NAT versus combo Ag/Ab testing performance for reducing the HCV window phase

Syria LAPERCHE, MD, PhD
Centre National de Référence des Hépatites B et C et du VIH en Transfusion
Institut National de la Transfusion Sanguine, Paris, France

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Background

- HCV Ag/Ab combos (or HCV Ag assays) an alternative for ensuring the blood safety in countries where HCV NAT cannot be introduced:

  40 to 50% of HCV-RNA pos /HCV Ab neg samples, positive with HCV combos assays

  Laperche S, et al Transfusion 2005
  Dean L, UK Health Protection Agency, Feb 2007
  Tuke et al. Transfusion 2008

  **BUT**

  - The cut off viremia level detected by combo assays in the window phase is unclear

  - Many studies have been done in genotype 1a SC panels from the US
Objectives

Through an international collaboration

- **Part 1:** Establish the **differences in the sensitivity** between HCV combo assays, HCV antigen assays, and NAT blood screening systems in the detection of viremia during the WP **according to the genotype and the VL** by testing random subsets of the observed HCV NAT yield cases from different regions of the world.

- **Part 2:** Evaluate the **analytical sensitivity** of HCV combo assays and NAT systems in dilutions of high viral load HCV NAT WP samples of genotypes: 1a, 1b, 2a, 2b, 3a and 4.
Participating laboratories

Dr E. Brojer, Dr P. Grabarczyk
Institute of Haematology and Transfusion Medicine, Warsaw, Poland

Dr El Elyabi, Dr F. Moftah
Shabrawishi Hospital, Finni Square, Dokki, Cairo, Egypt

Dr V. Kalibatas
Nacionalinis Kraujo Centras, Lithuania

M. Nubling
PEI, Langen, Germany

Dr. S. Stramer
ARC, USA

Dr J. Tanaka, Pr H. Yoshizawa
Hiroshima University, Hiroshima, Japan

H van Drimmelen
Delft Diagnostic Laboratory, Voorburg, the Netherlands

Pr MP Busch, Scientific Adviser
Blood Systems Research Institute, San Francisco, CA, USA

Dr Syria Laperche, NCR, INTS, coordination
### Part 1: Detection of early infection

Samples: HCV NAT yield donations

Minimum 2ml with known genotype and determined VL

<table>
<thead>
<tr>
<th>Country</th>
<th>N samples</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>17</td>
<td>1,3,4</td>
</tr>
<tr>
<td>France</td>
<td>5</td>
<td>1,3,4</td>
</tr>
<tr>
<td>Germany</td>
<td>26</td>
<td>1,2,3,5</td>
</tr>
<tr>
<td>Japan</td>
<td>35</td>
<td>1,2</td>
</tr>
<tr>
<td>Lithuania</td>
<td>19</td>
<td>1,3</td>
</tr>
<tr>
<td>Poland</td>
<td>70</td>
<td>1,3,4</td>
</tr>
<tr>
<td>USA</td>
<td>166</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>338</strong></td>
<td></td>
</tr>
</tbody>
</table>
Part 1: Detection of early infection

Assays

- **HCV Combos (INTS)**
  - Monolisa HCV Ag/Ab Ultra (BioRad)
  - Murex HCV Ag/Ab Combination (Abbott)

- **HCV Ag (PEI)**
  - Architect HCV Ag (Abbott)
# Part 1: Detection of early infection

## Method

**HCV Combos (INTS)**
- Monolisa HCV Ag/Ab Ultra (BioRad)
- Murex HCV Ag/Ab Combination (Abbott)

<table>
<thead>
<tr>
<th>Result</th>
<th>Combo 1</th>
<th>Single testing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td></td>
<td>0.9 &lt; S/CO &lt; 2</td>
<td><strong>Testing in duplicate</strong></td>
</tr>
<tr>
<td>&gt; or = 2</td>
<td><strong>Testing in duplicate</strong></td>
<td><strong>Testing in duplicate</strong></td>
</tr>
</tbody>
</table>

*S/CO Mean value*
Part 2: Analytical sensitivity

Samples

- **Dilution panel (DDL)**

Samples used for preparation of HCV dilution panels:

<table>
<thead>
<tr>
<th>Panel number</th>
<th>origin</th>
<th>gen</th>
<th>IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01</td>
<td>Egypt</td>
<td>1a</td>
<td>6.26E+05</td>
</tr>
<tr>
<td>P02</td>
<td>Egypt</td>
<td>1a</td>
<td>1.17E+06</td>
</tr>
<tr>
<td>P03</td>
<td>Japan</td>
<td>1b</td>
<td>6.90E+06</td>
</tr>
<tr>
<td>P04</td>
<td>Japan</td>
<td>1b</td>
<td>2.90E+07</td>
</tr>
<tr>
<td>P05</td>
<td>Japan</td>
<td>2a</td>
<td>1.70E+07</td>
</tr>
<tr>
<td>P06</td>
<td>Japan</td>
<td>2a</td>
<td>4.90E+07</td>
</tr>
<tr>
<td>P07</td>
<td>Japan</td>
<td>2b</td>
<td>7.40E+06</td>
</tr>
<tr>
<td>P08</td>
<td>Japan</td>
<td>2b</td>
<td>1.60E+06</td>
</tr>
<tr>
<td>P09</td>
<td>Lithuania</td>
<td>3a</td>
<td>3.00E+06</td>
</tr>
<tr>
<td>P10</td>
<td>Lithuania</td>
<td>3a</td>
<td>2.00E+07</td>
</tr>
<tr>
<td>P11</td>
<td>Egypt</td>
<td>4</td>
<td>1.08E+06</td>
</tr>
</tbody>
</table>

- **Panel 1**: half log dilutions from $3 \times 10^7$ to $3 \times 10^5$ IU/ml
- **Panel 2**: half log dilutions from $1 \times 10^5$ to $3 \times 10^3$ IU/ml
- **Panel 3**: half log dilutions from $1 \times 10^3$ to $10^{-1}$ IU/ml

- HCV RNA WHO, Eurohep and Hiroshima chimpanzee infectivity standards
Part 2: Analytical sensitivity
Method (1)

- **Panel 1 in duplicate**
  - Monolisa HCV Ag/Ab Ultra (BioRad) (INTS)
  - Murex HCV Ag/Ab Combination (Abbott) (INTS)
  - Architect HCV Ag (Abbott) (PEI)
  
  ➢ Cut off level of combos for each genotype

- **Panel 2 + standards in duplicate**
  - Combos (INTS)
  - Architect HCV Ag if the last dilution sample of panel 1 positive (PEI)
  - bDNA (Berkeley, USA)
  - Cobas Taq Man (Roche Molecular Systems) (INTS)

  ➢ Cut off level of combos for each genotype
cross VL calibration

- **Panel 3 + reference standards in 12 replicates**
  - Procleix Ultrio on Tigris IDT (Poland)
  - Taq Screen MPX on S201 system IDT (PEI)

  ➢ Cut off level of NAT for each genotype
The cut off crossing point of combo ELISA expressed as IU/ml or copies/ml will be determined by regression analysis after logit log transformation of the response curves. The cut off will be compared with the 95% and 50% detection limits found with NAT screening systems and will be expressed in IUs and copies/ml according to calibration in the bDNA 3.0 assays as the reference method and the Roche TaqMan assay for comparison.

Assuming that for each genotype the viral doubling time of HCV is comparable (0.45 days) the time difference between the 50% hit rate seroconversion point for Ultrio ID, Ultrio MP-NAT (1:8) and TaqScreen MP-NAT (1:6), the HCV Ag and combo ELISA cut off crossing points will be extrapolated for each genotype from the results of the dilution panels.
<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>Monolisa screening * N positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>17</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>France</td>
<td>5</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Germany</td>
<td>26</td>
<td>12 (46%)</td>
</tr>
<tr>
<td>Japan</td>
<td>35</td>
<td>22 (63%)</td>
</tr>
<tr>
<td>Lithuania</td>
<td>19</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>Poland</td>
<td>70</td>
<td>19 (27.1%)</td>
</tr>
<tr>
<td>USA</td>
<td>166</td>
<td>67 (40.4%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>338</td>
<td>130 (38.5%)</td>
</tr>
</tbody>
</table>

* Including grey zone (0.9-1), to be confirmed by repeat testing for some samples
### ARC Study (2006) of Monolisa HCV Ag/Ab Ultra (BioRad)

<table>
<thead>
<tr>
<th>HCV Sample Type</th>
<th>No. Reactive</th>
<th>Viral Loads (Copies/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 cutoff</td>
<td>0.5 cutoff</td>
</tr>
<tr>
<td>17 RNA +/Ab -</td>
<td>7 (41%)*</td>
<td>8 (47%)*</td>
</tr>
<tr>
<td>50 RNA +/Ab +</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20 RNA -/Ab -</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Evaluation of HCV NAT Positive/Anti-HCV Antibody Negative Samples - samples identified by MP-NAT in blood donors from U.S.

- **HCV Combo Assay #1** – 47 of 55 (85.5%)
- **HCV Combo Assay #2** – 19 of 55 (35%)

8 nonreactive samples
100-70,000 copies/mL;
1st reactive sample = 110,000
Research HCV Combo Assay
Correlation with HCV RNA titer

$R^2 = 0.9279$

CO = 44,000 RNA copies/mL
95% CI = 8300 - 229,000 copies/mL
1. Include additional samples to constitute an international panel of HCV NAT Yield samples

2. Establish cost effectiveness ratio of each method

3. Extend the study to HIV NAT yield samples
Sponsorship

- **Abbott** providing
  Murex HCV Ag/Ab Combination and Architect Ag assays.

- **Biorad** providing
  Monolisa HCV combo Ag/Ab Ultra assays

- **CHIRON-Novartis** providing
  Ultrio and Procleix assays
  a grant to INTS for the study
  a support for the preparation and shipment of samples