Use of Gyrolab in Biotechnology Process Development

Eric Bishop
MedImmune
Gaithersburg, MD USA
Why Bother ??

- If its not broke don’t fix it. Right??
  - ELISA is widely used for process related impurity measurements
  - Accepted by all regulatory agencies

- Need for increased sample throughput and efficiency
  - More projects
  - More analytical samples submissions
  - Space and human resource limitations
Potential Solution

- Gyrolab
  - Simplified sample preparation
  - Fully automated sample processing
  - Higher throughput
  - Faster results
Objectives

- Convert a manual process related impurity ELISA to the Gyrolab format
- Optimize the assay for Gyrolab
- Perform a head-to-head comparison of the methods
- Bridging studies between the assays
- Evaluate the attributes of each format
Method Development on the Gyrolab
Assay Development on the Gyrolab

- Creation of reagents and establishment of basic conditions
- Optimizing conditions for maximum sensitivity
- Establishing positive and negative controls
- Assay Qualification

- **Total Development Time ~ 1 Week**
Reagents and Basic Conditions

ELISA

Gyrolab

Biotin

Alexa-647

Streptavidin coated support
Dynamic Range Comparison

ELISA vs. Gyrolab Standard Curve

- Gyrolab (200nL)
- ELISA

Response Units vs. ng/ml
Gyrolab Format
Assay Performance
## Accuracy and Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution Factor</th>
<th>Mean Measured Unspiked (ng/mL)</th>
<th>Mean Measured Spiked (ng/mL)</th>
<th>%Spike</th>
<th>Adj. Recovery</th>
<th>%CV</th>
<th>Concentration (ng/mL)</th>
<th>% Nominal</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 ng/mL</td>
<td>2</td>
<td>80</td>
<td>975</td>
<td>98</td>
<td>160</td>
<td>7</td>
<td>168</td>
<td>112</td>
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<tr>
<td></td>
<td>4</td>
<td>44</td>
<td>434</td>
<td>87</td>
<td>176</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&lt;LLOQ</td>
<td>224</td>
<td>90</td>
<td>&lt;LLOQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 ng/mL</td>
<td>2</td>
<td>195</td>
<td>988</td>
<td>99</td>
<td>390</td>
<td>9.1</td>
<td>417</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>115</td>
<td>428</td>
<td>86</td>
<td>460</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>50</td>
<td>240</td>
<td>96</td>
<td>400</td>
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<tr>
<td>2000 ng/mL</td>
<td>2</td>
<td>832</td>
<td>1036</td>
<td>104</td>
<td>1664</td>
<td>2.5</td>
<td>1671</td>
<td>84</td>
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<tr>
<td></td>
<td>4</td>
<td>429</td>
<td>462</td>
<td>92</td>
<td>1716</td>
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<td></td>
<td>8</td>
<td>204</td>
<td>248</td>
<td>99</td>
<td>1632</td>
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<td>8000 ng/mL</td>
<td>2</td>
<td>3370</td>
<td>881</td>
<td>88</td>
<td>6740</td>
<td>1.5</td>
<td>6671</td>
<td>83</td>
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<tr>
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<td>1638</td>
<td>427</td>
<td>85</td>
<td>6552</td>
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<td>8</td>
<td>840</td>
<td>251</td>
<td>100</td>
<td>6720</td>
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<td></td>
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<tr>
<td>20000 ng/mL</td>
<td>2</td>
<td>8618</td>
<td>1235</td>
<td>124</td>
<td>17236</td>
<td>1.1</td>
<td>17108</td>
<td>86</td>
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<tr>
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<td>4245</td>
<td>560</td>
<td>112</td>
<td>16980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2180</td>
<td>329</td>
<td>Bad Spike</td>
<td>NA</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Day-to-Day Analysis

![Bar chart showing process related impurity across Day 1 and Day 2 samples.]

- **Day 1**
  - High sample value
- **Day 2**
  - Lower sample values compared to Day 1
Run-to-Run Analysis
ELISA vs. Gyrolab
Head-to-Head Assay Comparison

- Selected 38 in-process samples for testing
- Samples run on the manual ELISA and Gyrolab

Evaluation:
- Sample Results
- Turnaround time
- Analyst hours
- Reagent Consumption
Head-to-Head Sample Testing Results
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gyrolab</th>
<th>Manual ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of testing</td>
<td>1 Day</td>
<td>8 Days*</td>
</tr>
<tr>
<td>Total Analyst Time</td>
<td>2 hrs</td>
<td>50 hrs</td>
</tr>
<tr>
<td>Coating Ab Consumption</td>
<td>20.4 uL</td>
<td>275 uL</td>
</tr>
<tr>
<td>Detection Ab Consumption</td>
<td>14 uL</td>
<td>200 uL</td>
</tr>
<tr>
<td>Antigen Consumption</td>
<td>14 uL</td>
<td>100 uL</td>
</tr>
</tbody>
</table>

* 2 analysts for 3 days, 1 analyst for 5 days
## Overall Format Comparison

<table>
<thead>
<tr>
<th></th>
<th><strong>Gyrolab</strong></th>
<th><strong>ELISA</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Labor</strong></td>
<td>Manual sample preparation; Fully automated processing</td>
<td>Totally manual</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>1 hr. Sample prep + 50 minutes/CD (automated)</td>
<td>Overnight coating + 1 hr sample prep + 3 - 5 hours manual processing</td>
</tr>
<tr>
<td><strong>Dynamic range of the Standard Curve</strong></td>
<td>4 logs</td>
<td>2 logs</td>
</tr>
<tr>
<td><strong>Required Dilutions</strong></td>
<td>All samples diluted 1:2</td>
<td>Various dilutions based on process step</td>
</tr>
<tr>
<td><strong>Sample and Reagent Volumes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coating Antibody</td>
<td>4.1 μL / CD</td>
<td>28 μL / plate</td>
</tr>
<tr>
<td>Antigen</td>
<td>2.8 μL / CD</td>
<td>10 μL / plate</td>
</tr>
<tr>
<td>Detection Ab</td>
<td>2.8 μL / CD</td>
<td>20 μL / plate</td>
</tr>
<tr>
<td>Sample</td>
<td>100 μL</td>
<td>10 μL - 2 mL</td>
</tr>
<tr>
<td>Sample Throughput</td>
<td>78 samples/day</td>
<td>12 samples/day</td>
</tr>
<tr>
<td>Rate of Success on First Run</td>
<td>~ 90%</td>
<td>~ 50%</td>
</tr>
</tbody>
</table>
Pros and Cons of Assay Formats

- ELISA

  - Pros
    - Currently accepted standard for process related impurities
    - Common technology
    - Minimal equipment requirements

  - Cons
    - Manual processing
    - High failure rate on first run
    - High reagent consumption
    - Multiple analysts required
    - Low throughput
Pros and Cons of Assay Formats (cont’d)

- **Gyrolab**
  - **Pros**
    - Automated sample processing
    - High throughput
    - Low reagent consumption
    - Fewer analysts required
    - High rate of success
  
  - **Cons**
    - Newer technology
    - Large upfront investment
    - Initial analyst training
    - Must purchase diluents and consumables
Conclusions

- Gyrolab offers an effective way to increase efficiency in process residual testing.
  - Benefits were realized in:
    - Increased sample throughput
    - Decreased sample repeat rates
    - Decreased reagent consumption
    - Increased analyst productivity

- Gyrolab has been implemented as the standard assay format for process related impurity testing
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Questions ??