Use of Gyrolab in Biotechnology Process Development

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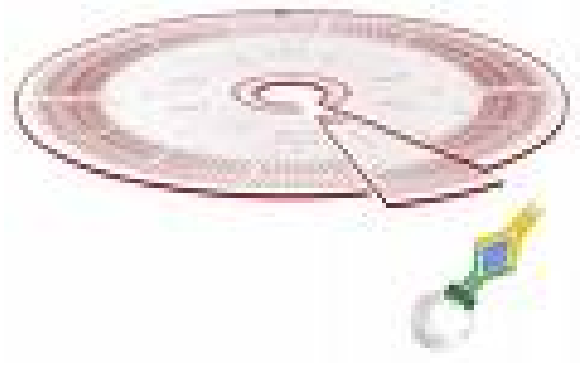
Why Bother ??

- 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

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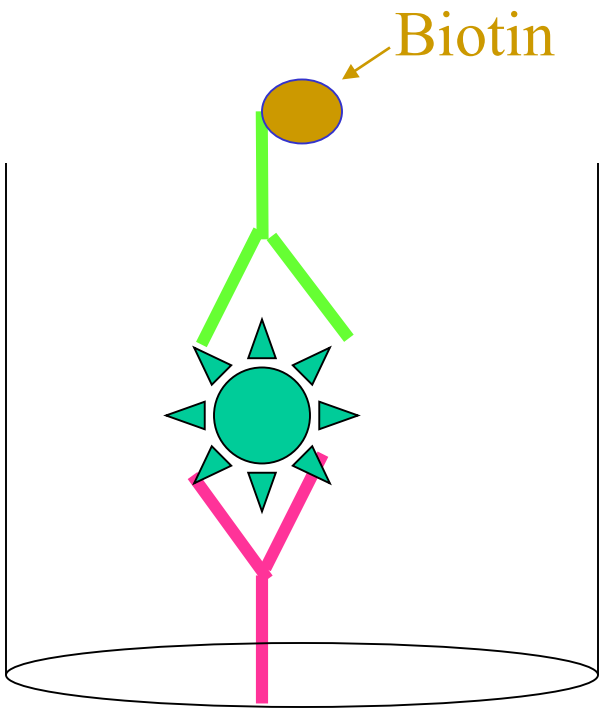
Method Development on the Gyrolab

Assay Development on the Gyrolab

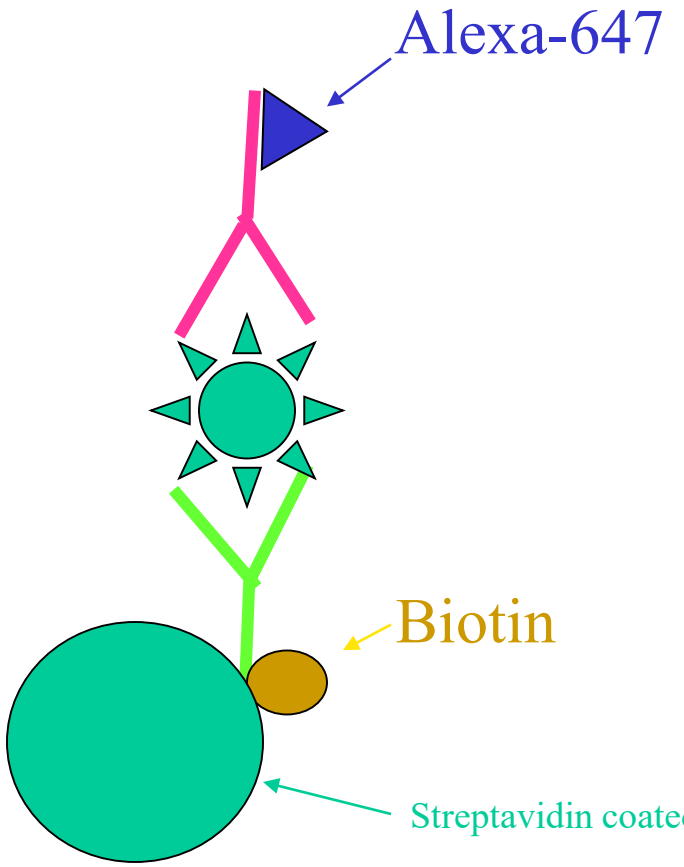
- Creation of reagents and establishment of basic conditions
- Optimizing conditions for maximum sensitivity
- Establishing positive and negative controls
- Assay Qualification
- **Total Development Time ~ 1 Week**

Reagents and Basic Conditions

ELISA



Gyrolab



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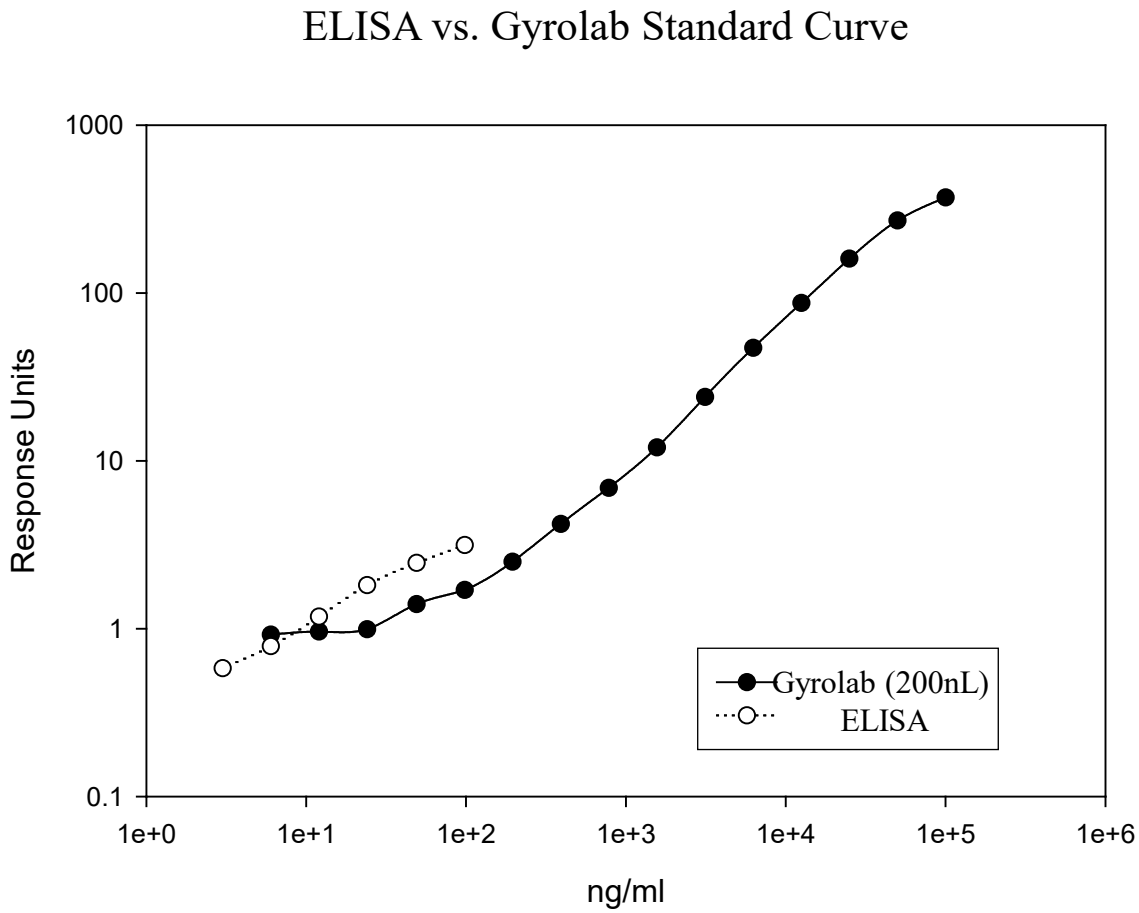
Dynamic Range Comparison



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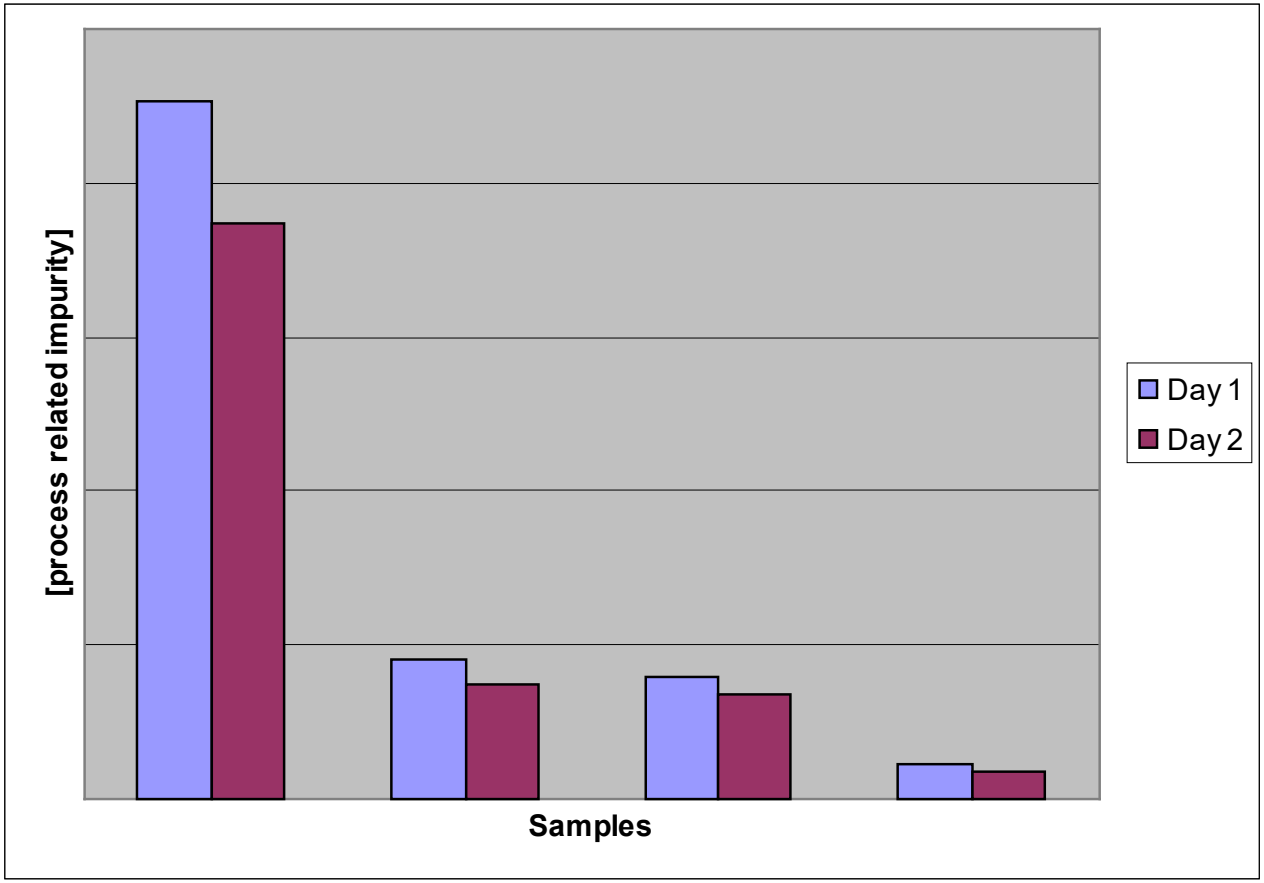
Gyrolab Format Assay Performance

Accuracy and Precision

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

Day-to-Day Analysis



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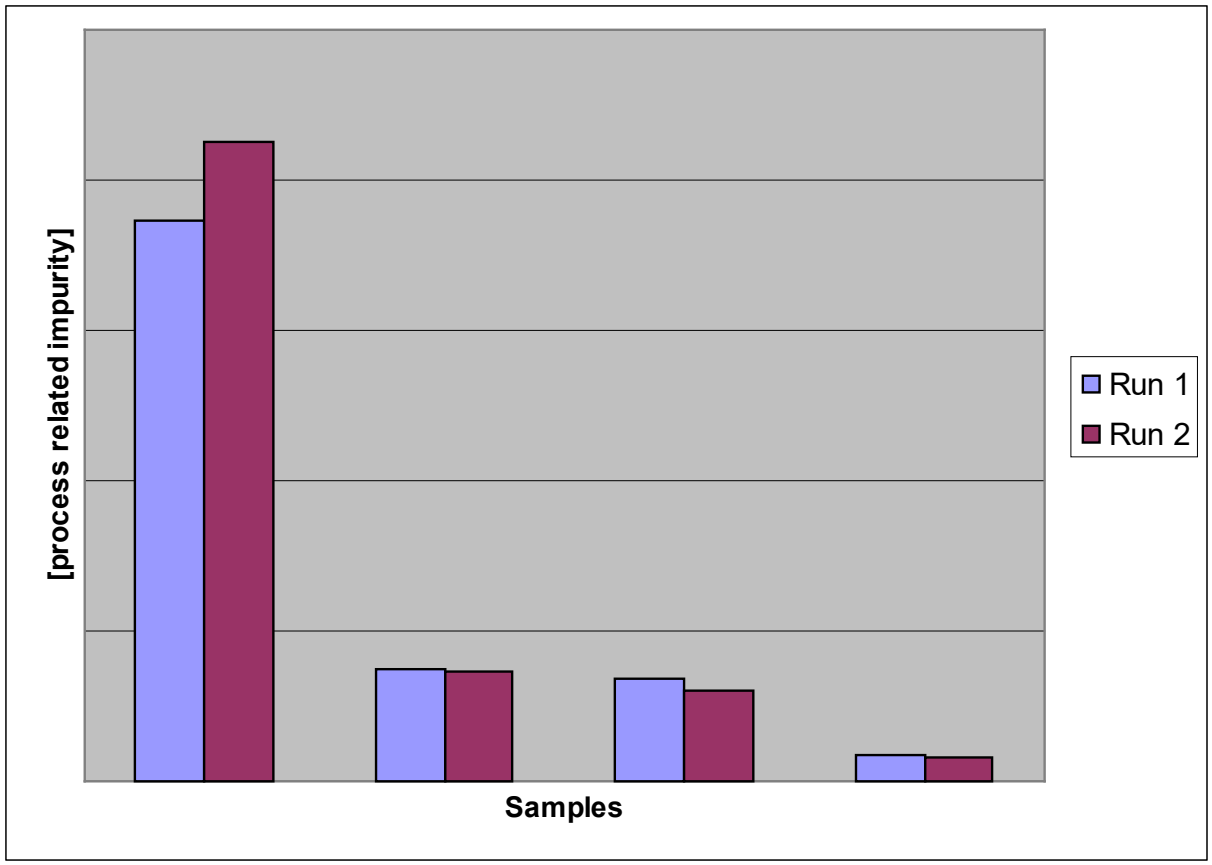
Run-to-Run Analysis



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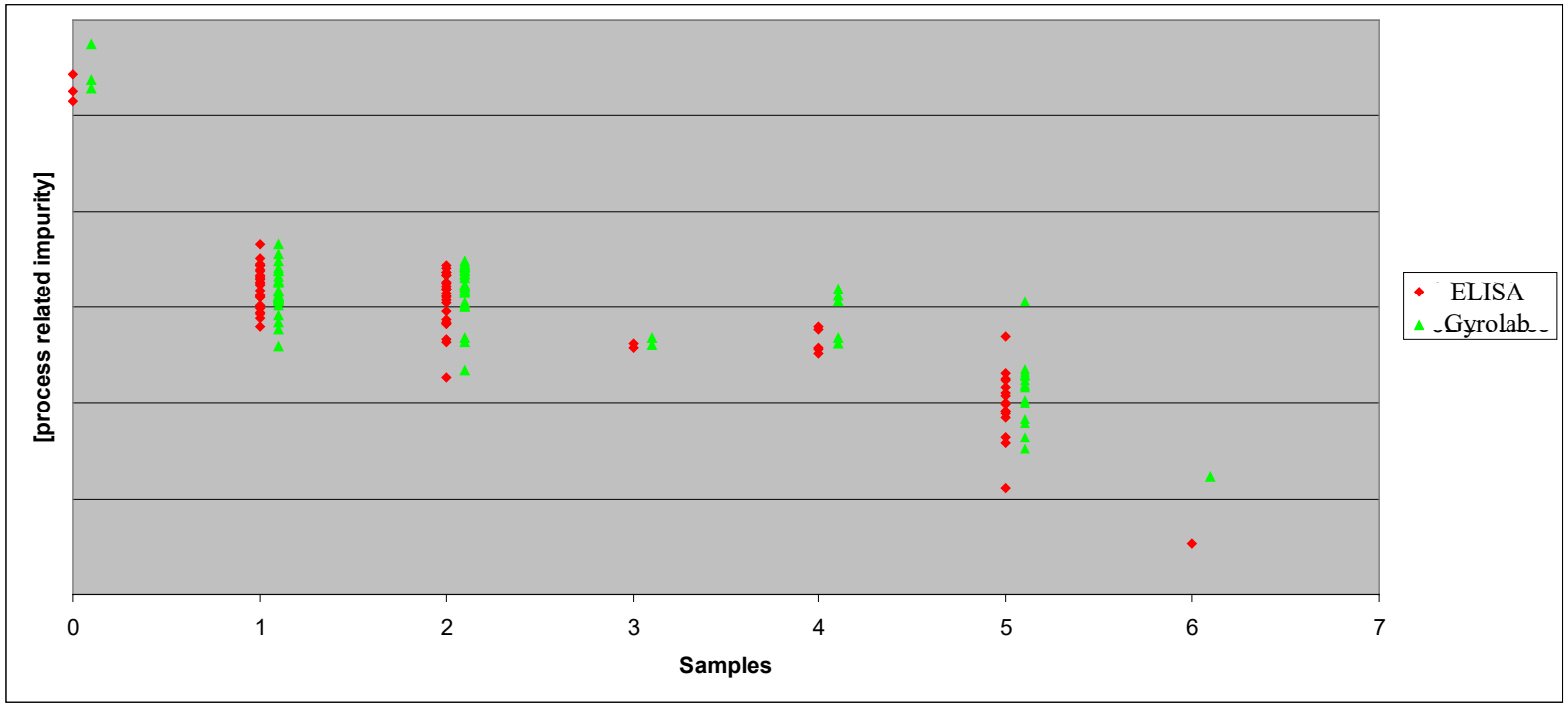
ELISA vs. Gyrolab

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



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Comparison (cont'd)

Parameter	Gyrolab	Manual ELISA
Days of testing	1 Day	8 Days*
Total Analyst Time	2 hrs	50 hrs
Coating Ab Consumption	20.4 uL	275 uL
Detection Ab Consumption	14 uL	200 uL
Antigen Consumption	14 uL	100 uL

* 2 analysts for 3 days, 1 analyst for 5 days

Overall Format Comparison

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

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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

Acknowledgements

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Questions ??