Validation of an ELISA development kit for apolipoprotein B measurement in dried blood spots

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Ischemic Cardiovascular Disease

• Leading cause of death worldwide in both developed and developing countries
• Age-standardized death rates due to ICD have declined steeply since 1980 in developed nations, but rates in Eastern Europe, Central Asia, are increasing
• 90% of ICD cases estimated to be preventable; predicting risk of ICD development therefore critical!
• Primary cause of ICD is atherosclerosis; chronic inflammatory state
Lipoproteins & atherosclerosis
What are Lipoproteins?
Markers of Ischemic Cardiovascular Disease

- Standard markers of CVD:
  - LDL-cholesterol (LDL-C)

- Overwhelming evidence that ApoB is a superior marker of CVD risk

1 copy of ApoB per LDL molecule
Importance of a 1:1 ApoB: LDL molecule ratio

- LDL cholesterol content can vary considerably among individuals with the same LDL particle concentration.
- The higher the actual number of dense LDL particles, the greater the risk of CVD development.
- American Diabetes Association, American College of Cardiology issued a joint consensus statement in 2008 that ApoB be the final test of the effectiveness of any LDL-cholesterol-lowering treatment.
Other advantages of ApoB

- More amenable to standardization than LDL-C measurement
- ApoB standardization has been achieved by an IFCC standardization project
- Almost all ApoB is bound to lipoproteins with no measurable concentration of ApoB in aqueous medium
- Levels of ApoB are not affected by food ingestion, therefore it can be measured in the non-fasting state
Prior DBS assays for ApoB

- Vladutiu et al. 1980 - Electrodiffusion
- Dudman 1985 - Radial immunodiffusion
- Ohta et al., 1988 - ELISA
- Micic et al., 1988 - double rocket immuno-electrophoresis

Cumbersome assays, used antibodies or antisera produced in-house or that are no longer available, or both.
Mabtech ApoB ELISA development kit

- Monoclonal antibody (capture) 20/17
- Biotinylated monoclonal antibody (detection) LDL 11
- Streptavidin-horseradish peroxidase
- *Purified ApoB standard
- Assay range: 8-800 ng/mL
DBS standards & controls

• Purified apoB standard not stable when used to make DBS standards
• Liquichek Lipids Control Level 2
  – ApoA1
  – ApoB
  – HDL cholesterol
  – LDL cholesterol
  – Total cholesterol
  – Triglycerides
Eugene200 validation samples

- Recruited 208 adults from Eugene & Springfield, OR from Nov 2014 - Feb 2015
- Collected matched fingerprick DBS, venous DBS, plasma, buffy coat, & saliva samples
Selected 50/208 samples with vDBS apoB values spanning the range of observed values.

- < 1.04 g/L - low risk coronary artery disease
- 1.22 - 1.4 g/L - high risk
- > 1.4 - very high risk
Stats for ApoB DBS sandwich ELISA

• Intra-assay CV:
  – High control: 1.33%
  – Low control: 1.08%
• Inter-assay CV: 11.2%
• Analytical Sensitivity: 0.02 g/L (n = 12 plates)
No significant difference between vDBS and fDBS ApoB measurements

Deming regression: $y = 1.32x$

- No proportional difference between vDBS and fDBS

95% CI slope: 1.0 - 1.6
Significant linear relationship between fDBS ApoB values & plasma ApoB values

Deming regression: $y = 1.54x + 1.27$
No bias (-0.005) and very few outliers based on Bland-Altman analysis
Linearity

Linearity in acceptable range between 80-120% (but would ideally like between 90-110%)

<table>
<thead>
<tr>
<th>Dilution</th>
<th>vDBS (n=6)</th>
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<tbody>
<tr>
<td>1:2</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>92-109</td>
</tr>
<tr>
<td>1:4</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>98-117</td>
</tr>
<tr>
<td>1:8</td>
<td>116</td>
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<td>106-123</td>
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**Spike & Recovery**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (mg/dL)</th>
<th>Expected (mg/dL)</th>
<th>% Recovery</th>
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<tbody>
<tr>
<td>#1</td>
<td>117.61</td>
<td>113.64</td>
<td>88.0</td>
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<tr>
<td>#2</td>
<td>128.10</td>
<td>127.64</td>
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<tr>
<td>#3</td>
<td>147.9</td>
<td>141.71</td>
<td>104.4</td>
</tr>
<tr>
<td>#4</td>
<td>117.7</td>
<td>119.14</td>
<td>98.8</td>
</tr>
<tr>
<td>#5</td>
<td>102.25</td>
<td>100.6</td>
<td>101.6</td>
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DBS matrix does not appear to interfere with analyte recovery.
ApoB concentrations decrease with increasing number of freeze-thaw cycles

% Recovery relative to 1 freeze-thaw cycle

# Freeze-thaw cycles
ApoB concentrations decreased at storage temps > -20 °C
Physiological range of hematocrit had no effect on ApoB concentration

<table>
<thead>
<tr>
<th>Hematocrit %</th>
<th>% Recovery (vs. 50% hematocrit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>118%</td>
</tr>
<tr>
<td>40</td>
<td>126%</td>
</tr>
<tr>
<td>60</td>
<td>100%</td>
</tr>
<tr>
<td>80</td>
<td>112%</td>
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Limitations

• No “gold standard”
  – Obtained plasma quality controls from Northwest Lipid Metabolism And Diabetes Research Laboratories (repository for WHO/IFCC reference material for apo B)

• Assay involves numerous dilution steps
Conclusions

- DBS-based ELISA assay for ApoB appears promising for population-level research
- Simple to perform, & can stockpile reagents
- Further investigation of assay linearity
- Assess performance with reference laboratory QC standards
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