Mini-pool NAT HIV 1 breakthrough transmission cases and probability of interdiction by current small pool or individual donation NAT screening systems

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Background

• NAT screening in mini pools of 16 to 96 donations was first introduced in the late 1990s

• Viral load required for HIV transmission by NAT-negative window phase donations has been studied in animal models

• Minimal infectious dose, and transfusion and host determinants of TT-HIV not established in humans

• Published case reports exist of HIV window period (WP) donations that were not detected by MP-NAT, as well as reports of similar donations from non-NAT tested units that would have been missed by MP-NAT
Reports of HIV MP16-96 negative (non) transmission cases

2. R. Phelps et al. Transfusion 2004; 44:929-933
Redefining the HIV-infectious window period in the chimpanzee model: evidence to suggest that viral nucleic acid testing can prevent blood-borne transmission

High Specific Infectivity of Plasma Virus from the Pre-Ramp-Up and Ramp-Up Stages of Acute Simian Immunodeficiency Virus Infection

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FIG. 3. vRNA+ plasma samples used to produce the ramp-up-stage plasma pool and outcome of challenge of recipient animals with the serially diluted ramp-up-stage plasma pool. (A) Plasma vRNA levels in donor animals that were vaginally inoculated twice in one day with 105 TCID50 of SIVmac251 or weekly from 0 to 13 weeks with 103 TCID50 of SIVmac251 until infection was detected. Each sample used to make up the ramp-up-stage pool is circled. (B) Plasma vRNA levels in SIV-naïve recipient animals after i.v. infusion of the ramp-up-stage plasma pool. While 1 animal inoculated i.v. with 2 SIV RNA copies (animal 32970) did not become infected, 2 of 2 animals inoculated i.v. with 20 SIV RNA copies (animals 33815 and 35036) did become infected. These two animals had a typical pattern of viremia after the plasma transfer.
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FIG. 3. vRNA+ plasma samples used to produce the ramp-up-stage plasma pool and outcome of challenge of recipient animals with the serially diluted ramp-up-stage plasma pool. (A) Plasma vRNA levels in donor animals that were vaginally inoculated twice in one day with $10^5$ TCID$_{50}$ of SIVmac251 or weekly from 0 to 13 weeks with $10^3$ TCID$_{50}$ of SIVmac251 until infection was detected. Each sample used to make up the ramp-up-stage pool is circled. (B) Plasma vRNA levels in SIV-naive recipient animals after i.v. infusion of the ramp-up-stage plasma pool. While 1 animal inoculated i.v. with 2 SIV RNA copies (animal 32970) did not become infected, 2 of 2 animals inoculated i.v. with 20 SIV RNA copies (animals 33815 and 35036) did become infected. These two animals had a typical pattern of viremia after the plasma transfer.

Macaque ID$_{50} = 1-10$ SIV virions / inocula
Aims

Compile data from these HIV MP-NAT “breakthrough” and untested cases

Estimate minimal infectious dose, derived from empirical data, for human transfusion cases

Estimate the probability that current individual donation (ID) or 6, 8 and 16 triplex MP NAT systems would have interdicted these transmission events
Methods-I

• Literature review of TT HIV cases through 12/08 yielded 10 case reports
  – 11 blood products prepared from these 10 donations were infectious and 4 were not

• Viral loads of implicated donations measured or estimated
  – 6 donations, low VL was measured
    • in two of these cases a more accurate estimation of VL was obtained by limiting dilution analysis against viral standard dilutions quantified in copies/ml by bDNA 3.0 assay
  – 1 platelet apheresis donation was in the eclipse phase (4 days after exposure)
    • concentration of 1 copy/ml was back-estimated by regression analysis from the viral load in a subsequent donation using a viral doubling time of 0.85 days
  – 3 donations, the viral load was not available
    • assumed that these donations contained a viral load at the MP-NAT 50% hit rate
Methods-II

• Detection limits of HIV NAT assays estimated by probit analysis
  – copies/ml quantified in bDNA 3.0 assay (1 IU = ~ 0.5 copy), using database of NAT results on standard dilutions (Delft Diagnostic Laboratory)
  – 50% detection limits (in cps/ml):
    • 4.6 for Ampliscreen
    • 1.9 for NucliSens-Ampliscreen
    • 2.3 for PROCLEIX® HIV-1/HCV (Duplex)
    • 3.3 for PROCLEIX® ULTRIO®
    • 4.2 for TaqSscren®

• Total virions infused estimated based on component volume x viral load (VL)
MP-NAT non-reactive HIV-1 WP donations (1997-2008) and outcome of infection in recipients

<table>
<thead>
<tr>
<th>1st author of report</th>
<th>country</th>
<th>unit</th>
<th>screening assay</th>
<th>pool size</th>
<th>estimated cps/mL</th>
<th>estimated mL plasma transfused</th>
<th>estimated copies transfused</th>
<th>recipient infection</th>
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<td>FFP</td>
<td>Duplex</td>
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<td>Duplex</td>
<td>16</td>
<td>37(^*)</td>
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</table>

\(^*\) Storage time of 12 and 7 days respectively
\(^\text{50\%}\) MP-NAT hit rate estimate
\(^**\) Estimated by back extrapolation based on doubling time
# HIV transmission outcomes by blood component type

<table>
<thead>
<tr>
<th>Blood product</th>
<th>Virions infused</th>
<th>Transmission rate</th>
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</thead>
<tbody>
<tr>
<td>FFP</td>
<td>~4100</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>PLTs</td>
<td>~600-3000</td>
<td>3/3 (100%)*</td>
</tr>
<tr>
<td>RBCs</td>
<td>~500-3500</td>
<td>6/9 (67%)</td>
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</table>

* excludes one case of non-tx from a platelet apheresis unit donated in the eclipse phase

50% minimum infectious dose estimated at ~400 virions, compared to ~3-10 virions in SIV macaque infectivity studies

reduction of infectivity during storage or genuinely lower minimum dose of HIV than SIV?
HIV viral inocula in transmitting vs non-transmitting transfusions

Reverse probit analysis estimate of 50% MID
828 copies (414 virions)*

- Tx case inocula (imputed): (8280), (8280), 7200, 6150, 4920, 1500, (1472), 1460, 1250, 1000, (912)
- Non-Tx case inocula (imputed): 1960, (1472), 1200, (240)

* If exclude cases with imputed VL, 50% ID = 858 copies (429 virions)
Methods-III

• The probabilities of 1) non-detection of the estimated viral loads in the implicated donations and 2) infection given the infused viral copy numbers were estimated by probit analysis

• 95% detection limits* in c/mL of the triplex NAT screening systems:
  • 20.4 for ULTRIO® ID-NAT
  • 163 for ULTRIO® MP-8 NAT
  • 326 for ULTRIO® MP-16 NAT
  • 137 for cobas TaqScreen® MP-6 NAT

• Transmission risk is the product of these two probabilities (Weusten et al, Transfusion 2002, 42;537-548)

* according to probit analysis on DDL data base with NAT results of several laboratories
# Probability of detection of infectious MP-NAT breakthrough infections by ID or small pool NAT

<table>
<thead>
<tr>
<th>1st author of report</th>
<th>estimated cps/mL</th>
<th>recipient infection</th>
<th>ULTRIO® ID-NAT (20.4 c/mL)</th>
<th>ULTRIO® MP-8 (163 c/mL)</th>
<th>ULTRIO® MP-16 (326 c/mL)</th>
<th>S201 MP-6 (137 c/mL)</th>
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<td>69%</td>
<td>45%</td>
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<tr>
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<td>99%</td>
<td>62%</td>
<td>37%</td>
<td>64%</td>
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<tr>
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<td>62%</td>
<td>37%</td>
<td>64%</td>
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<tr>
<td>Stramer</td>
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<td>62%</td>
<td>37%</td>
<td>64%</td>
</tr>
<tr>
<td>Delwart</td>
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<td>87%</td>
<td>97%</td>
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<tr>
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<td>72%</td>
<td>48%</td>
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<tr>
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<tr>
<td>Ferreira</td>
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<td>99%</td>
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<tr>
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<td>85%</td>
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<tr>
<td><strong>Average Probabilities</strong></td>
<td></td>
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<td><strong>99.5±0.05%</strong></td>
<td><strong>77.5±15%</strong></td>
<td><strong>57.9±22 %</strong></td>
<td><strong>79.5±14%</strong></td>
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* Stat. not significant p=0.745
AS2  Adonis Stassinopoulos, 3/18/2009
AS3  Numbers don't add up!!
     Adonis Stassinopoulos, 3/21/2009
Probability that ID-NAT or MP6-8-16-NAT would have interdicted MP16-96 negative HIV WP transmissions

<table>
<thead>
<tr>
<th>NAT option</th>
<th>% transmission interdicted</th>
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<tbody>
<tr>
<td><strong>ULTRIO® ID</strong></td>
<td>99.5±0.05%</td>
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<td><strong>ULTRIO® MP8</strong></td>
<td>77.5±15%*</td>
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<td><strong>ULTRIO® MP16</strong></td>
<td>57.9±22 %</td>
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<td><strong>TaqScreen® MP6</strong></td>
<td>79.5±14%*</td>
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* Stat. not significant p=0.745
Summary

• Our review of the literature shows that HIV-1 contaminated (RNA+) blood products from donors in the early window phase are not always infectious.
  – 2 FFP units and 3 whole blood derived platelet concentrates containing ~600-4100 virions were all infectious
  – 3/9 (33%) red blood cell concentrates (RBCs) containing ~500-3500 virions were not infectious

• Based on probit analysis 50% infectious dose estimated at 828 copies (or 414 virions)
  – If exclude cases with imputed VL of 50% NAT LOD = 858 copies (or 429 virions)

• This minimum infectious dose is 100-fold higher than that estimated from infectivity experiments in which immediately frozen SIV plasma inocula from acutely infected macaques were end-point titered and transfused into naive macaques (Ma Z-M.et al, J Virology)
  – possible explanation for reduced HIV-1 infectivity in the human cases is virus degradation upon storage of RBCs for > 7 days at 4 °C
Limitations

• The outcome of our analysis may have been affected by:
  – reporting bias - non-transmission cases traced following look-back are often not reported
  – variety of assays were used to quantify low VL in the implicated donations for which plasma was available
  – Imputation of VL in the few cases w/o residual plasma may have assigned VLs that were too high, leading to an erroneously high estimated rate of detection by small pool or ID-NAT

Conclusions

• Despite these limitations, our analysis suggests that a majority of observed MP-NAT breakthrough cases could have been detectable if the currently available triplex small pool or ID-NAT systems had been used

• Continued systematic reporting and compilation of HIV look-back case data will be important to confirm and extend the results of this exploratory analysis