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

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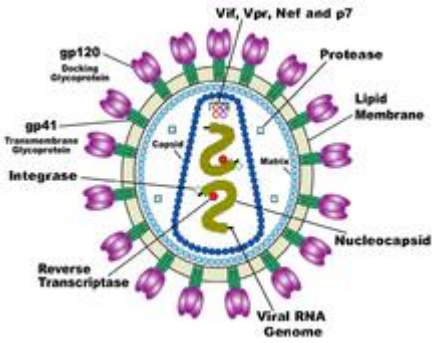
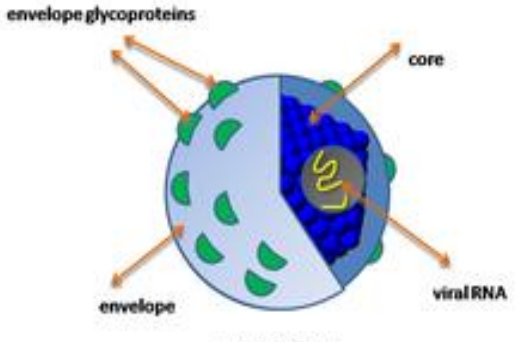
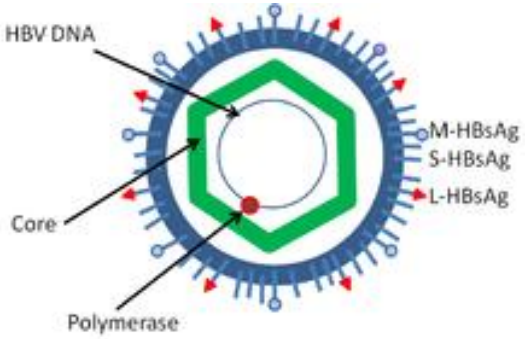
Human Immunodeficiency virus



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 India[1]

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

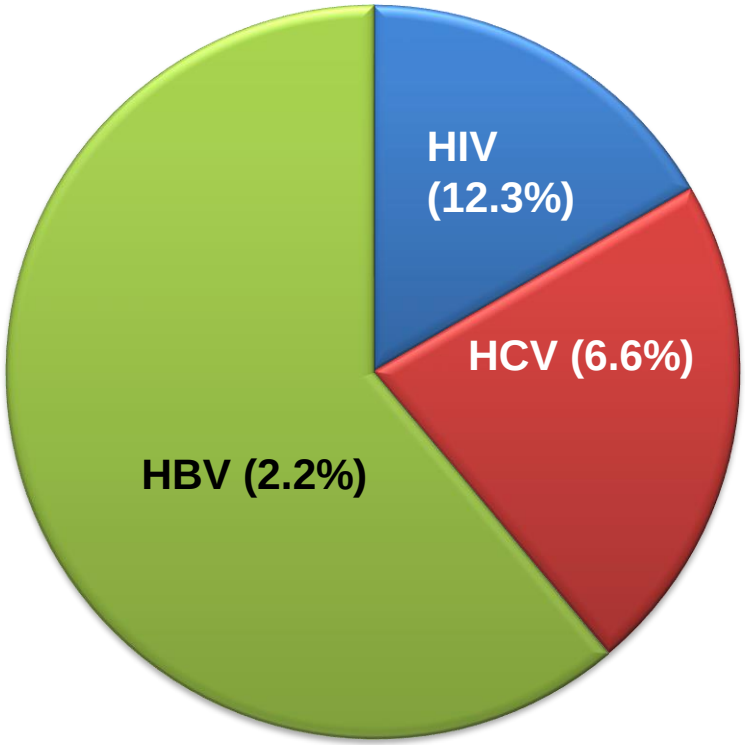
General

HIV
0.36%

HCV
0.9%

HBV
2.5-4.0%

( ) % global disease burden
General population



Blood Donors

HIV
0.3%

HCV
0.4%

HBV
1.1%

1. Computerized Management Information System Bulletin, National AIDS Control Organization Dept of AIDS Control. 2009; 15 & 86 Annual Report 2010-11,
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%)

	% Prevalence in Donors: Global*			India
	First Time	Repeat	Fold Decrease	
HIV	0.73	0.24	3	1.2
HCV	11	1	11.09	2.25
HBV	5.3	2.2	2.5	~2.5

**Roth et al., International Survey on NAT testing of blood donations: 1999-2009. Vox Sanguinis 2012;102:82-90.*

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

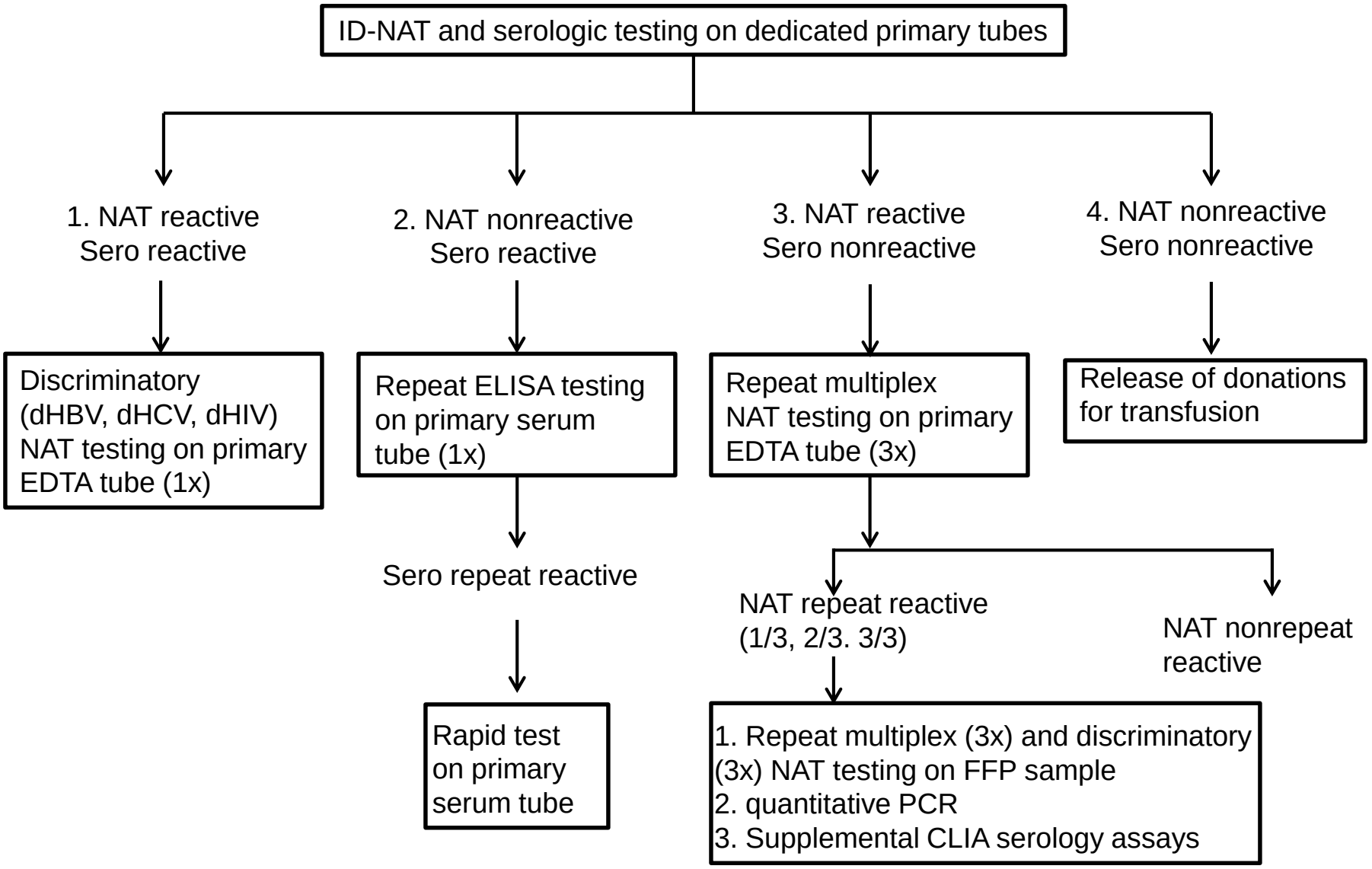
- DMCH in North India is a Private, tertiary care, teaching hospital BB
- 34,000 annual donations
- Thalassemic region (4%), requiring 20% of donation; Repeat recipients
- Higher prevalence of HCV

- 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



DMCH Ultrio Plus assay Screening Experience (N=39295)

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

	dHIV	dHCV	dHBV
National Sero (%)	0.14	0.33	0.94
DMCH Sero (%)	0.08	0.97	0.82
Total	31	380	323
Concordant	23	296	266
Sero yields	7	61	20
NAT yields	1	25	37
NAT yield Ratio	1:39295	1:1571	1:1062

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

National data: Assessment of NACO supported BBs, Preliminary Report 2016
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.

HIV Reaction Rate

classification	n	rate	%
total HIV-1 NAT yield*	1	1:39295	3.2
anti-HIV ELISA & NAT concordant	23	1:1708	74.1
anti-HIV/NAT- or nonspecific	7	1:5613	22.6
total HIV infections	31	1:1267	100

- *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

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

‡ 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

	HCV yield rate			Residual risk RBC	
	n	rate	%	only anti-HCV	with ID-NAT
Pre-anti-HCV-WP	15[$]	1:2630	3.9	1:2594	1:269,138
Unresolved dHCV Yields	10[#]	1:3930	2.6		
ELISA & NAT Concordant	296*	1:132	78.4		
Probable resolved or nonspecific	61	1:644	15.9		

- [$]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

classification	n	rate	%
total HBV-NAT yield	34	1:1156	11
HBsAg ELISA & NAT concordant	254	1:155	82.5
HBsAg+/NAT- or nonspecific	20	1:1965	6.5
total HBV infections	308	1:128	100

- 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

HBV yield rates				residual risk RBC	
classification	n	rate	%	with ID-NAT	HBsAg only
Pre-HBsAg WP	3	1:13,098	10.3	1:20773	1:8033
a-HBs breakthrough#	1	1:39,295	3.4		
Post-HBsAg WP	1	1:39,295	3.4	1:65,711	1:11,094
OBI a-HBs-	20	1:2183	68.9		
OBI a-HBs+	4	1:9824	13.8		
total classified	29	1:1355	100	1:15,783	1:4659
unclassified	5	1:7859		ignored	
#excluded from risk analysis calculations					
total HBV-NAT yield	34	1:1156	11.0%		

- 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

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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