Evaluation of the yield of HBV DNA-positive, seronegative donors using an automated HIV-1/HCV/HBV triplex NAT assay

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J Linnen, J Blunt (Gen-Probe/Chiron)
Paul Coleman (Abbott Labs)
Wolfram Gerlich (Univ of Giessen, Germany)
JP Allain (Univ of Cambridge, UK),
Ultrio Study Objective

- Evaluate the “yield” of HBV DNA positive donations that are HBsAg and anti-HBc nonreactive using a combination of individual donation and mini-pool (MP) NAT of 16 donations with the Ultrio reagents and TIGRIS platform to supplement the existing Gen-Probe Ultrio license for an HBV DNA screening claim.
- Determine rate/characteristics of HBV yield donors.
- HBV screening claim approved by FDA on 8/12/08 for MPs up to 16 donations.
Scope

- 3 of the 5 ARC NTLs involved (Charlotte, Detroit and St. Louis)
  - All donations tested for HIV-1/HCV/HBV (triplex UltraTrio assay) on the TIGRIS instruments using both
    - ID NAT for a target of 600,000 donations
    - MP NAT for a minimum of 2 million donations
  - HIV-1/HCV (duplex) NAT was discontinued during the evaluation
    - Other NTLs continued using HIV-1/HCV (duplex) using the manual platform (eSAS)
  - Testing of WNV assay continued without change
    - TIGRIS or eSAS
Expected Yield
Confirmation of MP NAT Reactivity for ID NAT Yield

- ID NAT: up to 5 window period (WP) donations
  - IND studies: yield of 1:100,000-1:200,000
- MP NAT: 1 additional WP donation
- Dilutional studies to determine reactivity of ID NAT yield samples using pool sizes of: 4, 8, 16
Results (1/28/08-1/5/09)

- Donations tested by MP NAT = 3,118,368
  - 1640 Rx MPs resolved to a Rx donation = 0.05%
  - Unresolved pool rate = 0.21%, or 413/194,898
- Donations tested by ID NAT = 576,490
  - 945 Rx IDs = 0.16%
- Total tested = 3,694,858
  - 2585 Utrio Rx dtns = 0.07%
  - 2119 (82%) discriminated = 0.06%
  - 455 nondiscriminated, or 0.01% (1:8120) of total tested
    - MP NAT = 65 (1:47,974)
    - ID NAT = 390 (86%, 1:1478)
    - 431 eligible for follow up of which 120 have been submitted for reentry
  - 11 QNS for discriminatory testing
**Discriminated Results (1/28/08-1/5/09)**

- 2119 (82%) discriminated, or **0.06%** of total tested
- 2083 (98%) concordant serologic results from 2060 donors
  - 426 HBV, 231 HIV, 1426 HCV
- 3 HIV of which **2 confirmed** (**1:1,847,429**); 1 false pos
- 42 HCV of which **15 confirmed** (**1:246,324**); 27 false pos
- 30 HBV of which **9 confirmed** (**1:410,540**); 21 false pos
  - 8 MP pos (**1:389,796**), 1 ID pos (**1:576,940**)
  - 6 anti-HBs pos donors with likely vaccine breakthrough (**1:270,956** assuming 44% donors vaccinated; **1:228,680** MP only)
    - 2/5 developed HBsAg; 4/5 developed anti-HBc
  - 3 anti-HBs neg window period donors (**1:689,707** assuming 56% donors unvaccinated; **1:873,143** MP only)
    - 1/2 developed HBsAg; 2/2 developed anti-HBc
## Results of Dilutions for 019 Yield Donor S/CO values

<table>
<thead>
<tr>
<th></th>
<th>Undilute</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
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<tbody>
<tr>
<td>ARC dHBV TIGRIS</td>
<td>26.55</td>
<td>0.06</td>
<td>0.14</td>
<td>0.19</td>
</tr>
<tr>
<td>GP dHBV eSAS</td>
<td>23.28</td>
<td>19.49</td>
<td>0.03</td>
<td>0.03</td>
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<tr>
<td>GP Ultrio eSAS</td>
<td>13.10</td>
<td>0.08</td>
<td>9.81</td>
<td>0.09</td>
</tr>
<tr>
<td>Donor</td>
<td>Donor Status</td>
<td>Sex</td>
<td>Age</td>
<td>City/State</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>-----</td>
<td>-----</td>
<td>------------</td>
</tr>
<tr>
<td>013</td>
<td>Repeat 11/18/05, MP+, anti-HBs+</td>
<td>M</td>
<td>27</td>
<td>Ann Arbor/MI</td>
</tr>
<tr>
<td>042</td>
<td>Repeat 2/10/07, MP+, anti-HBs+</td>
<td>M</td>
<td>28</td>
<td>Olmsted Falls/OH</td>
</tr>
<tr>
<td>003</td>
<td>Repeat 5/21/05, MP+, anti-HBs--; strong seroconv</td>
<td>F</td>
<td>37</td>
<td>Marietta/GA</td>
</tr>
<tr>
<td>019</td>
<td>Repeat 1/15/08, ID+ (MP-), anti-HBs-</td>
<td>F</td>
<td>44</td>
<td>White House/TN</td>
</tr>
<tr>
<td>011</td>
<td>Repeat 3/29/07, ID+ (MP+), anti-HBs+</td>
<td>F</td>
<td>17</td>
<td>Centralia/IL</td>
</tr>
<tr>
<td>Donor</td>
<td>Donor Status</td>
<td>Sex</td>
<td>Age</td>
<td>City/State</td>
</tr>
<tr>
<td>-------</td>
<td>--------------</td>
<td>-----</td>
<td>-----</td>
<td>------------</td>
</tr>
<tr>
<td>055</td>
<td>MP+, anti-HBs-</td>
<td>M</td>
<td>20</td>
<td>Batesville/MS</td>
</tr>
<tr>
<td>074</td>
<td>Repeat 05/17/08</td>
<td>F</td>
<td>24</td>
<td>Coral Springs/FL</td>
</tr>
<tr>
<td>001</td>
<td>Repeat 03/31/08</td>
<td>M</td>
<td>22</td>
<td>Boston/MA (NY)</td>
</tr>
<tr>
<td>029</td>
<td>Repeat 04/03/07</td>
<td>M</td>
<td>19</td>
<td>Colerain/NC</td>
</tr>
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</table>
## Virologic/Serologic Markers in HBV DNA Yield Donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Anti-HBs mIU/mL @ index</th>
<th>Time (days) followed</th>
<th>Viral Load Range (c/mL)</th>
<th>Duration (days) DNA pos</th>
<th>HBsAg first pos day (duration)</th>
<th>Anti-HBc first pos day</th>
<th>Anti-HBc IgM</th>
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</thead>
<tbody>
<tr>
<td>013</td>
<td>+; 43</td>
<td>243</td>
<td>100-200</td>
<td>≥ 34</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>042</td>
<td>+; 33</td>
<td>133</td>
<td>800-45,000</td>
<td>≥ 75</td>
<td>75 (54)</td>
<td>107</td>
<td>+</td>
</tr>
<tr>
<td>003</td>
<td>+; 3</td>
<td>320</td>
<td>200-4800</td>
<td>= 44</td>
<td>-</td>
<td>70</td>
<td>+</td>
</tr>
<tr>
<td>011</td>
<td>+; 11</td>
<td>189</td>
<td>100-50,000</td>
<td>≥ 137</td>
<td>108 (60)</td>
<td>168</td>
<td>+</td>
</tr>
<tr>
<td>055</td>
<td>-</td>
<td>&gt;175</td>
<td>&gt;10^8</td>
<td>≥ 75</td>
<td>41 (123)</td>
<td>70</td>
<td>+</td>
</tr>
<tr>
<td>074</td>
<td>-</td>
<td>&gt;116</td>
<td>200</td>
<td>≥ 73</td>
<td>-</td>
<td>73</td>
<td>+</td>
</tr>
<tr>
<td>001</td>
<td>+; 100 (day 45)</td>
<td>&gt;115</td>
<td>100</td>
<td>≥ 49</td>
<td>-</td>
<td>69</td>
<td>+</td>
</tr>
</tbody>
</table>
Anti-HBs Concentrations in HBsAg and Anti-HBc Negative Blood Donors (N=520)

Vaccine breakthrough HBV yield donors:
HBV DNA Viral Loads among HBsAg and anti-HBc Positive Donors (N=500)

HBV yield partners:
HBV Additional Studies

- Performed by 3 groups
  - Prof Wolfram Gerlich
    - Institute for Medical Virology, Univ of Giessen, Germany
    - Viral loads, genotype, subtype, sequence analysis
  - Dr. Paul Coleman
    - Abbott Laboratories
    - Anti-HBc IgM (ARCHITECT), sequence analysis
  - Prof JP Allain
    - Laboratory of Molecular Virology, Depart Haematology, Univ of Cambridge, UK
    - Viral loads, genotype, sequence analysis
    - Sequencing
      - Full length, preS/S, BPC/PC
<table>
<thead>
<tr>
<th>Donor or Partner</th>
<th>DNA Viral Load (copies/mL)</th>
<th>HBsAg Conc. (ng/mL)</th>
<th>Genotype</th>
<th>Subtype</th>
<th>Mutation (No. and Location)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor - 003</td>
<td>1,200, 86</td>
<td></td>
<td>WT A2</td>
<td>Pending</td>
<td>5 - sN59D; sT143A; sS210N; sL22S; sK122E</td>
</tr>
<tr>
<td>Partner - 003</td>
<td>2.7 x10^9</td>
<td>67,300</td>
<td>WT A2</td>
<td>Pending</td>
<td>2 – preS V172M and rtC6Y</td>
</tr>
<tr>
<td>Donor - 011</td>
<td>2,700, 13</td>
<td></td>
<td>WT F1</td>
<td>Pending</td>
<td>2 - S and preS domain stop codons @ sY71 Stop and preS F24L</td>
</tr>
<tr>
<td>Partner - 011</td>
<td>2.4 x10^10, 2.6 x 10^8</td>
<td>100,600</td>
<td>WT F1</td>
<td>Pending</td>
<td>1 - T173M Not present in other F1 strains</td>
</tr>
<tr>
<td>Donor - 013</td>
<td>35</td>
<td></td>
<td>WT B2</td>
<td>adw2</td>
<td>1 - sF220L (rtL229V) Not present in other B2 strains</td>
</tr>
<tr>
<td>Partner - 013</td>
<td>8.0 x10^9, 1.8 x 10^6</td>
<td></td>
<td>WT B2</td>
<td>adw2</td>
<td>1 - sF220L (rtL229V) Not present in other B2 strains</td>
</tr>
<tr>
<td>Donor - 042</td>
<td>65, 230, 43</td>
<td></td>
<td>WT A2</td>
<td>ayw3</td>
<td>2 - sT125M and sP127T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WT D3</td>
<td>ayw3</td>
<td>2 - sG44R and sT143A</td>
</tr>
<tr>
<td>Donor - 001</td>
<td>11</td>
<td></td>
<td>C2 (N China, Korea)</td>
<td>adw2</td>
<td>1 – G145R; vaccine escape mutation</td>
</tr>
<tr>
<td>Partner - 001</td>
<td>1.9 x 10^8</td>
<td></td>
<td>C2)</td>
<td>adw2</td>
<td>1 – G145R; vaccine escape mutation</td>
</tr>
</tbody>
</table>
HBV Bootstrap Analysis for 6 cases with full genome sequences

where $\geq 70$ is $p<0.05$

0.01 substitutions/site
Virologic/Serologic Profiles

• Breakthrough cases with likely infection via a sexual partner
  – All partners tested and confirmed to have high levels of HBV DNA ($>10^7$ copies/mL), HBsAg, anti-HBc but **no** anti-HBs, and when sequencing complete, same virus as the donor
  – Donors with and without HBsAg development
• Breakthrough case occupational exposure
• Acute infection in the absence of vaccination
UFS Yield Donor 003FQ75130

- dHBV
- HBsAg
- anti-HBc IgM
- Viral Load
- anti-HBc
- anti-HBs

Days

Copies/mL

S/CO

CO/S

mIU/mL

Viral Load

anti-HBc

anti-HBs

Days

Copies/mL

S/CO

CO/S

mIU/mL

Days

Copies/mL

S/CO

CO/S

mIU/mL

Copies/mL

S/CO

CO/S

mIU/mL

Days

Copies/mL

S/CO

CO/S

mIU/mL

Days

Copies/mL

S/CO

CO/S

mIU/mL
UFS Yield Donor 055N 30971

- **dHBV**
- **HBsAg**
- **anti-HBc IgM**
- **Viral Load**

Graph showing the levels of different parameters over days:

- **S/CO** (Samples/Control) from 0.00 to 1.00
- **CO/S** (Controls/Samples) from 0.00 to 1.00
- **Copies/mL**
- **mIU/mL**

Days range from 0 to 175.
## Comparison of HBV DNA Yields in US Studies

<table>
<thead>
<tr>
<th>Study Description</th>
<th>HBV DNA Yields</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC Roche Trials (1, 6, 24)</td>
<td></td>
</tr>
<tr>
<td>1:352,451</td>
<td></td>
</tr>
<tr>
<td>1:425,730</td>
<td></td>
</tr>
<tr>
<td>1:72,336</td>
<td></td>
</tr>
<tr>
<td>(2/704,902) COBAS AmpliScreen trial</td>
<td></td>
</tr>
<tr>
<td>(7/2,980,103) + extended COBAS AmpliScreen testing</td>
<td></td>
</tr>
<tr>
<td>(1/72,336) MPX trial</td>
<td></td>
</tr>
<tr>
<td>1:381,555</td>
<td>(8/3,052,439) Combined</td>
</tr>
<tr>
<td>1:610,488</td>
<td>(5/3,052,439) Combined (– PRISM HBsAg RRs)</td>
</tr>
<tr>
<td>1:282,984</td>
<td></td>
</tr>
<tr>
<td>(4/1,131,937) vs GSC 3.0 and PRISM HBsAg</td>
<td></td>
</tr>
<tr>
<td>ABC GP Trial (1, 8) 9 sites; 5/07 to 7/16/08</td>
<td></td>
</tr>
<tr>
<td>1:410,540</td>
<td>(9/3,694,858) vs PRISM HBsAg</td>
</tr>
<tr>
<td>ARC GP Trial (1, 16) 1/29/08 to 1/5/09</td>
<td></td>
</tr>
</tbody>
</table>
Summary

- Performance of duplex (eSAS) and triplex (ULTRIO®-TIGRIS) is comparable regarding MP NAT specificity and HIV-1/HCV NAT yield
- 9 HBV DNA pos/HBsAg and anti-HBc neg donations produced comparable yields to other HBV yield studies performed in the US
  - 1:410,540 yield rate
  - 8 of 9 yield donors were detected by MP NAT; 1:389,796 yield rate
    - 7 with long-term follow up
      - All with detectable HBV DNA for 34-137 days
      - 3 with HBsAg at 41-75 days after DNA (duration 54-123 days)
      - 6 with anti-HBc SC at days 69-168
  - 6 of 9 were immunized individuals having anti-HBs at index or shortly thereafter; although these represent acute infections, dynamics of infection differ, and infectivity of such donations is unknown; 1:270,956 yield rate (or 1:228,680 for MP only)
  - 3 of 9 were window period donors; 1:689,707 yield rate (or 1:873,143 for MP only)
Conclusions re HBV NAT

• ID NAT modeling studies (nonvaccinated donors)
  – Lowest residual risk; highest yield = 1:466,000 – 1:713,000
  – Logistically not feasible for either platform
  – Modeling cannot predict total yield due to vaccinated donors with differing kinetics early in infection

• MP NAT modeling studies (nonvaccinated donors)
  – MPX: MP 6 highest yield = 1:830,000 (= 1:713,000)
  – Ultrio: MP 8 = MP 16 = 1:1,345,000 – 1:2,000,000

• MP NAT observed yield (both vaccinated and nonvaccinated)
  – MP NAT at various pool sizes = 1:300,000-1:600,000
  – Data do not suggest benefit of MP 8 vs MP 16
  – ARC study; MP 16 detected 8 of 9 units
    • 9th yield unit only detected by ID NAT (not detected MPs 4, 8 or 16)

• High yield of MP 16 indicates value in comparison to ultra-sensitive HBsAg assays
  – Total yield = 1:410,540
  – MP 16 yield = 1:389,796
  – Vaccinated MP 16 yield = 1:228,680; infectivity ?
  – Nonvaccinated MP 16 yield = 1:873,143
Are anti-HBs positive units infectious?
<table>
<thead>
<tr>
<th></th>
<th>HBsAg</th>
<th>Anti-HBc</th>
<th>Anti-HBc IgM</th>
<th>Anti-HBs (mIU/mL)</th>
<th>HBV DNA (IU/mL)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor (index)</td>
<td>Neg</td>
<td>Pos</td>
<td>NT</td>
<td>12-29</td>
<td>180</td>
<td>D</td>
</tr>
<tr>
<td>Recip* #1</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>NT</td>
<td>D</td>
</tr>
<tr>
<td>4 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8 days</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>185</td>
<td>D</td>
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<tr>
<td>Recip #2</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>1.1x10^8</td>
<td>D</td>
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<tr>
<td>7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
</tbody>
</table>

* Neg pretransfusion
TTV: No HBV infection in recipients regardless of anti-HBc status when the anti-HBs S/N > 10 (10-60 mIU/mL)

Hollinger, Transfusion 2008
Of 12 infectious components, 11 (92%) were window period and 1 (8%) was anti-HBc positive (jumbo FFP)
## Results of JRC LB Study

<table>
<thead>
<tr>
<th>Anti-HBc in donors</th>
<th>Infectious</th>
<th>Anti-HBs in donors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>Low titer</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>Yes (12)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No (51)</td>
<td>16 (100%)</td>
</tr>
</tbody>
</table>
Additional Slides for questions
Additional Results (JPA) and Conclusions to Date

- Full sequences obtained for 6 (013, 003, 055, 074, 011, 042)
  - 1 each F, B, D and 3 each A2; half representing genomes not typically found in the US (majority in US is A2 in Caucasians, A1 in Blacks and B/C in Asians)
- Pre-S/S or BCP/PC sequences for 8 (above + 019 + 001)
  - 7 WT (compared to ref strains in GenBank); all major hydrophilic regions (MHRs) of the S protein conserved. Also, core, pol and X genes conserved; no stop codons
  - 1 S protein vaccine escape mutation (001)
- 4 (013, 003, 011, 042) are likely breakthrough infections, since no MHR mutations and all WT; one (001) vaccine escape
  - Inability of background levels of vaccine-derived anti-HBs to neutralize infectious virus
  - Low level anti-HBs might be sufficient to complex HBsAg making it undetectable but not sufficient enough, or too low, to prevent infection and the detection of HBV DNA
  - Public health implications; vaccine does prevent chronic HBV but not infection (all of these donors did clear virus and had truncated HBsAg responses, if present); more frequent boosters may be warranted
- 3 likely window period cases (019, 055 and 074)
**Impact of ID NAT**

- **Charlotte pot. yield (N = 23)**
- **Charlotte non-discrim. (N = 194)**
- **Detroit pot. yield (N = 4)***
- **Detroit non-discrim. (N = 133)**
- **St. Louis pot. yield (N = 3)**
- **St. Louis non-discrim. (N = 104)**

* One case not listed, contamination event.

**Total non-discriminated**

- **ID NAT** - 365
- **MP NAT** - 66

* ID NAT

Date (by collection date): Jan Wk 5, Feb Wk 2, Mar Wk 2, Apr Wk 2, May Wk 2, Jun Wk 1, Jul Wk 3, Aug Wk 3, Sep Wk 2, Oct Wk 3, Nov Wk 1, Dec Wk 3, Jan Wk 1.
Specificity Comparisons

Duplex eSAS – MP NAT (16); 3 NTLs to 12/07
  ➢ 33,868,522 screened = 99.9974% (99.9972%-99.9975%)*

Ultrio TIGRIS – MP NAT (16); 3 NTLs
  ➢ 3,118,368 screened = 99.9973% (99.9966%-99.9978%)*

Ultrio TIGRIS – ID NAT; 3 NTLs
  ➢ 576,490 screened = 99.9297% (99.9225%-99.9364%)*

*95% CIs by the binomial distribution
## Concordant Ultrio/Serology
### 2083 Results/2060 Donors

<table>
<thead>
<tr>
<th>No. Reactive</th>
<th>HBV</th>
<th>HIV</th>
<th>HCV (+anti-HBc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St Louis</td>
<td>63*</td>
<td>23</td>
<td>333*</td>
</tr>
<tr>
<td>Detroit</td>
<td>78</td>
<td>22*</td>
<td>222*</td>
</tr>
<tr>
<td>Charlotte</td>
<td>285* (67%)</td>
<td>186* (81%)</td>
<td>871* (61%)</td>
</tr>
<tr>
<td>Total</td>
<td>426</td>
<td>231</td>
<td>1,426**</td>
</tr>
<tr>
<td>Rate</td>
<td>1:8673</td>
<td>1:15,995</td>
<td>1:2591</td>
</tr>
</tbody>
</table>

*12 donations reactive for both HIV and HCV Ab + NAT
5 donations reactive for both HBV and HCV Ab + NAT
6 donations reactive for both HBV and HIV Ab + NAT

**327 (23%) HCV reactives also anti-HBc reactive
Ultrio Nonreactive – Serology Reactive (Confirmed Positive); N=5666 from 5662 Donors

<table>
<thead>
<tr>
<th></th>
<th>HBsAg (+)</th>
<th>Anti-HCV (+)</th>
<th>Anti-HIV (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID NAT (-) (N=576,490)</td>
<td>134 (25)</td>
<td>455 (71)</td>
<td>339 (2)</td>
</tr>
<tr>
<td>MP NAT (-) (N=3,118,368)</td>
<td>655 (153)</td>
<td>2615 (446)</td>
<td>1468 (27)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>789 (178)</td>
<td>3070 (517)</td>
<td>1807 (29)</td>
</tr>
</tbody>
</table>

Rate False Pos Serology (ID or MP NAT -)
- 1:5289
- 1:6212
- 1:1501
- 1:1438
- 1:1710
- 1:2164

Rate of Conf’d Pos Serology (ID or MP NAT -)
- 1:23,060-
- 1:20,381
- 1:8120
- 1:6992
- 1:288,245-
- 1:115,495
Comparison of HBV DNA Reactivity in Anti-HBc Reactive Donors
(with removal of all HBsAg, HCV and HIV reactives)

<table>
<thead>
<tr>
<th></th>
<th>22 Reactive Ultrio MP NAT of 6573 anti-HBc RRs</th>
<th>12 Reactive Ultrio ID NAT of 1447 anti-HBc RRs</th>
<th>34 Total Reactive of 8020 anti-HBc RRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) dHBV Pos (No. PCR* Pos/No. Tested)</td>
<td>18 (0.33%) (5/9 = 56%)</td>
<td>9 (0.62%) (6/7 = 86%)</td>
<td>27 (0.34%) (11/16 = 69%)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>10 (0.15%)</td>
<td>8 (0.55%)</td>
<td>19 (0.23%)</td>
</tr>
</tbody>
</table>

*Multiprep Ampliscreen HBV ID NAT
HBV Sensitivity of NAT Systems according to the PIs

- ID NAT Ultrio/TIGRIS = 10.40 IU/mL
- MP NAT 8 Ultrio/TIGRIS = 83.20 IU/mL
- MP NAT 16 Ultrio/TIGRIS = 166.40 IU/mL
- ID NAT MPX/s 201 = 3.80 IU/mL
- MP NAT 6 MPX/s 201 = 22.80 IU/mL
### Table 1  HBV DNA Yield Samples

**Index and Follow up Results**

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<table>
<thead>
<tr>
<th>DRAW DATE</th>
<th>INDEX SAMPLE – HBsAg &amp; anti-HBc NR</th>
<th>FOLLOW UP SAMPLE: DAYS REACTIVE (DAYS OF FOLLOW UP)</th>
<th>DONOR HISTORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 6/11/2003</td>
<td>51 61,000</td>
<td>7 (54) 26-54 (54) 26-54 (54) 0-54 (54) 0-7 (54)</td>
<td>27 YEAR OLD MALE REPEAT DONOR-MULTIPLE MALE SEXUAL PARTNERS</td>
</tr>
<tr>
<td>2 4/24/2004</td>
<td>NT 37,000</td>
<td>14 (177) NR to 177 28-177 (177) &lt;14-177 (177) 0-21 (177)</td>
<td>50 YEAR OLD MALE REPEAT DONOR, NO RISK FACTORS IDENTIFIED</td>
</tr>
<tr>
<td>3 7/24/2003</td>
<td>NT 2300</td>
<td>7-21 (134) 28-63 (63) 28-134 (134) 63-91 (91) 0-28 (134)</td>
<td>29 YEAR OLD MALE REPEAT DONOR ACUPUNCTURE 8 WEEKS PRIOR TO DONATION</td>
</tr>
<tr>
<td>4 9/6/2002</td>
<td>NT 2000</td>
<td>17-200 (200) 55-200 (200) 48-200 (200) NR to 200 0-200 (200)</td>
<td>26 YEAR OLD MALE REPEAT DONOR, NO KNOWN RISK</td>
</tr>
<tr>
<td>DRAW DATE</td>
<td>INDEX SAMPLE – HBsAg &amp; anti-HBc NR</td>
<td>FOLLOW UP SAMPLE: DAYS REACTIVE (DAYS OF FOLLOW UP)</td>
<td>DONOR HISTORY</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------</td>
<td>-----------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>5 10/24/2006</td>
<td>NR 270</td>
<td>NR NA</td>
<td>UNABLE TO ENROLL IN FOLLOW UP</td>
</tr>
<tr>
<td>6 6/11/2005</td>
<td>NR 200</td>
<td>NR NA</td>
<td>39 YEAR OLD MALE REPEAT DONOR REPORTED VACCINATION HISTORY 3 DAYS PRIOR TO DONATION. COULD NOT CONTACT FOR F/U;</td>
</tr>
<tr>
<td>7 9/25/2002</td>
<td>2340 200</td>
<td>NR to 167 29-167 (167) 22-167 (167) 0-167 (167) 0-22 (167)</td>
<td>49 YEAR OLD FEMALE REPEAT DONOR, HISTORY OF VACCINE BUT WITH NEGATIVE TITER 8 WEEKS PRIOR TO INDEX</td>
</tr>
<tr>
<td>8 MPX* 7/10/2005</td>
<td>RR &lt;LOD</td>
<td>NR to 59 NT NR to 59 0 (0) 0-59 (59)</td>
<td>PHILLIPPINE IMMIGRANT; NO HISTORY OF VACCINATION</td>
</tr>
</tbody>
</table>

* Nonreactive by MP COBAS AMPLISCREEN; Reactive IDT using multiprep procedure
Table 2 Window Period Samples
Repeat Viral Load, Genotype and PRISM Results
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<table>
<thead>
<tr>
<th>Donor #</th>
<th>Draw Date</th>
<th>HBV DNA Quantitation original copies/mL</th>
<th>HBV DNA Quantitation 2007 repeat copies/mL</th>
<th>HBV genotype</th>
<th>PRISM S/CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/11/2003</td>
<td>61,000</td>
<td>31,000</td>
<td>H</td>
<td>1.22, 1.16, 1.16</td>
</tr>
<tr>
<td>2</td>
<td>4/24/2004</td>
<td>37,000</td>
<td>3850</td>
<td>C</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>7/24/2003</td>
<td>2300</td>
<td>2480</td>
<td>B</td>
<td>1.90, 1.99, 1.97</td>
</tr>
<tr>
<td>4</td>
<td>9/6/2002</td>
<td>2000</td>
<td>ND</td>
<td>ND</td>
<td>1.11, 1.23, 1.09</td>
</tr>
<tr>
<td>5</td>
<td>10/24/2006</td>
<td>270</td>
<td>&lt;200</td>
<td>NA</td>
<td>0.22</td>
</tr>
<tr>
<td>6</td>
<td>6/11/2005</td>
<td>200</td>
<td>&lt;200</td>
<td>NA</td>
<td>0.81</td>
</tr>
<tr>
<td>7</td>
<td>9/25/2002</td>
<td>200</td>
<td>ND</td>
<td>NA</td>
<td>0.22</td>
</tr>
<tr>
<td>8</td>
<td>7/10/2005</td>
<td>&lt;LOD</td>
<td>&lt;200</td>
<td>NA</td>
<td>0.19</td>
</tr>
</tbody>
</table>