Introduction of Hepatitis E Virus (HEV) NAT for Human plasma (pooled and treated for virus inactivation) - 1646

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On behalf of the European Pharmacopoeia Department

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HEPATITIS E VIRUS (HEV): CHARACTERISTICS

- Small, non-enveloped virus
- Single strand RNA genome of 7.2 kb and 3 ORFs

Jay H. Hoofnagle & al. NEJM, 2012
HEPATITIS E VIRUS (HEV): CHARACTERISTICS

- 1955: Infection first documented during an outbreak in India: 29,300 cases;
- 1980s: Identified as “epidemic Non-A Non-B Hepatitis” outbreak in India, Afghanistan, and Pakistan;
- 1983: M. Balayan visualised HEV using immune electron microscopy to examine his own stool samples, collected after self-administration of infectious material.

- **Genotype 1 and 2**: Affects human only, endemic hepatitis and associated with waterborne and faecal-oral transmission;
- **Genotype 3 and 4**: Affects pigs and humans - zoonotic transmission.
HEV GEOGRAPHIC DISTRIBUTION

Jay H. Hoofnagle & al. NEJM, 2012
HEV CLINICAL FEATURES

- Incubation period: 3 to 8 weeks
- Viremia: 4- 6 weeks

Jay H. Hoofnagle & al. NEJM,2012
HEV CLINICAL FEATURES

- Asymptomatic infections
- Mainly self limiting illness: jaundice, anorexia, abdominal pain, nausea, vomiting, fever...)

Developing countries:
- Mortality rates from 0.2% to 4.0%
- Mortality rate higher in infants < 2 years of age
- Maternal mortality rate from 10% to 25%
- Older people (>60 years) more severely affected, male predominance

- Reported cases of prolonged infection in immunocompromised patients in Europe (transplant patients) and immunosuppressed persons (HIV co-infection)
- Acute HEV in the context of pre-existing chronic disease
CONCERNS: POST-TRANSFUSION HEV INFECTION

- High rate of asymptomatic HEV infections -> concerns of transmission via blood donation
- Post-transfusion HEV reported in many countries but currently no reported transmission by pooled plasma

(Courtesy of S. Baylis)
- Infections of blood donors (and transfusion transmitted infection) in Japan after consumption of grilled pork liver/meat [Matsubayashi et al., 2008]
- Viraemic plasma donor in Germany [Adloch et al., 2009]
- Acute hepatitis in recipient (67 yrs) [Matsubayashi et al., 2004] (gt 4)
- Hepatitis, mild jaundice (gt 3) in a recipient of a viraemic red cell donation in UK [Boxall et al., 2006]
- Persistent Infection (gt 4) in lymphoma patient (21yrs) after transfusion of RBC during chemotherapy [Tamura et al., 2007]
- Case of acute hepatitis (gt 3f) in a recipient in France [Colson et al., 2007]
- Acute hepatitis (gt 4) in a recipient of a platelet concentrate in Japan [Matsubayasi et al., 2008]
SEROPREVALENCE

- Germany

  4,352 subjects tested  16.8 % positive

  18-34 years  6.1% positive
  50-60 years  >20% positive
  60-64 years  26.4% positive

- England

  Seroprevalence of ~ 13 %

  Estimation of ~ 60 000 cases per year
RNA concentration: ≤ 1000 copies/ml
Low IgG levels in fractionation pools (except Asia)

<table>
<thead>
<tr>
<th>Source of Pools</th>
<th>No. Positive / No. Analysed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>3/34</td>
</tr>
<tr>
<td>Europe/North America</td>
<td>0/3</td>
</tr>
<tr>
<td>North America</td>
<td>1/4</td>
</tr>
<tr>
<td>Middle East</td>
<td>0/11</td>
</tr>
<tr>
<td>Asia</td>
<td>4/23</td>
</tr>
<tr>
<td>Overall</td>
<td>8/75</td>
</tr>
</tbody>
</table>

S. Baylis & al., Vox Sang. 2012

Courtesy of S. Baylis
HEV RNA IN SINGLE DONATIONS (1)

<table>
<thead>
<tr>
<th>Country</th>
<th>Rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>1: 4,415</td>
<td>Baylis et al., Vox Sang 2012*</td>
</tr>
<tr>
<td>Sweden</td>
<td>1: 8,278</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>&lt;1: 50,456</td>
<td></td>
</tr>
</tbody>
</table>

* Donors for S/D plasma
HEV RNA IN SINGLE DONATIONS (2)

Courtesy of S. Baylis

<table>
<thead>
<tr>
<th>Sample code</th>
<th>IgM*</th>
<th>IgG*</th>
<th>ALT*</th>
<th>Viral load (log_{10} IU/ml)*</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+/-</td>
<td>-</td>
<td>Neg.</td>
<td>3.22</td>
<td>Sweden</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+/-</td>
<td>Neg.</td>
<td>3.26</td>
<td>Germany</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>Neg.</td>
<td>5.35</td>
<td>Germany</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>Neg.</td>
<td>4.39</td>
<td>Sweden</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
<td>Neg.</td>
<td>4.95</td>
<td>Sweden</td>
</tr>
<tr>
<td>6</td>
<td>+/-</td>
<td>-</td>
<td>Elevated</td>
<td>4.54</td>
<td>Germany</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>Neg.</td>
<td>4.19</td>
<td>Germany</td>
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<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>Neg.</td>
<td>4.76</td>
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<td>9</td>
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<td>-</td>
<td>Neg.</td>
<td>3.86</td>
<td>Sweden</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>Elevated</td>
<td>4.64</td>
<td>Sweden</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>Elevated</td>
<td>3.20</td>
<td>Sweden</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>Neg.</td>
<td>5.68</td>
<td>Sweden</td>
</tr>
</tbody>
</table>

ALT, alanine transaminase.

*Positive samples (+) defined as S/Co ≥1 (according to the kit specifications); equivocal samples (+/-) gave an S/Co ≥1 on initial round of testing and S/Co < 1 on repeat testing using alternative batches of kit. Negative samples (−). Positive control for IgG was performed using the WHO International Reference Reagent for anti-HEV IgG (95/584). Negative control for IgM was performed using the WHO International Reference Reagent for anti-HEV IgM (95/605). Negative control for IgG was performed using the WHO International Reference Reagent for anti-HEV IgG (95/584).

b > 80 IU/l.

c RNA titres determined by real-time RT-PCR in comparison to the WHO International Standard for HEV RNA – code number 6329/10 [1].

- Viraemic titers usually ~2 to >5 log_{10} IU/ml
- However, viraemic titers may exceed 7 log_{10} IU/ml
- Only 3 of 12 viraemic donations were ALT positive
Proposal from Germany (PEI) supported by the work done by S. Baylis and co-workers

- Knowledge of the prevalence of HEV RNA in plasma pools and single donations
- HEV NAT for Human plasma (pooled and treated for virus inactivation)- Monograph 1646 and not for plasma for fractionation

Safety measure

👰 Plasma for fractionation undergo further processing including steps for efficient virus removal
👰 no inactivation/removal step for non-enveloped virus, such as HEV during the production of S/D plasma
👰 Smaller size of pools for the production of S/D plasma
• **Hepatitis E virus RNA.** The plasma pool is tested using a validated nucleic acid amplification technique (2.6.21). A positive control with $2.5 \log_{10}$ IU of hepatitis E virus RNA per millilitre and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors. The pool complies with the test if it is found non-reactive for hepatitis E virus RNA.
TIMELINE

• Jan – Mar 2013   Pharmeuropa public consultation
• Mar – May 2013   Pharmeuropa National Authority comments
      (Pharmeuropa 25.1.: http://pharmeuropa.edqm.eu, until 31/05/2013)
   ⚫ If comments in line with the proposed revision and Group 6B agrees with the comments

• Oct 2013        Discussion of comments in Group 6B
• Nov 2013        Proposal to Ph. Eur. Commission
   ⚫ If the Ph.Eur Commission approves the revision

• 1 Jul 2014      Publication
• 1 Jan 2015      Implementation

Alongside the revision of the monograph, a Biological Standardisation Programme project (BSP127) for the establishment of a HEV RNA for NAT Biological Reference Preparation will be carried out.
Many Thanks for Your Attention