

Strategy, Struggles, and Success of Monitoring Process Related Impurities with Automation

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Caliper Users Group - 17Jun09

Wyeth
BioPharma

FDA Guidance for Industry Q6B: Specifications

2.3.1

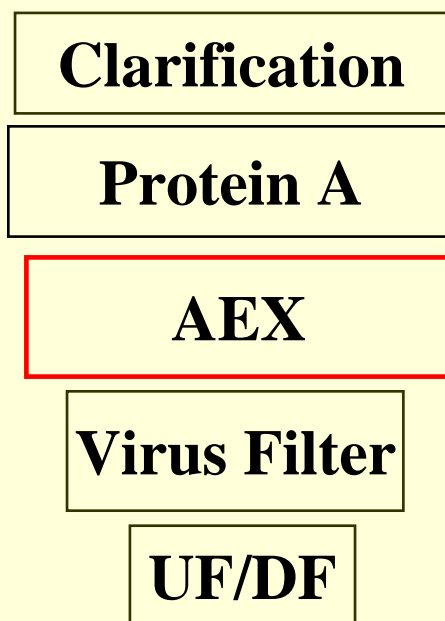
Adequate design of a process and knowledge of its capability are part of the strategy used to develop **a manufacturing process that is controlled and reproducible**, yielding a drug substance or drug product that meets specifications. In this respect, limits are justified based on critical information gained from the entire process spanning the period from early development through commercial scale production.

4.1.3

Process-related impurities (section II.A.4) in the drug substance may include cell culture media, host cell proteins, DNA, monoclonal antibodies or chromatographic media used in purification, solvents, and buffer components. **These impurities should be minimized by the use of appropriate, well controlled manufacturing processes.**

Wyeth mAb Platform Purification Process

Two Column Platform



Typical Purity Targets

Protein A

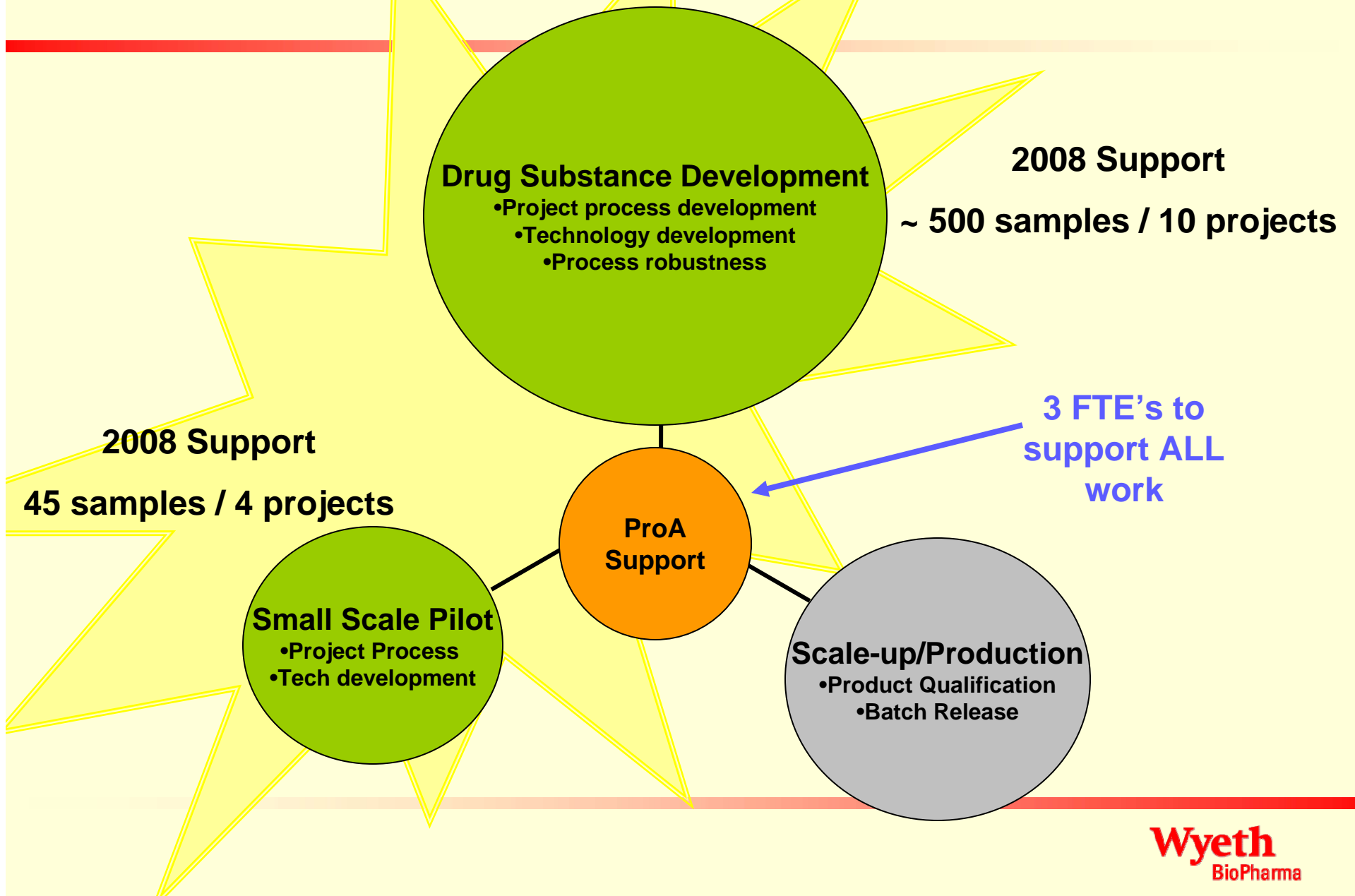
HCP

DNA

MuLV

HMW

Breakdown of Protein A Impurity Support



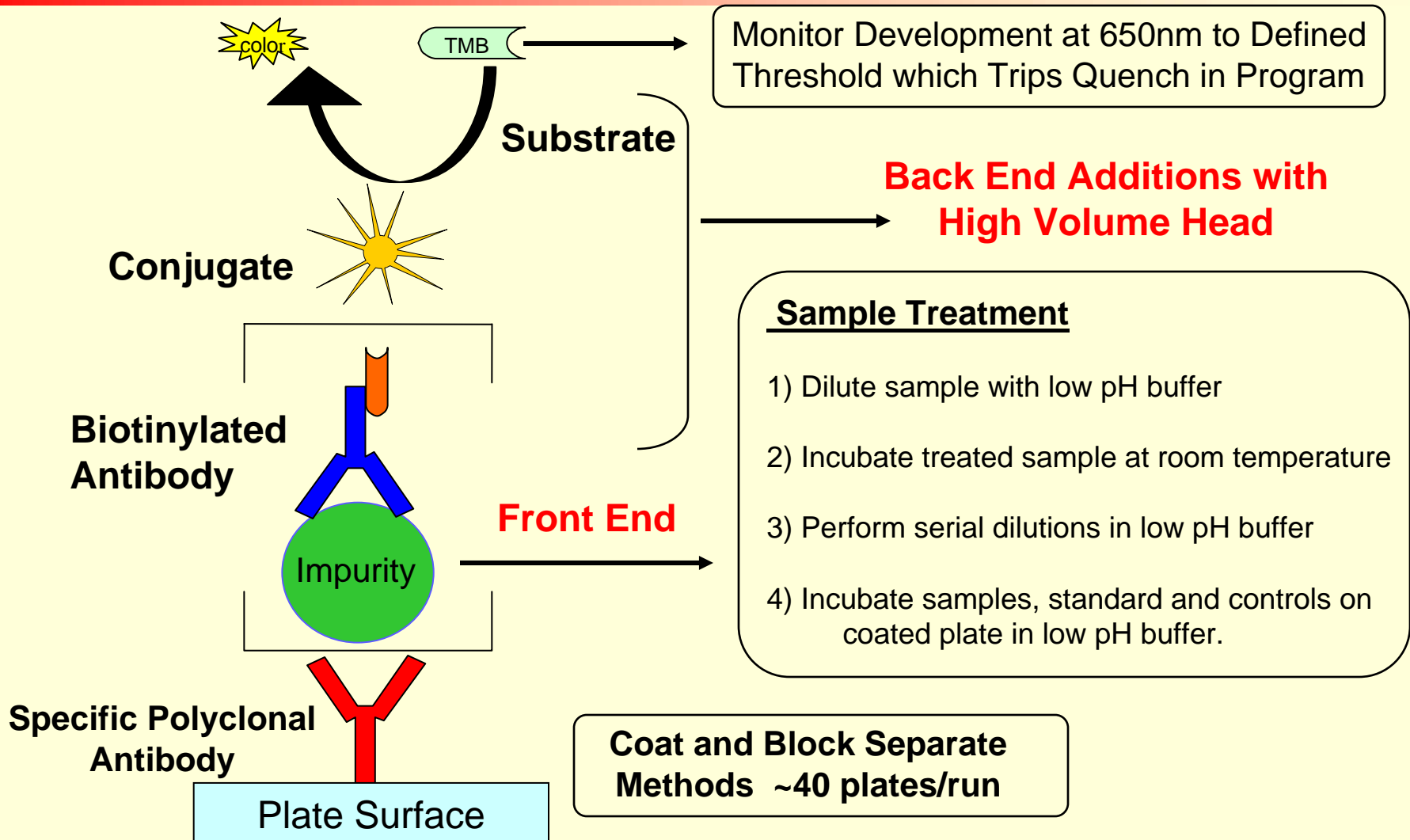
Why Caliper?

- **Space (4'x6' vs 10' linear bench space)**
 - ▶ Utilizes vertical space
- **Liquid handler has features not offered by other companies**
 - ▶ Three ways of dispensing liquid to plates for flexibility
 - ▶ Gripper for moving things on deck or handling lids
- **Integration of equipment is completely independent of liquid handling (deck space is not used)**

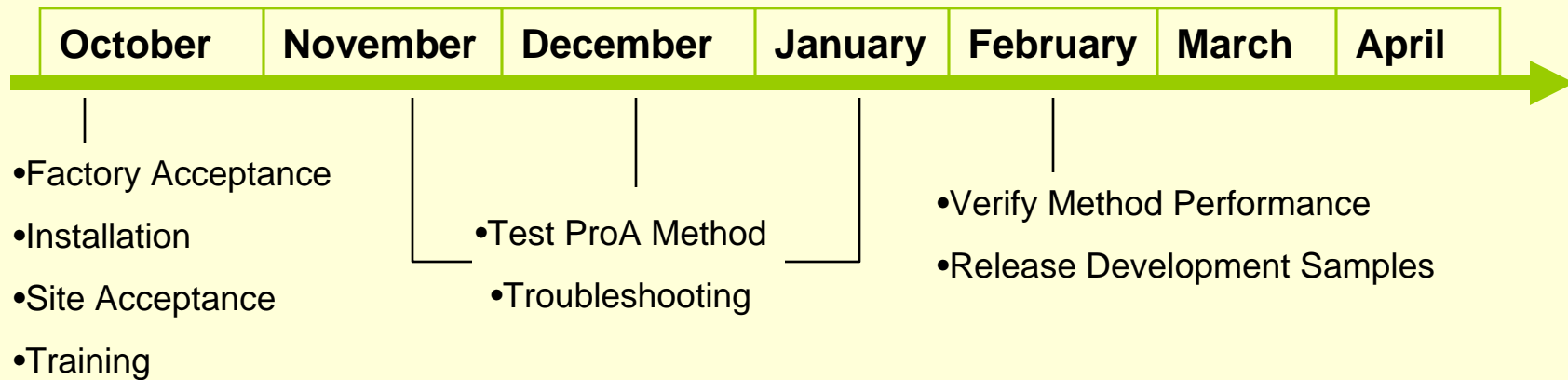


- **Customization and robot-centric strategy allows for flexibility in the future**
- **Local company allows for fast response and easy training**
- **Formal training and customized written method part of the package**

Impurity ELISA Adapted to Automation



Expectations



- **Minor optimization of supplied method**
- **Time to learn error handling**
- **Test the system to verify performance**

GOAL – Release Development Samples 1Q'09

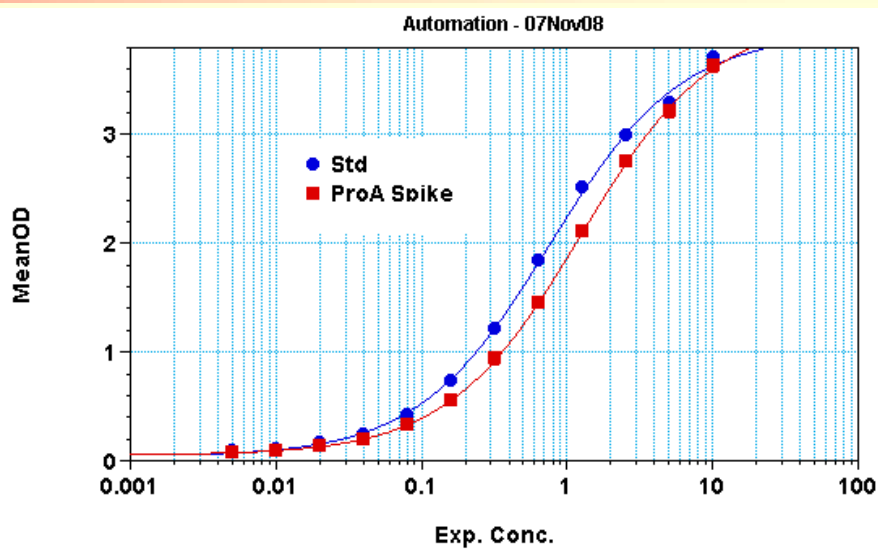
Initial Automation Assessment

Pros

- Produced very representative standard curves
- Precision of impurity spikes (n=6) was acceptable

Cons

- Impurity spikes read off standard curve show very low recovery
- Issue with over development of HRP/TMB reaction



Expected	Observed	CV%	% of Expected
10.24	10.369	26.1	101.3
5.12	3.601	11.1	70.3
2.56	1.82	4.6	71.1
1.28	0.868	3.2	67.8
0.64	0.419	4.8	65.4
0.32	0.216	8	67.5
0.16	0.106	6.1	66.3
0.08	0.054	8.8	67
0.04	0.027	11.7	68.2
0.02	0.015	7.4	76.6
0.01	0.009	11.7	91
0.01	0.007	11.6	133.8

Troubleshooting and Optimization

It HAS to be poor mixing!

Why are those plates over-developing?

Is it the new plate washer?

Maybe the light-sensitive reagents are exposed for too long?

Look at that assay buffer sheeting off the outside of those tips!



Accuracy and Precision of Z8 in Assay Buffer using 100ul Dispense

CV of Replicates	Mean	Replicates					% of Manual
		1	2	3	4	5	
0.5%	0.340	0.3406	0.3375	0.3395	0.3386	0.3416	99%
0.5%	0.335	0.3379	0.3345	0.3337	0.3346	0.3349	97%
1.0%	0.339	0.3416	0.3393	0.3363	0.3436	0.3358	98%
1.1%	0.341	0.338	0.3456	0.3397	0.3372	0.3449	99%
0.9%	0.335	0.3348	0.3398	0.334	0.3313	0.3347	97%
0.5%	0.337	0.335	0.3358	0.3367	0.3394	0.3372	98%
0.1%	0.335	0.3354	0.3352	0.3343	0.3348	0.3349	97%
0.5%	0.338	0.3394	0.3392	0.3369	0.3363	0.3401	98%
	Mean	0.3378	0.3384	0.3364	0.3370	0.3380	
	CV between Cannulas	0.8%	1.0%	0.7%	1.1%	1.1%	

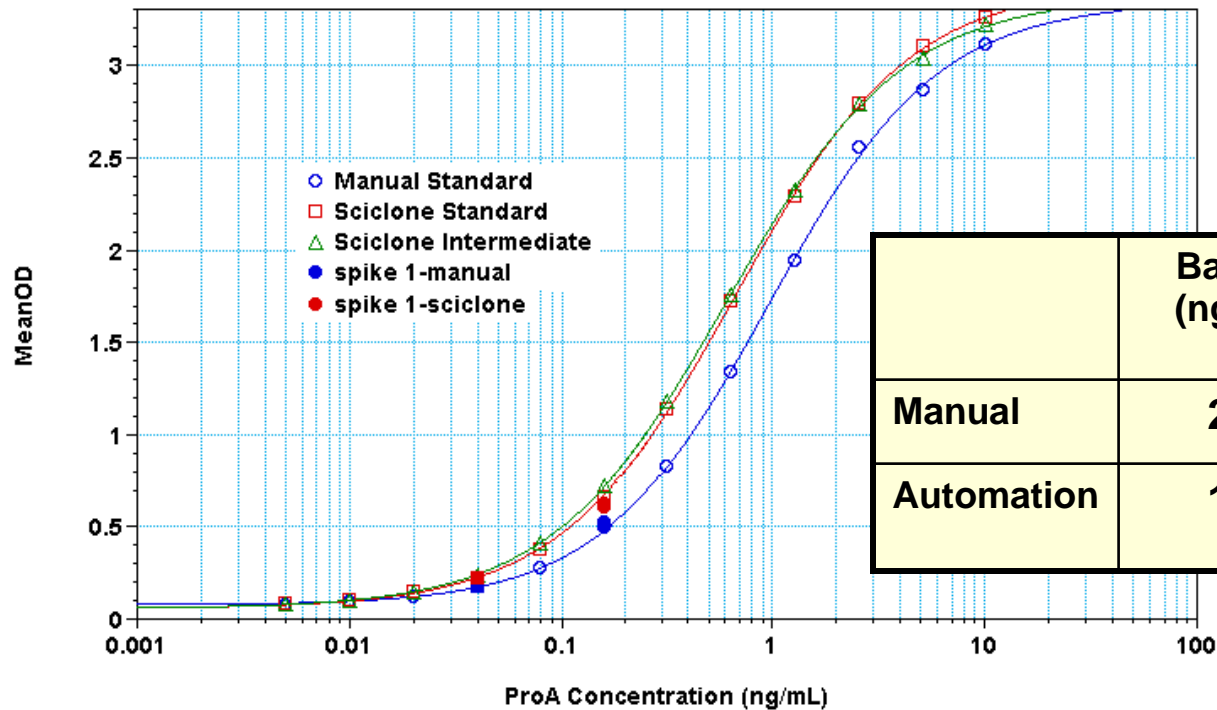
Overall CV for all Z8
0.3375
0.003
1%

Default Liquid Class

Excellent Precision at 100 ul

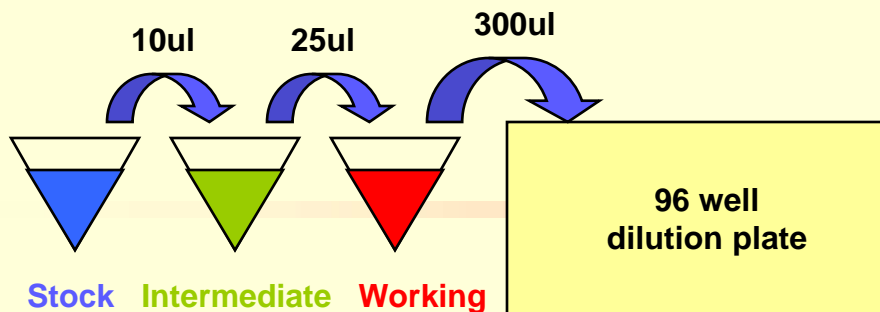
Front End Breakdown and Investigation

Plate Run Manually Using Standards Prepared by Automation



mAb in-Process Samples

	Batch 1 (ng/mL)	Batch 2 (ng/mL)
Manual	216	450
Automation	149	336

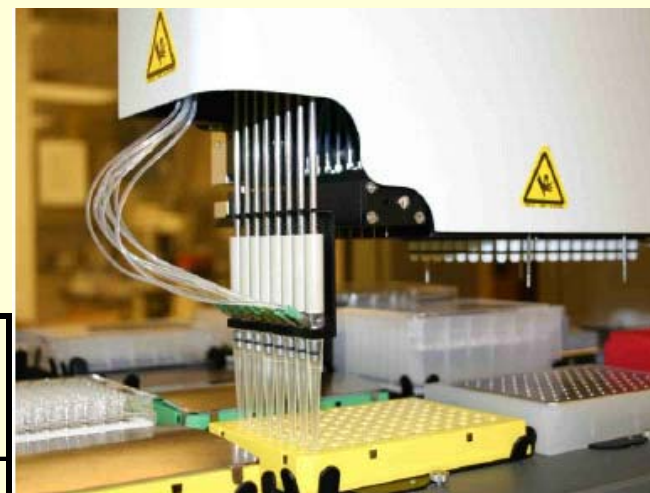


Everything is ~30% off Accuracy of Z8???

Gravimetric Measurements

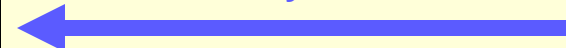
Issues with Gravimetric Measurements

- Can not test low volumes
- Can not assess all 8 channels simultaneously
- Extremely tedious to check all 8 channels
- Error introduced from balance

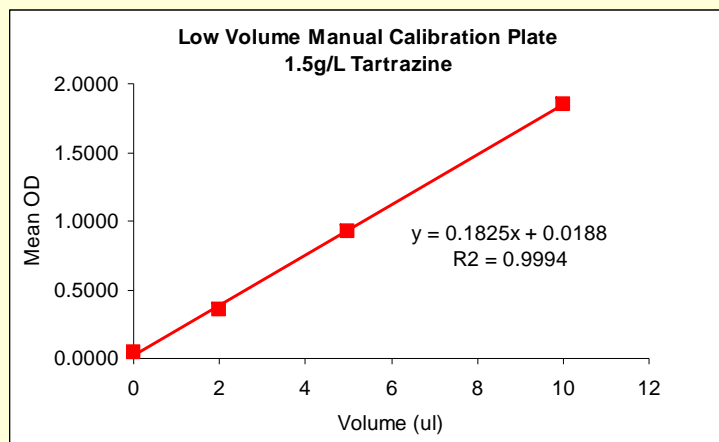


Gravimetric Accuracy of Channel 1- Z8				
	volume measured			
	25	50	100	200
1	0.0171	0.0414	0.1021	0.1935
2	0.0196	0.0441	0.0949	0.194
3	0.0208	0.0444	0.0952	0.194
4	0.0205	0.0444	0.0951	0.1938
5	0.0207	0.045	0.0954	0.1935
Mean	0.01974	0.04386	0.09654	0.19376
density	0.9968			
Vf	19.8	44.0	96.8	194
% Recovery	79%	88%	97%	97%

Poor Accuracy at Low Volumes

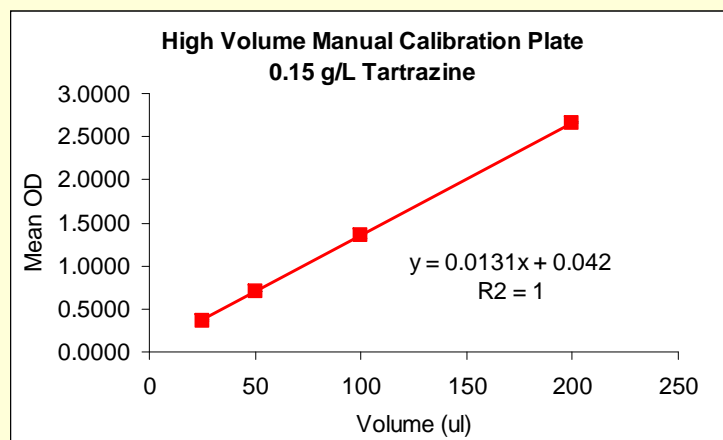


Calibration with Tartrazine



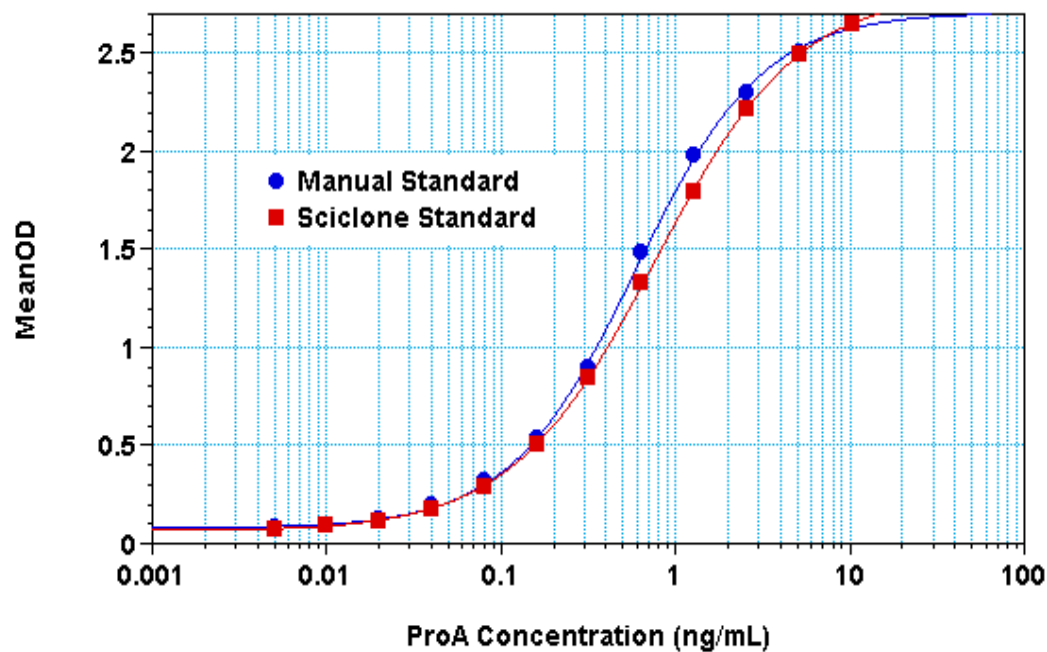
Calibration Point	Mean	SD	CV	Observed
2ul	0.3180	0.0158	5.0%	1.6
5ul	0.8396	0.0306	3.6%	4.5
10ul	1.7257	0.0199	1.2%	9.4
25ul	0.3502	0.0033	0.9%	23.5
50ul	0.6742	0.0045	0.7%	48.2
100ul	1.3225	0.0105	0.8%	97.7

New Default Liquid Class Created with Observed Values



CORRECTED	Mean	SD	CV	Observed
2ul	0.4020	0.0183	4.5%	2.1
5ul	0.9562	0.0289	3.0%	5.1
10ul	1.8765	0.0388	2.1%	10.2

Confirmation of New Liquid Class



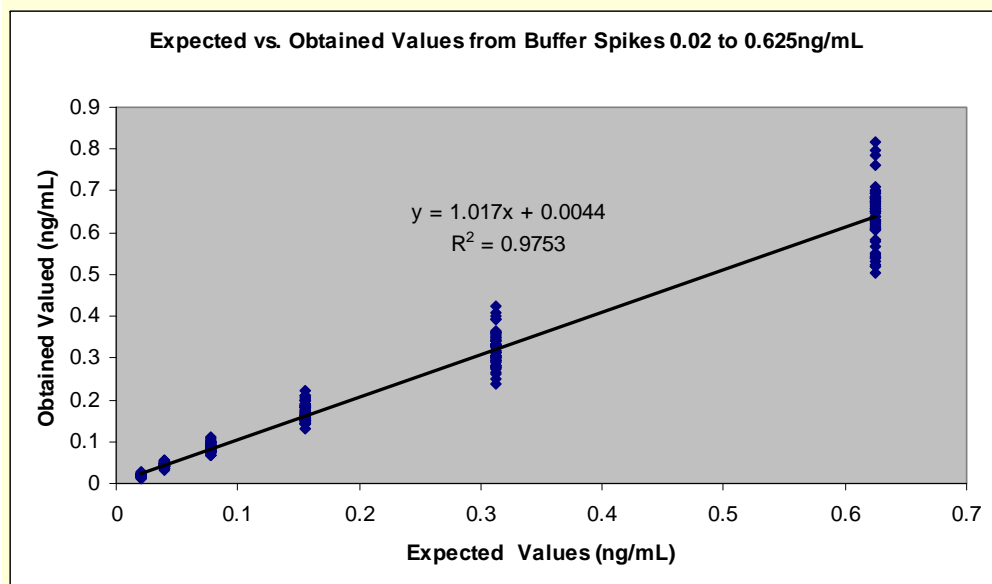
Verify Method by Comparing to Manual ELISA Data

12 plates run over 3 days with Standard, Impurity Spikes, and In-process Sample

- **Linearity and Range**
- **Accuracy of Buffer Spike Recovery**
- **Precision of Sample Results**

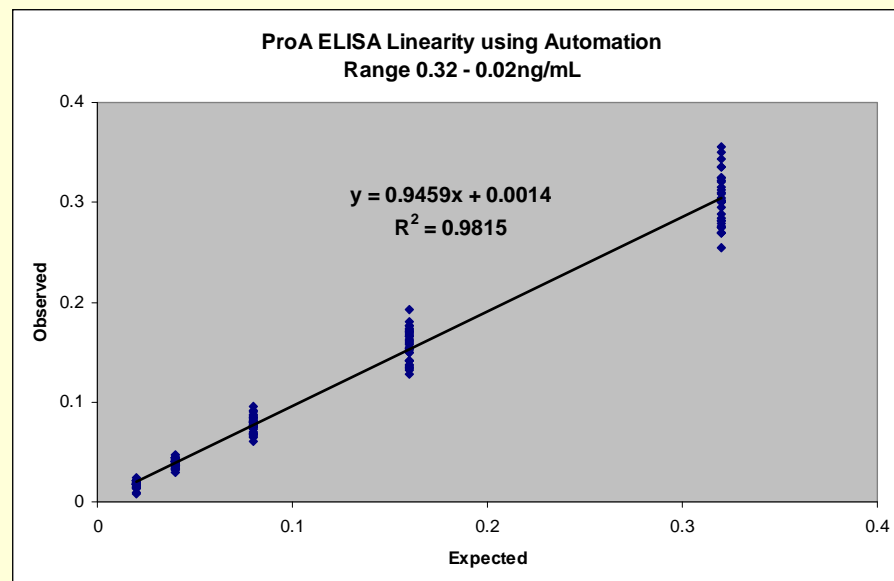
Linearity and Range (non-product)

Manual Data



Expected Results (ng/mL)	Mean	Std Dev	CV	Mean Recovery
0.625	0.639	0.070	11.0%	102%
0.313	0.322	0.040	12.4%	103%
0.156	0.169	0.022	13.0%	108%
0.078	0.086	0.010	11.6%	110%
0.039	0.043	0.005	11.6%	111%
0.020	0.019	0.003	15.8%	96%

Data from Automation



Expected Results (ng/mL)	Mean	CV	Mean Recovery
0.625	0.585	8%	91%
0.313	0.302	9%	94%
0.156	0.157	10%	98%
0.078	0.078	10%	97%
0.039	0.039	10%	98%
0.020	0.019	13%	93%

Precision of Sample

In-Process Sample Results

Frozen in single-use vials for control

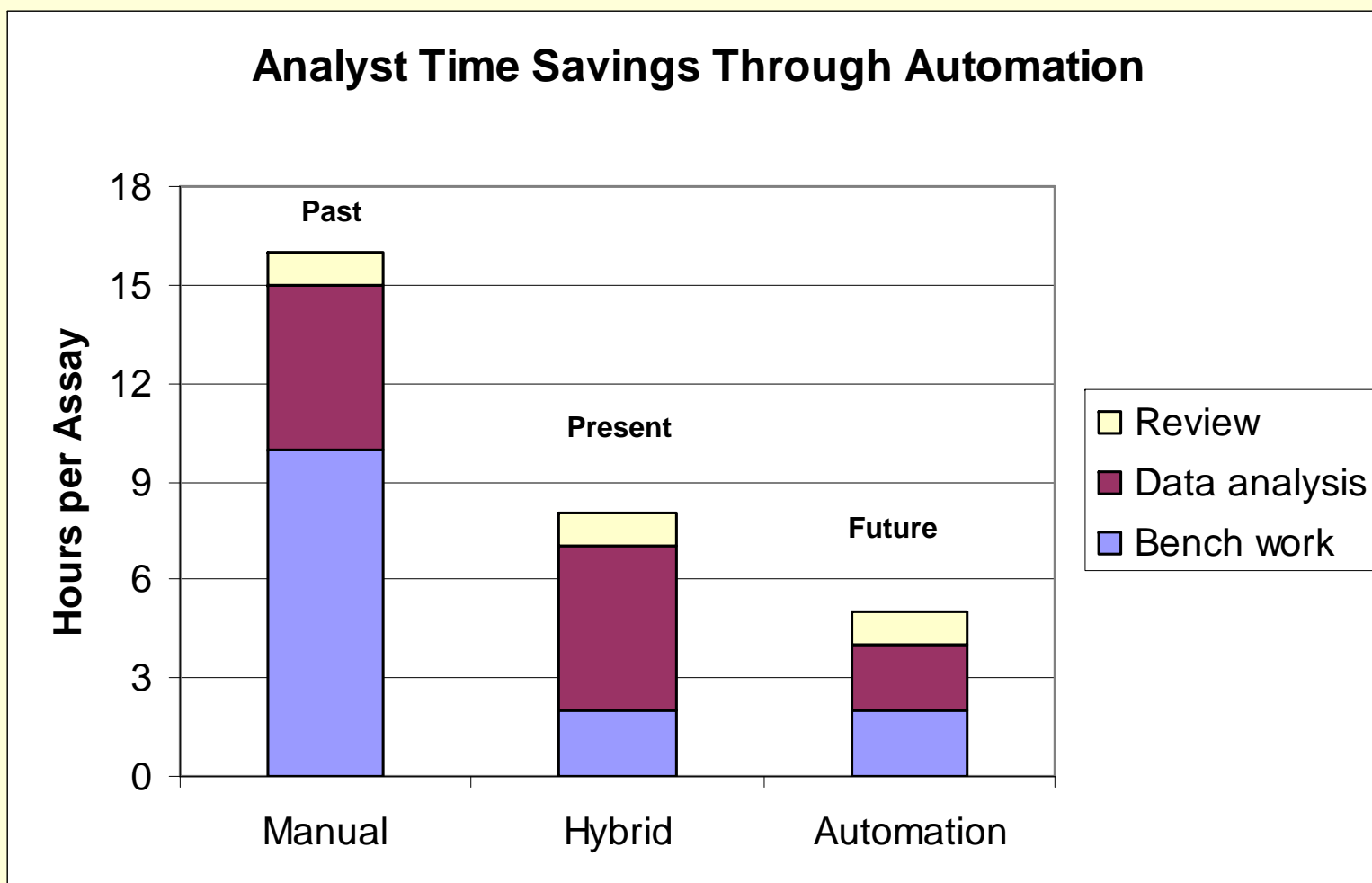
Assay Reference Number	ProA Peak Sample (ng/mL)		
	1	2	3
ELISA090401-1	356	367	356
ELISA090401-2	282	249	283
ELISA090401-3	307	313	323
ELISA090401-4	326	285	305
ELISA090402-1	392	369	371
ELISA090402-2	333	282	294
ELISA090402-3	288	274	290
08Apr09-1	304	285	296
08Apr09-2	339	321	303
ELISA090409-1	306	301	344
ELISA090409-2	299	282	273
		312	
		33.9	
		11%	

Manual	ng/mL
41874-41-1	378
41568-28	335
41608-94-1	296
41608-94-1	302
41608-94-2	288
41608-94-2	273
41608-94-3	326
	314
	35.4
	11%

Mission Accomplished

**Analyzed First Samples by
Automation on April 17, 2009!**

Sample Release and Improved Efficiency



Summary and Lessons Learned

- **Increased through-put and gained analyst opportunity through automation of impurity ELISA**
 - ▶ Up-front collaboration effort on method paid off for timeline and customer confidence
 - ▶ One person assigned to effort without other distractions
- **Verified the method performed by automation gives similar results to manual**
- **Calibration of default settings with water should be checked on installation for customer confidence**
- **Calibration should be covered in more detail during training**
- **Consider automated data analysis integration for high-throughput**

Acknowledgements

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Additional Slides

Wyeth
BioPharma

Precision Specification for Z8

Sciclone Z-8 (0.5 - 200 μ l)

Z-8 Volume Range:

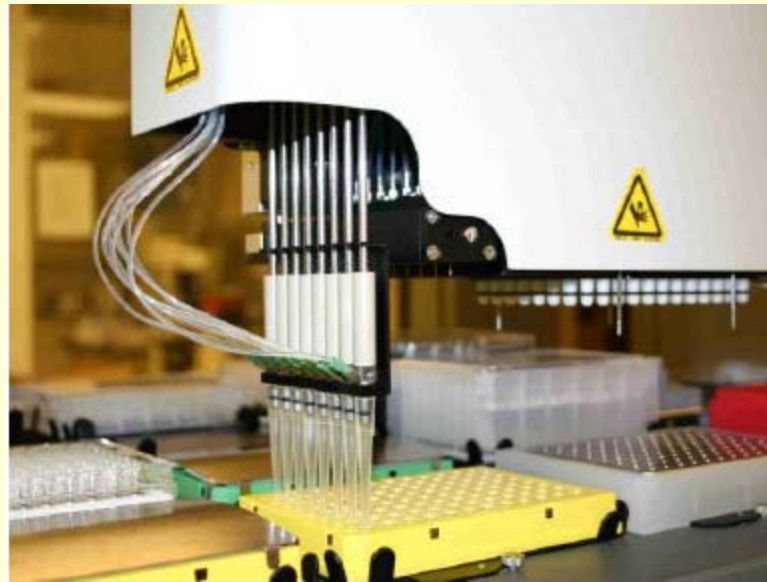
0.5 μ l - 200 μ l

Z-8 Precision:

Disposable tip:

0.5 μ l – 5 μ l <5%CV

5.0 μ l – 200 μ l <3%CV



Wyeth Z8 is fitted with the 250ul syringes however, the factory QC of Z8 was done with the 100ul syringe set.

Default settings in water

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R2 in Action

