WHO Parvovirus B19 Genotype Panel

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In Oct 2009, a plasma-derived parvovirus B19 (B19V) genotype (gt) panel was recognized by the WHO ECBS on the basis of results from a collaborative study jointly conducted by PEI/NIBSC/CBER.

In May 2009, Sally Baylis and I presented data from the study at the SoGAT XXI meeting in Brussels.

Today, I will provide an update regarding the development and use of this panel.
Parvovirus B19 and Plasma Screening

- Extremely high viremic levels, $>10^{12}$ IU/ml, of parvovirus B19 (B19V) can be found in acutely infected, but asymptomatic donors.

- B19V transmission has occurred in recipients, mostly by pooled plasma-derived products and rarely by single-donor blood components.

- In 1999, safety of Pooled Plasma S/D Treated correlated with those lots having $<10^4$ copies (IU)/ml of B19V DNA in manufacturing pools. This became the threshold level for pools destined for all plasma derivatives in the U.S. and for certain products in Europe.
Regulatory Requirements for B19V NAT Testing in Europe

• In 2004 introduction of Ph Eur requirements for B19V DNA testing for plasma pools used in the manufacture of:
  – Human plasma (pooled and treated for virus inactivation)
  – Human anti-D immunoglobulin
  – Human anti-D immunoglobulin for intravenous administration
  – Human albumin/normal immunoglobulin added to anti-D immunoglobulin

• Quantitative test limit of 10 IU/μl
B19V NAT Screening in the U.S.

- The B19V transmission associated with Pooled Plasma S/D Treated (a phase 4 study) was discussed at the Sep 1999 BPAC. For plasma for further manufacturing, B19V NAT screening was recommended as an in-process, not donor-screen, test.
  - Currently fractionators are performing plasma minipool testing (240-512 units per minipool) for excluding original donations having about $10^6$ IU/ml B19V DNA or greater.
  - B19V DNA limit of $\leq 10^4$ IU/ml for manufacturing pools destined for all plasma derivatives (FDA Guidance for Industry, July 2009)
Genetic Variation of Parvovirus B19

- B19V genome was previously considered to be highly conserved, i.e., to vary by <2%.
- Recently more divergent B19V variants (e.g., V9, A6, Lali) were identified.
- 3 distinct virus gts have been classified and sequences can vary by ~15%.
  - subgroups recently identified for gts 1 and 3.
- In the VP1/2 region, variation is ~1% at the amino acid level between virus that represent a single serotype.
Regulatory Requirement or Recommendation for Detection of B19V Variants

• 8th Report of the International Committee on the Taxonomy of Viruses (2005) classified gt 2 and 3 variants as strains of B19V.

• Hence, need to detect all 3 gts.
  – Now included as a mandatory Ph Eur test requirement
  – Recommended in the FDA Guidance for B19V NAT

• But problems of implementation and issues with specificity of commercial kits available in Europe
  – No licensed commercial kits available yet in the U.S.

• No reference materials for gts 2 and 3
  – WHO IS (both 1st and 2nd) and CBER/FDA standard are gt 1.
The need for a B19V Genotype Panel

- At the Extraordinary Meeting of SoGAT held at NIBSC in Mar 2007 to discuss standardization for B19V genotypes, the proposal to prepare a gt panel was initiated by Sally Baylis et al and was agreed upon by meeting participants.
- Beginning in late 1999, some fractionators initiated (albeit gradually) the use of minipool B19V NAT screening as an in-process quality control test to lower the viral load in manufacturing pools. CBER/FDA needs such a panel for fractionators to use in validation.
4-Member Genotype Panel Preparation (I)

- 3 Window-period plasma donations for preparing 3 positive members
  - Gt 1: same originating plasma, $\sim 10^{12}$ IU/ml, for WHO 1\textsuperscript{st} IS (99/800) and 2\textsuperscript{nd} IS (99/802) for B19V DNA from NIBSC
  - Gt 2: $\sim 10^{11}$ IU/ml, IM-81 strain (GenBank AY903437) from Baxter BioScience under MTA \cite{Blümel et al, J Virol 2005}
  - Gt 3a: $\sim 5 \times 10^{11}$ IU/ml, P1 (GenBank FJ265736) from Talecris Biotherapeutics under MTA \cite{Rinckel et al, Transfusion 2009}
Genetic Diversity of B19V Genotypes
(Phylogenetic analysis based upon sequences from VP1u region)

Genotype 1

1st WHO IS (99/800)
AY504945
AF162273
AY386330

Genotype 2

A6 (AY064476)
Lali (AY044266)
IM-81 (AY903437)

Genotype 3

D91.1 (AY083234)
V9 (AJ249437)
P1 (FJ265736)
4-Member Genotype Panel Preparation (II)

- One negative member derived from pooled plasma (also used as diluent for viral stocks)
  - Preparation of a defibrinated negative human plasma pool (contract manufactured by SeraCare) derived from 25 screened Source Plasma units (~20 L in total) kindly provided by National Genetics Institute (NGI). All donations were tested by NGI and found negative for the following markers:
    - Anti-HIV 1/2, anti-HCV, HBsAg, anti-HBc (IgG and IgM, Abbott Corzyme), & anti-B19V (IgG and IgM, Biotrin)
    - ID-NAT: HIV-1, HCV, HBV, B19V, HAV, & WNV
  - Pooled plasma testing
    - ID-NAT for HIV-1, HCV, HBV, HAV, & B19V by both NGI and Talecris; negative for all viral markers
4-Member Genotype Panel Preparation (III)

• Formulation of intermediate viral stocks, $\sim 10^{10} \& \sim 10^8$ IU/ml, for all 3 gts

• Quantification of 3 intermediate stocks, $\sim 10^8$ IU/ml, by 4 laboratories (PEI, NGI, Talecris, and CBER)
  – 6 quantitative NAT methods

• Formulation of 3 final bulks targeted to contain $\sim 10^6$ IU/ml
  – Each bulk monitored before fill by CBER

• Filling of 4-member gt panel in Mar 2008
  – $\sim 3000$ vials filled per member (1.1 ml fill per vial)* and stored at $\leq -70 \, ^\circ C$ (CBER’s contract filler, SeraCare)

* Only 1 member filled per week
The panel was evaluated in parallel with the 2nd WHO IS for B19V (99/802, gt 1).

The study commenced in Oct 2008.

Participants were requested to use assays targeting conserved sequences of B19V and to test the 4-member panel samples in 4 independent assays.

- Samples labeled as Members #1-4, which correspond to gts 1-3 and the negative plasma control member. However, identity of each member was unknown to participants during the study.

35 laboratories from 13 different countries participated

- Plasma fractionators, blood establishments, kit manufacturers, reference/clinical diagnostic/regulatory/research laboratories
B19V Genotype Panel Collaborative Study (II)

- 34 sets from quantitative NAT assays; 10 from qualitative.
- Primers/probes used were from NS1 or VP1/2 regions in approx. equal numbers.
- Data were mainly from in-house tests but some from commercial tests (several under development).
- The negative panel member was consistently reported as non-reactive.
Potency of Panel Member #1 (M1) relative to Assigned Unitage of the IS (99/802)

(Results from qualitative assays are shown as grey boxes.)
Potency of M2 relative to IS (99/802)
Potency of M3 relative to IS (99/802)

Potency of Panel Member M3 Relative to IS

IU (log10/ml)
Overall Means of Potencies (log_{10} IU/ml) relative to Concurrently Tested IS for Quantitative and Qualitative Assays

<table>
<thead>
<tr>
<th>Sample</th>
<th>Assay</th>
<th>N</th>
<th>Mean</th>
<th>95% CI</th>
<th>Min - Max</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>M1</td>
<td>Qual</td>
<td>10</td>
<td>5.95</td>
<td>5.68-6.22</td>
<td>5.10-6.57</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>Quan</td>
<td>34</td>
<td>5.98</td>
<td>5.94-6.02</td>
<td>5.74-6.20</td>
<td>0.46</td>
</tr>
<tr>
<td>M2</td>
<td>Qual</td>
<td>10</td>
<td>5.84</td>
<td>5.26-6.41</td>
<td>3.78-6.68</td>
<td>2.90</td>
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<tr>
<td></td>
<td>Quan</td>
<td>34</td>
<td>5.87</td>
<td>5.74-5.99</td>
<td>4.52-6.36</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>Quan*</td>
<td>32</td>
<td>5.94</td>
<td>5.86-6.02</td>
<td>5.43-6.36</td>
<td>0.93</td>
</tr>
<tr>
<td>M3</td>
<td>Qual</td>
<td>9</td>
<td>5.47</td>
<td>4.75-6.18</td>
<td>3.69-6.32</td>
<td>2.63</td>
</tr>
<tr>
<td></td>
<td>Quan</td>
<td>31</td>
<td>5.97</td>
<td>5.87-6.07</td>
<td>5.18-6.58</td>
<td>1.40</td>
</tr>
</tbody>
</table>

* Excluding Laboratories 09 and 28
Stability Testing of M1-M3  
(Stored at ≤70 °C)

<table>
<thead>
<tr>
<th>Time after filling (Months)</th>
<th>B19V DNA (log_{10} IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>5*</td>
<td>5.86</td>
</tr>
<tr>
<td>8*</td>
<td>5.79</td>
</tr>
<tr>
<td>9</td>
<td>6.04</td>
</tr>
<tr>
<td>10</td>
<td>6.00</td>
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<tr>
<td>12</td>
<td>6.04</td>
</tr>
<tr>
<td>15</td>
<td>5.93</td>
</tr>
<tr>
<td>25</td>
<td>5.94</td>
</tr>
</tbody>
</table>

Samples of each panel member were extracted (0.2 or 1.0* ml each) at the times indicated. Each extract was tested in triplicate, in parallel with the IS.
Long-Term Stability Testing of Two Liquid/Frozen Candidate Preparations after Storage at ≤−70 °C for <9 Years in NIBSC

• Two candidate preparations, CC (CBER B19V Standard, gt1) and DD (from Sanquin, gt1), were stored in NIBSC since 1999 for the original collaborative study to establish the 1st B19V IS (AA, 99/800)*

• 4 vials each of CC and DD were sent by NIBSC to both PEI and CBER in Feb 2009 and tested by calibrating against the 2nd B19V IS (BB, 99/802). No loss of potency was observed.
  – 6.21 (95% CI, 6.16 – 6.26) and 8.05 (8.0 – 8.10) log$_{10}$ IU/ml from 8 runs for CC and DD, respectively
  – Original study: 6.0 and 7.9 log$_{10}$ IU/ml for CC and DD

* Saldanha et al, ECBS 2000 and Vox Sang 2002
Conclusions (I)

• The 4-member, plasma-derived B19V panel has been evaluated concurrently with the 2nd WHO IS, in an international collaborative study, with a wide range of quantitative and qualitative NAT procedures.
  – Most developed in-house but some were commercial assays.

• Good agreement was observed for the 2nd WHO IS (gt 1) and the panel member M1(gt 1) with only a small number of outlying estimates for M2 (gt 2) and M3 (gt 3a).
  – The study confirmed the validity of the assigned value for the 2nd WHO IS, i.e., $10^6$ IU/ml. The confidence intervals were narrower than those obtained in the original study.
Conclusions (II)

• The overall geometric means of the quantitative assays were very close to the target value of $10^6$ IU/ml for all 3 positive panel members.

• However, the applicability of using a gt1 virus (i.e., the WHO IS) to calibrate plasma samples containing gt 2 and 3 viruses has not been determined.

• Thus, there will be no unitage assigned to individual panel members.

• The panel is to be used to ensure that the detection of all 3 gts is adequate and to confirm the absence of cross-reactivity of assays for the negative member, M4.
Conclusions (III)

• Stability studies suggest that the panel of B19V samples is stable for long-term use when stored at \(\leq-70\, ^\circ C\).

• This 1\textsuperscript{st} WHO International Reference Panel for Parvovirus B19 Genotypes is available from NIBSC (09/110) and CBER (Panel 1).

• The availability of the panel will facilitate validation of B19V NAT assays for detecting all 3 genotypes.

• The panel is not intended to replace the WHO IS for B19V DNA
  – The geometric means and the min. and max. ranges for each panel member are to be provided for information only.
Acknowledgements

• NGI, CSL Behring, Baxter BioScience & Talecris Biotherapeutics for providing materials essential for the panel

• Collaborative study participants

• Study planning, testing, evaluating and reporting
  – Sally A Baylis, PEI
  – Alan B Heath and David J Padley, NIBSC
  – Li Ma and Mei-ying W Yu, CBER/FDA

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