Blood Banking system
• Disease Prevalence
• Donor Base
• Blood supply & Need
• National policy on blood safety standards
• Case Study: DMCH
• Current drawbacks
Categories of Blood Banks

• Highly decentralized-fragmented system with more than 2760* blood banks

• Blood Banking Services: (a) Hospital associated (75%) (2070), (b) Independent (25%)

• (a) Hospital associated: Government (70%-1449 BB), Private (30%)
  – Central Government BBs:
    • General public; Armed forces; Central Government employed (railways); autonomous Central Govt BBs- AIIMS
  – State Government:
    • State employees;
    • Municipal Hospitals open for all
  – Private BBs:
    • Commercial (60%); Charitable Trust (40%)

• (b) Independent Blood banks:
  • Red cross, Lion’s, Rotary

*Assessment of NACO supported BBs, Preliminary Report 2016, p1
Blood Banking: Current Indian Scenario

- Of the 2760 blood banks
- 36% (1000) BB - >10,000 units/year (>30/day)
- 54% (1500) BB 3000 – 5000 units/year (10-15/day)
- 10% BB - <1000 units/year (<4 /day)

- Low volume collection; Automation is a problem. Reliance on manual testing, & rapid tests
- Quality compliance issues
Estimated HIV, HCV, HBV Prevalence
General Population vs. Blood Donors in India¹

Based on a population of 1.2 Billion and 10 Million Donations ²

General

- HIV 0.36%
- HCV 0.9%
- HBV 2.5-4.0%

Blood Donors

- HIV 0.3%
- HCV 0.4%
- HBV 1.1%

( ) % global disease burden
General population

2. NACO, Department of AIDS Control, Ministry of Health & Family Welfare, Government of India, 2011; Assessment of NACO supported BBs, Preliminary Report 2016, p1
Donor Base at Blood Banks

- Overall <50% Voluntary donations; high % of first-time, & replacement donors

(a) Govt. Hospitals and Independent BBs:

- Voluntary & Replacement (50%) donors at Govt. BB
- Voluntary only at Independent BB
- Off-Site Camps at Colleges, Workplaces, Religious institutions, Central locations
  - Vans, on-site space

(b) Private hospitals cannot conduct donor camps:

- Voluntary (5%)
- Replacement donors (95%)

<table>
<thead>
<tr>
<th></th>
<th>% Prevalence in Donors: Global*</th>
<th>India</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Time</td>
<td>Repeat</td>
</tr>
<tr>
<td>HIV</td>
<td>0.73</td>
<td>0.24</td>
</tr>
<tr>
<td>HCV</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>HBV</td>
<td>5.3</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Factors affecting Blood Collection

• Only 10 million units collected annually against 12 million (1% of total population) requirement

• Factors for low collection:
  – lack of awareness
  • High deferral
  – high prevalence of life-style diseases: Cardiac problems (12%)
  – genetic diseases: Diabetes (5% of the population)
  – high Infectious burden: Occult HBV, Seasonal infections
  – anemia

• Female to Male 1:10
Blood Banking: Current needs

• Blood & Blood product requirement is high:
  – Genetics – Thalassemia, hemophilia
  – Maternal mortality: 0.24% of live births through postpartum hemorrhage & malnutrition. Practice is Preventative blood transfusion - MDG #5/SDG#3 (0.07%)
  – Infant mortality: 4.1% of live births - MDG #4 (2.5%)
  – Infectious burden requiring blood products: Dengue, MDG #6
  – Cancer- poor early diagnosis
  – Trauma

• 41% of collected blood is fractionated; Mostly into two fractions of PRBC and plasma with platelets
• 350 mL collected for females and not fractionated
• Ratio of Components:Whole Blood usage is 25:75 vs. Global - 90:10
Blood Banking compliances

- Mandatory screening for detection of five diseases: HIV-1/2, HCV, HBV, Syphilis; & Malaria (visual smear)
- The target of detection not defined
- Retest of initial reactives is mandated
- 80% (1126 BB) of Government hospital BBs are provided reagents by NACO; also can obtain permission to procure reagents from external sources
- Private and Independent BBs procure their choice of commercial reagents, Various brands depending on availability
- Variety of ELISA, CLIA, and Rapid test reagents amongst Government, Private and Independent BBs
- High variance in results between BBs; and from time to time in the same BB
Disease Reporting & Follow-up

- Greater emphasis on HIV through the presence of NACO
- HIV reactive results from all BBs are conveyed to NACO and State AIDS agencies
- Hepatitis cases referred to ICTC (Integrated counselling & testing centers)
- NAT testing is not mandated. NAT only positives are not notified to agencies
- Follow-up testing for reactive donors is difficult to enforce. Donor database to defer infected donors is not in place.
- Trace-back for infected recipient is also difficult, additionally legal complications deter such investigations.
Challenges of NAT implementation

• Nationally ~11% of donated blood is NAT tested

• For the BB
  – Complexity of NAT testing needs: Specimen collection & handling; Reagent handling; Operator Training, Special environment needs, Special amplified product handling

• For the Customer
  – Lack of risk-benefit awareness
  – Availability
  – Affordability

• NAT is provided by two manufacturers
  Grifols: ID-NAT
  Roche: MP 6 NAT
  A few sites use homebrew MP-NAT with variable pool size
Case Study: DMCH Private Hospital Blood Bank

- DMCH in North India is a Private, tertiary care, teaching hospital BB
- 34,000 annual donations
- Thalassemic region (4%), requiring 20% of donation; Repeat recipients
- Higher prevalence of HCV

- Donor base:
  - 92% First-time, 7.5% Repeat;
  - 51% Voluntary, 49% Replacement
  - 95.4% Male, 4.6% Female

- Serology Screening assays: ELISA of BioMerieux, Bio-Rad, Genedia, Ranbaxy
- Initial sero reactives repeat tested with the same reagent, if RR retested by the Rapid assay (Tridot assays)
- Adopted ID-NAT Ultrio Plus assay in 2013

- Strengths of the study: NAT yields confirmed in reference lab by Supplemental serology and NAT assays
- Weakness: Serology yields not confirmed at a reference lab, significant number of NAT yields not available for confirmation
Algorithm for Serology & NAT testing

ID-NAT and serologic testing on dedicated primary tubes

1. NAT reactive
   Seroreactive
   Discriminatory (dHBV, dHCV, dHIV)
   NAT testing on primary EDTA tube (1x)

2. NAT nonreactive
   Seroreactive
   Repeat ELISA testing on primary serum tube (1x)
   Sero repeat reactive
   Rapid test on primary serum tube

3. NAT reactive
   Serononreactive
   Repeat multiplex NAT testing on primary EDTA tube (3x)
   NAT repeat reactive (1/3, 2/3, 3/3)
   1. Repeat multiplex (3x) and discriminatory (3x) NAT testing on FFP sample
   2. Quantitative PCR
   3. Supplemental CLIA serology assays

4. NAT nonreactive
   Serononreactive
   Release of donations for transfusion
   NAT nonrepeat reactive
DMCH Ultrio Plus assay Screening Experience (N=39295)

Concordant: Sero-NAT reactive, Serology Yields, NAT Yields

<table>
<thead>
<tr>
<th></th>
<th>dHIV</th>
<th>dHCV</th>
<th>dHBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>National Sero (%)</td>
<td>0.14</td>
<td>0.33</td>
<td>0.94</td>
</tr>
<tr>
<td>DMCH Sero (%)</td>
<td>0.08</td>
<td>0.97</td>
<td>0.82</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>380</td>
<td>323</td>
</tr>
<tr>
<td>Concordant</td>
<td>23</td>
<td>296</td>
<td>266</td>
</tr>
<tr>
<td>Sero yields</td>
<td>7</td>
<td>61</td>
<td>20</td>
</tr>
<tr>
<td>NAT yields</td>
<td>1</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td><strong>NAT yield Ratio</strong></td>
<td><strong>1:39295</strong></td>
<td><strong>1:1571</strong></td>
<td><strong>1:1062</strong></td>
</tr>
</tbody>
</table>

- High seroprevalence of HCV
- High HCV & HBV NAT yields

National data: Assessment of NACO supported BBs, Preliminary Report 2016
<table>
<thead>
<tr>
<th>classification</th>
<th>n</th>
<th>rate</th>
<th>%</th>
</tr>
</thead>
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<td>total HIV-1 NAT yield*</td>
<td>1</td>
<td>1:39295</td>
<td>3.2</td>
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<td>anti-HIV ELISA &amp; NAT concordant</td>
<td>23</td>
<td>1:1708</td>
<td>74.1</td>
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<td>anti-HIV/NAT- or nonspecific</td>
<td>7</td>
<td>1:5613</td>
<td>22.6</td>
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<td>total HIV infections</td>
<td>31</td>
<td>1:1267</td>
<td>100</td>
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</table>

- *The single NAT HIV-1 yield was 10/10 reactive by the Ultrio Plus assay, which has a LoD of 18.5 IU/ 11 Cps/mL.
- It was CLIA non-reactive; Q-PCR reactive, <20Cps/mL
**HCV test results on 16 ELISA nonreactive NAT yields**

of 25 Total NAT yield 9 dual HBV/HCV NAT reactives were unresolved because of lack of sample

<table>
<thead>
<tr>
<th>WP NAT yield case</th>
<th>Ultrio Plus primary tube</th>
<th>Ultrio Plus FFP unit</th>
<th>dHCV FFP unit</th>
<th>Roche HCV-RNA IU/mL</th>
<th>Blood bank anti-HCV ELISAs*</th>
<th>Biorad retest anti-HCV ELISA S/CO¶</th>
<th>Abbott anti-HCV CLIA</th>
<th>Infection status</th>
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<tbody>
<tr>
<td>1</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3686</td>
<td>NR</td>
<td>NR</td>
<td>0.14 NR</td>
<td>WP</td>
</tr>
<tr>
<td>2</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>1 058,809</td>
<td>NR</td>
<td>NR</td>
<td>0.07 NR</td>
<td>WP</td>
</tr>
<tr>
<td>3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>2 352,582</td>
<td>NR</td>
<td>NR</td>
<td>0.03 NR</td>
<td>WP</td>
</tr>
<tr>
<td>4</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>93,157</td>
<td>NR</td>
<td>NR</td>
<td>0.04 NR</td>
<td>WP</td>
</tr>
<tr>
<td>5</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>19,035</td>
<td>NR</td>
<td>NR</td>
<td>0.06 NR</td>
<td>WP</td>
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<tr>
<td>6**</td>
<td>2/3</td>
<td>3/3</td>
<td>3/3</td>
<td>ND†</td>
<td>NR</td>
<td>NR</td>
<td>0.05 NR</td>
<td>possible WP</td>
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<tr>
<td>7</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>47,569</td>
<td>NR</td>
<td>0.06 NR</td>
<td>2.04 R</td>
<td>concordant</td>
</tr>
<tr>
<td>8</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>9 748,951</td>
<td>NR</td>
<td>0.09 NR</td>
<td>1.85 R</td>
<td>concordant</td>
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<tr>
<td>9</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>2182</td>
<td>NR</td>
<td>0.68 NR</td>
<td>13.75 R</td>
<td>concordant</td>
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<tr>
<td>10¶</td>
<td>2/2</td>
<td>3/3</td>
<td>3/3</td>
<td>26,594</td>
<td>NR</td>
<td>3.71 R</td>
<td>13.80 R</td>
<td>concordant</td>
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<tr>
<td>11¶</td>
<td>3/3</td>
<td>3/3</td>
<td>2/2</td>
<td>10,195</td>
<td>NR</td>
<td>5.12 R</td>
<td>11.51 R</td>
<td>concordant</td>
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<tr>
<td>12¶</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>10,051</td>
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<td>1.60 R</td>
<td>12.48 R</td>
<td>concordant</td>
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<tr>
<td>13¶</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>52,397</td>
<td>NR</td>
<td>3.49 R</td>
<td>13.77 R</td>
<td>concordant</td>
</tr>
<tr>
<td>14¶</td>
<td>2/2</td>
<td>3/3</td>
<td>3/3</td>
<td>959,429</td>
<td>NR</td>
<td>6.07 R</td>
<td>14.52 R</td>
<td>concordant</td>
</tr>
<tr>
<td>15¶</td>
<td>2/2</td>
<td>3/3</td>
<td>3/3</td>
<td>ND†</td>
<td>NR</td>
<td>1.12 R</td>
<td>10.77 R</td>
<td>concordant</td>
</tr>
<tr>
<td>16¶</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>ND†</td>
<td>NR</td>
<td>1.18 R</td>
<td>13.46 R</td>
<td>concordant</td>
</tr>
</tbody>
</table>

† ND = Not detectable, LoD 12 IU/mL  ** dHBV and dHCV NAT reactive.
¶ = Retrospectively anti-HCV reactive with BioRad ELISA as well as rapid test
## HCV Infection Rate & Residual Risk

<table>
<thead>
<tr>
<th></th>
<th>HCV yield rate</th>
<th>Residual risk RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>rate</td>
</tr>
<tr>
<td>Pre-anti-HCV-WP</td>
<td>15$</td>
<td>1:2630</td>
</tr>
<tr>
<td>Unresolved dHCV Yields</td>
<td>10#</td>
<td>1:3930</td>
</tr>
<tr>
<td>ELISA &amp; NAT Concordant</td>
<td>296*</td>
<td>1:132</td>
</tr>
<tr>
<td>Probable resolved or nonspecific</td>
<td>61</td>
<td>1:644</td>
</tr>
</tbody>
</table>

- $Of these 10 were detected by CLIA
- #One HCV WP was 9/10 Ultrio Plus reactive (LoD 4.6 IU/mL) with 7 IU/mL viral load, but ND by qPCR (LoD 12 IU/mL)
- *Of the concordants, 4 were unresolved IR by NAT
- Of the 13 that were genotyped, 11 were type 3, and 2 were type 1
- 100 fold decrease in risk by NAT

*Kumar, R. et al., A nucleic acid amplification repeat testing algorithm reveals high risk of Hepatitis B and C transmission with serologic blood screening practice in Punjab, India. ISBT Science series, 2017,1-9.*
HBV Reaction Rate

<table>
<thead>
<tr>
<th>classification</th>
<th>n</th>
<th>rate</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>total HBV-NAT yield</td>
<td>34</td>
<td>1:1156</td>
<td>11</td>
</tr>
<tr>
<td>HBsAg ELISA &amp; NAT concordant</td>
<td>254</td>
<td>1:155</td>
<td>82.5</td>
</tr>
<tr>
<td>HBsAg+/NAT- or nonspecific</td>
<td>20</td>
<td>1:1965</td>
<td>6.5</td>
</tr>
<tr>
<td>total HBV infections</td>
<td>308</td>
<td>1:128</td>
<td>100</td>
</tr>
</tbody>
</table>

- Of the three viruses HBV was the most prevalent
- The NAT yields were substantial at 11%
- For NAT yields 33 were Male donors, with a single Female, 20 First-time, & 28 Married donors; 28 samples were total anti-HBc reactive
## HBV Infection Rate & Residual Risk

<table>
<thead>
<tr>
<th>classification</th>
<th>n</th>
<th>rate</th>
<th>%</th>
<th>with ID-NAT</th>
<th>HBsAg only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-HBsAg WP</td>
<td>3</td>
<td>1:13,098</td>
<td>10.3</td>
<td>1:20773</td>
<td>1:8033</td>
</tr>
<tr>
<td>a-HBs breakthrough#</td>
<td>1</td>
<td>1:39,295</td>
<td>3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-HBsAg WP</td>
<td>1</td>
<td>1:39,295</td>
<td>3.4</td>
<td>1:65,711</td>
<td>1:11,094</td>
</tr>
<tr>
<td>OBI a-HBs-</td>
<td>20</td>
<td>1:2183</td>
<td>68.9</td>
<td>1:15,783</td>
<td>1:4659</td>
</tr>
<tr>
<td>OBI a-HBs+</td>
<td>4</td>
<td>1:9824</td>
<td>13.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total classified</td>
<td>29</td>
<td>1:1355</td>
<td>100</td>
<td>1:15,783</td>
<td>1:4659</td>
</tr>
<tr>
<td>unclassified</td>
<td>5</td>
<td>1:7859</td>
<td></td>
<td></td>
<td>ignored</td>
</tr>
<tr>
<td>total HBV-NAT yield</td>
<td>34</td>
<td>1:1156</td>
<td>11.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Notes:
- A 2.5 fold decrease in residual risk by NAT for WP
- For OBI characterized by low viral load NAT decreased residual risk by 5.9 fold
- 2 Samples Genotyped, and were type D

*Kumar, R. et al., A nucleic acid amplification repeat testing algorithm reveals high risk of Hepatitis B and C transmission with serologic blood screening practice in Punjab, India. ISBT Science series, 2017, 1-9.*
DMCH Study Conclusions

• Through serology repeat testing (1x) 21% of sero IR were identified to be false positive and units were released for use
• Increased sensitivity by interrogating a larger sample volume through repeat triplicate NAT testing algorithm
• 15 of 28 HBV NAT reactive units by triplicate testing had undetectable VL by qPCR
• Increased Specificity by testing with Tube and FFP specimen, critical in the absence of follow-up of reactive donors
• In conjunction with NAT testing, despite using multiple brands of ELISA serology reagents of low specificity and sensitivity, it is still possible to provide safer blood
• With high HCV prevalence, & 20% of units used for thalassemic repeat recipients, Risk reduction through NAT tested blood is critical.
Conclusions - Blood Safety in India

• Absence of Definition in Policy for stringent testing and absence of Uniformity of reagents across all BBs is skewing the disease seroprevalence data

• Absence of Confirmatory requirements of reactives, & manual testing affecting quality of results, increasing wastage

• Lack of Donor follow-up is affecting disease control, and not having e-database of infected donors for preclusion is increasing the burden on BBs

• Very high prevalence in multiple transfusion recipients points to the inadequacy of current testing

• Public awareness of safe blood; availability & affordability of NAT tested blood is critical
Thank You