Analytical Validation of Cardiac Troponin I Assays for use in the Horse

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Outline

• Background & Objectives
• Methods & Materials
• Results
• Conclusions
• Questions?
Background & Objectives
Troponins

• Globular proteins found in striated muscle
• Regulate myosin-actin interaction in muscle contraction
• Components: I, T, C
• Sequence highly conserved in mammals
• Tissue-specific isoforms
Cardiac Troponin I (cTnI)

- Test of choice for physicians
- Analytically sensitive/specific
- Correlation with extent of injury
- Cheap, quick, easy assays
- Human tests
cTnI Assays

- Many manufacturers & designs
- Dedicated equipment required
- Standard & high-sensitivity assays
- Used in equine medicine
- One study using a fully validated assay*

*Slack et al, (2012), JVIM 26(5), 1202-8
cTnI Assays – Cont.

Why Validate?

• Epitope conservation not perfect
• Cross-reactivity is possible
• Analytic sensitivity & specificity varies
• Laboratory protocol not established
  • Sampling & quality control
Objective

1. Analytically validate commercial cTnI assays for use in the horse.
Methods & Materials
Test materials

- Clinically normal donor horse
- Serum collected under anesthesia
- Myocardium & skeletal muscle collected at postmortem
- Stored at -80°C
Protein Isolation

• Troponin complex isolated using Potter protocol*

• Modification: 200:1 dilution vs. 20:1

• Western blot analysis using anti-troponin I antibody*
  
  • Wells loaded with: murine cTnI, equine cardiac isolate, equine skeletal muscle

Validation protocol

• ASVCP guidelines

• Samples: cTnI spiked serum (dilutions)

• Parameters Assessed:
  • Linearity (reported range)
  • Limit of quantification (LoQ)
  • Comparative recovery
  • Short- & long-term precision
cTnI Analysis

• Recruited commercial laboratories
• Six commercial assays evaluated (A-F)
• Samples shipped overnight on dry ice
• Laboratories blinded to sample identity
Linearity

- Assays A-E
- 5 concentrations, 3 replicates
- Plot: Actual vs. Expected results
- Linear regression ($R^2$)
Comparison of Recovery

• Assays A-F

• 5 concentrations, 3 replicates

• High sensitivity assay A - referent

• Recovery = percentage of referent
Limit of Quantification

- Assays A & B
- Serial dilution cTnI spiked serum
- Lowest cTnI concentration with <20% coefficient of variation
Precision

- Assays A & B
- High and low concentrations
- 20 replicates
- Short-Term = 8 hour, within run
- Long-Term = 20 days
- Coefficient of Variation
## Assays used in this Study

<table>
<thead>
<tr>
<th>Assay</th>
<th>Name</th>
<th>Manufacturer</th>
<th>LoD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>ARCHITECT STAT High Sensitivity Troponin I</td>
<td>Abbott</td>
<td>0.0012</td>
</tr>
<tr>
<td>B</td>
<td>Dimensions Vista® Troponin I</td>
<td>Siemens</td>
<td>0.015</td>
</tr>
<tr>
<td>C</td>
<td>ARCHITECT STAT Troponin I</td>
<td>Abbott</td>
<td>0.009</td>
</tr>
<tr>
<td>D</td>
<td>Access AccuTnI</td>
<td>Beckman Coulter</td>
<td>0.01</td>
</tr>
<tr>
<td>E</td>
<td>Vitros ECi ES Troponin-I</td>
<td>Ortho Clinical Diagnostics</td>
<td>0.012</td>
</tr>
<tr>
<td>F</td>
<td>IMMULITE® 1000 Troponin I</td>
<td>Siemens</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* Manufacturer’s stated Limit of Detection (LoD) in µg/L from human studies.
Results
Linearity

- Assay E did not detect equine cTnl
- Assay F had low recovery
- Assays A-D: linearity:
  - $R^2$ 0.965-0.996
Linear Regressions

Assay A

Assay B

Assay C

Assay D
Comparison of Recoveries

- High Sensitivity Assay A used as referent
- 1-10 fold difference in recovery

<table>
<thead>
<tr>
<th>Assay A referent</th>
<th>Assay B</th>
<th>Assay C</th>
<th>Assay D</th>
<th>Assay F</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% (24.6*)</td>
<td>290% (71.45)</td>
<td>91.3% (22.47)</td>
<td>-</td>
<td>2.7% (0.67)</td>
</tr>
<tr>
<td>100% (17.98)</td>
<td>323% (58.05)</td>
<td>86.4% (15.54)</td>
<td>238% (42.73)</td>
<td>2.2% (0.55)</td>
</tr>
<tr>
<td>100% (11.6)</td>
<td>276% (32.04)</td>
<td>90.1% (10.45)</td>
<td>268% (31.12)</td>
<td>3.4% (0.39)</td>
</tr>
<tr>
<td>100% (4.49)</td>
<td>283% (12.69)</td>
<td>60.6% (2.72)</td>
<td>212% (9.53)</td>
<td>- (&lt;0.2)</td>
</tr>
<tr>
<td>100% (0.022)</td>
<td>68.2% (0.015)</td>
<td>0.9% (0.0002)</td>
<td>182% (0.04)</td>
<td>- (&lt;0.2)</td>
</tr>
</tbody>
</table>

*µg/L
Limit of Quantification (LoQ)

- Assay A = 0.0015 ± 0.00002 µg/L (CV = 17.6%)
- Assay B = 0.031 ± 0.002 µg/L (CV = 7.5%)
# Precision

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Assay</th>
<th>Concentration</th>
<th>Mean cTnI result*</th>
<th>CV**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-Term</td>
<td>Assay A</td>
<td>Low</td>
<td>0.0016 ± 0.2</td>
<td>0.136</td>
</tr>
<tr>
<td>(8 hours)</td>
<td>Assay A</td>
<td>High</td>
<td>2.0 ± 0.01</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>Assay B</td>
<td>Low</td>
<td>0.058 ± 0.003</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>Assay B</td>
<td>High</td>
<td>26.2 ± 0.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Long-Term</td>
<td>Assay B</td>
<td>Low</td>
<td>0.044 ± 0.009</td>
<td>0.209</td>
</tr>
<tr>
<td>(20 days)</td>
<td>Assay B</td>
<td>High</td>
<td>25.6 ± 0.32</td>
<td>0.012</td>
</tr>
</tbody>
</table>

*ng/L  
**Coefficient of Variation
Conclusions
Conclusions

• Assays A & B:
  • Performed within acceptable limits (ASVCP)
  • Suitable for use in equine medicine

• Assays C & D
  • Acceptable linearity
  • Potentially useful (with further testing)

• Assays E & F
  • Not suitable for use in equine medicine
  • Epitope conservation
Conclusions

• Human cTnI assays
  • Some clinically acceptable for equine use
  • MUST UNDERGO ANALYTICAL VALIDATION!
Acknowledgements

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  • Equine Guelph
  • Office of Research
  • Siemens Diagnostic

• Summer Students, Technicians
Questions?