EVALUATION OF A NOVEL PLATFORM FOR DETERMINING GENOTYPES OF HCV*

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HCV Genotyping: Significance and Challenges

- Key parameter in evaluation of HCV infected persons
  - Genotype has significance in therapeutic decision-making/prognosis
  - Likely to remain of considerable utility for at least medium term
  - Confirmation of genotype typically required pre-treatment (payment)
  - Marked increase in test utilization since DAA introduced

- Balancing cost/efficiency of testing vs accuracy
  - Viral heterogeneity presents challenges in accurate identification
  - Using more conserved regions (simplicity of testing constructs)
  - Using more variable regions (accuracy of identification)
HCV GENOTYPING: HCV GENOTYPE v 2.0 (LiPA)

- Effectively the ‘gold standard’ in routine laboratory testing

- Limited throughput, several manual operations, significant liquid waste volume
- Subjective reader-dependent interpretation of ‘difficult’ patterns
- Overall success rate to subtype with unaltered interpretation guideline 93-95%
HCV Genotyping: E-SENSOR® HCV\textsubscript{G} DIRECT*

1. The target DNA is mixed with the signal probe solution. If the applicable target DNA is present, hybridization to the signal probes occurs immediately.

2. The solution is pumped through the cartridge's microfluidic chamber and the target DNA/signal probe complex completes the reaction with the pre-assembled capture probe.

3. Voltage is swept across each electrode and target DNA is analyzed by electrochemical detection.

*Manufactured by GenMark Dx, Carlsbad, CA
Clinical Performance Study

- Goal was to stringently compare performance against current standard
- Sample set consisted of de-identified clinical samples (stored at -70°C)
- All samples previously analyzed by LiPA at LabCorp CET
- Total of 437 samples included in the final dataset divided into 2 cohorts
- Cohort designation based on LiPA result (genotype, subtype, pattern)
  - LiPA definitively genotyped/subtyped (Cohort A)
  - LiPA inconclusive/incomplete (Cohort B)
Clinical Study (Cohort A)

- Samples (n=269) yielded definitive results by LiPA
- Genotypically balanced cohort (35-40 samples per category*)
- Sample set biased to include typical and atypical LiPA patterns
- NS5B sequencing only performed on discordant samples

*Only 5 samples were available that had been identified as genotype 5
Clinical Study (Cohort B)

- Samples (n=168) yielded incomplete/problematic results by LiPA
  - Indeterminate (banding pattern not consistent with recognized pattern)
  - Highly atypical banding patterns (typically called to genotype despite lacking key band(s))
  - Genotype 1 no subtype designation
  - Genotype 2 no subtype designation
  - No core bands present result in an ambiguous result (1 possible 6 or 1 possible 4)
  - Identified as a mixture of two genotypes

- 3-5% of routine clinical samples fall into this category

- NS5B sequencing performed on all samples to generate reference result
## RESULTS: COHORT A

<table>
<thead>
<tr>
<th>Resolved Type</th>
<th>n</th>
<th>Correct</th>
<th>Discordant</th>
<th>No subtype</th>
<th>No call</th>
<th>Correct</th>
<th>Discordant</th>
<th>No subtype</th>
<th>No call</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>42</td>
<td>39</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>0</td>
<td>0</td>
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<tr>
<td>1b</td>
<td>41</td>
<td>40</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2a/c</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>0</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>39</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>38</td>
<td>0</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>36</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>36</td>
<td>0</td>
<td>N/A</td>
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<tr>
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<td>5</td>
<td>5</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>34</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>31</td>
<td>0</td>
<td>N/A</td>
<td>3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>269</strong></td>
<td><strong>265</strong></td>
<td><strong>4</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
<td><strong>263</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
<td><strong>6</strong></td>
</tr>
</tbody>
</table>

- LiPA correctly called 265/269 (98.5%) to genotype/subtype
- HCVg correctly called 263/264 (99.6%) of samples to genotype/subtype; 4 no-calls

- All 4 LiPA erroneous calls were genotype 1 viruses called as genotype 5
- 2/6 HCVg no-calls were genotype 1 viruses called as 5 by LiPA
- 3/6 HCVg no-calls were genotype 6 viruses (6h, 6n, 6q)
- 1/6 HCVg no-calls was a genotype 3 virus (3a)
## RESULTS: COHORT B

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Correct</th>
<th>Discordant*</th>
<th>No subtype</th>
<th>No call</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2 (2)</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>1a</td>
<td>52</td>
<td>43</td>
<td>8 (7)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1b</td>
<td>20</td>
<td>11</td>
<td>9 (3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>2a/c</td>
<td>13</td>
<td>7</td>
<td>3 (0)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2b</td>
<td>43</td>
<td>28</td>
<td>13 (12)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>3</td>
<td>7</td>
<td>N/A</td>
<td>7</td>
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<tr>
<td>4</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>Mixed</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>168</strong></td>
<td><strong>108</strong></td>
<td><strong>43 (24)</strong></td>
<td><strong>4</strong></td>
<td><strong>11</strong></td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate discordant at the genotype level including erroneous mixed calls

- Genotype 1c (1) and 1g (1)
- Genotype 2j (1)

- HCVg correctly called **108/157 (68.8%)** of samples to subtype; **131/157 (83.4%)** to genotype; **11** no-calls
- Both assay systems overcalled ‘mixed’ genotypes (LiPA n=53; HCVg n=24; NS5B n=8 )
- HCVg markedly improved resolution of ‘problematic’ genotype 1 samples (54/74)
CONCLUSIONS

• eSensor HCVg assay accurately/efficiently determines HCV genotypes
  – Resolves majority of LiPA untypable samples (approx 70%)
  – Readily automatable, expandable system
  – Objective determination of results
  – Highly dependable instrumentation; minimal maintenance

• Experience in the laboratory since implementation
  – 99.5% samples generate a definitive result (evaluation of no-call samples underway)
  – <0.2% cartridge failure rate
  – Significant decrease in labor utilization and in time to result
......Questions???