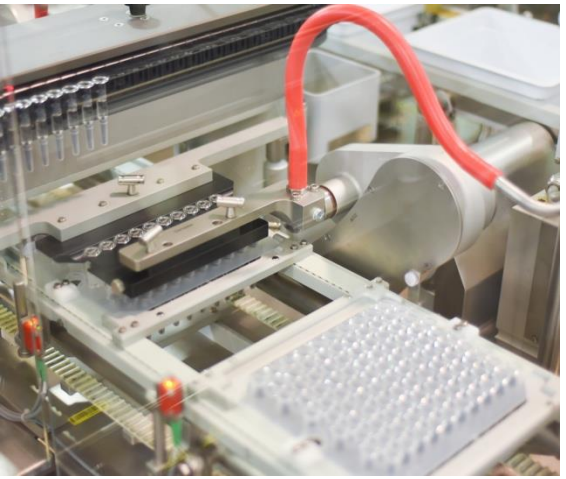
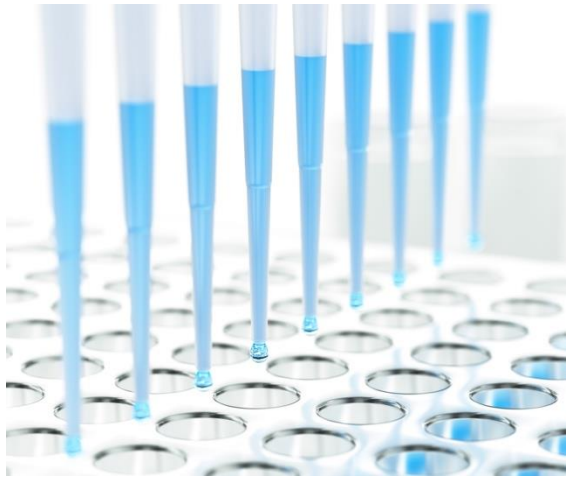




Catalent®
BIOLOGICS

Automating Cell-Based Assays: Reducing Variability & Time to Results

LUKE MERCER
MANAGER, BIOASSAYS

more products. better treatments. reliably supplied.™

AGENDA

1. **Introduction To Catalent**
2. Cell-Based Assays Variability and Automation
3. HEK293 Potency Assay
4. Conclusion

INTEGRATED & STANDALONE CDMO SOLUTIONS TO ACCELERATE YOUR BIOLOGIC TO MARKET

CELL LINE DEVELOPMENT

CLINICAL SUPPLY SERVICES & SOLUTIONS
FORMULATION & PROCESS DEVELOPMENT
EXPANDED ACCESS/COMMERCIAL LAUNCH

DRUG SUBSTANCE & DRUG PRODUCT MANUFACTURING
GLOBAL COMPARATOR SOURCING

INTEGRATED & STANDALONE ANALYICAL SERVICES

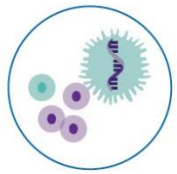
PRODUCT OPTIMIZATION
SCALE-UP
PROCESS VALIDATION
TECH TRANSFER

LINE EXTENSIONS
LIFECYCLE MANAGEMENT
BACK-UP SUPPLY


ANTIBODIES


RECOMBINANT PROTEINS


PLASMIDS


CELL & GENE THERAPIES


MRNAS

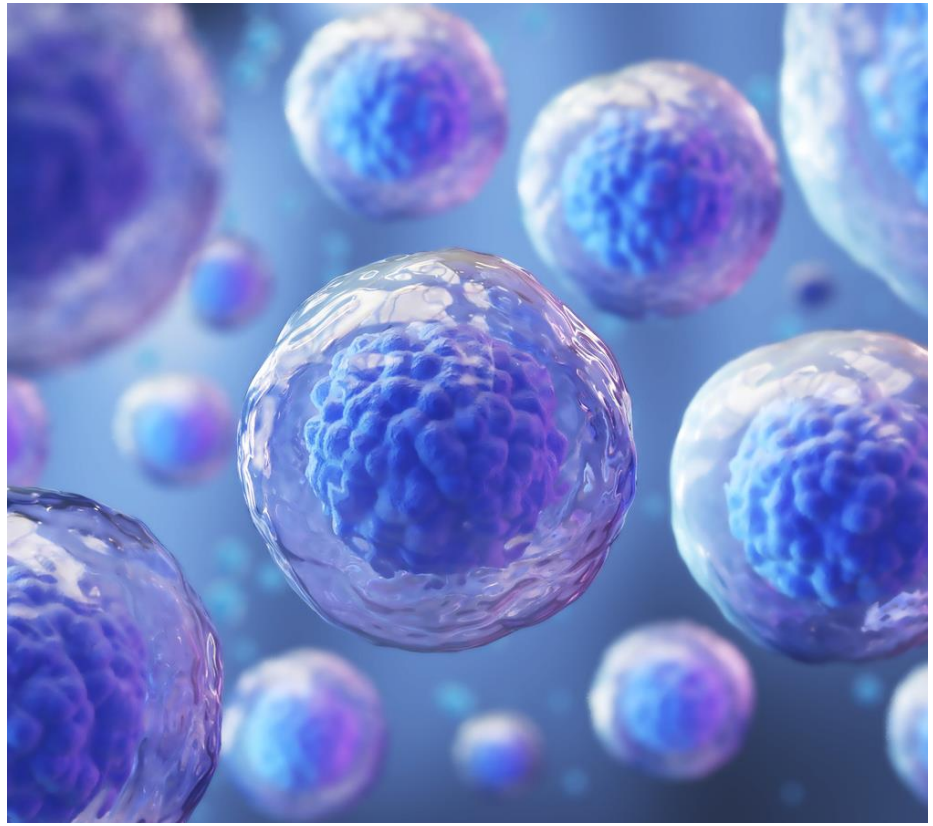
Biologics Analytical Services
Single Source for Integrated & Standalone Capabilities

GMP Analytical Services
- Method development, transfer & optimization
- Phase-appropriate validation
- In-process, release & stability testing
- Binding & cell-based assays
- Extractables & leachables

GLP Support for Clinical Studies

Experience with broad classes of large molecules:

- Monoclonal, polyclonal & bispecific antibodies
- Bioconjugates & ADCs
- Oligonucleotides
- Recombinant proteins
- Fusion proteins
- Pegylated peptides
- Cell and gene therapies
- Aptamers
- Vaccines
- Oligosaccharides



225+ scientists across the sites

100,000+ ft^3 of stability chambers

800+ assays/ techniques offered

300+ client programs supported

AGENDA

1. Introduction To Catalent
2. **Cell-Based Assays Variability and Automation**
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Cell-Based Potency Assays

Critical to show biological activity

High potential for variability

Time consuming setup and low sample throughput

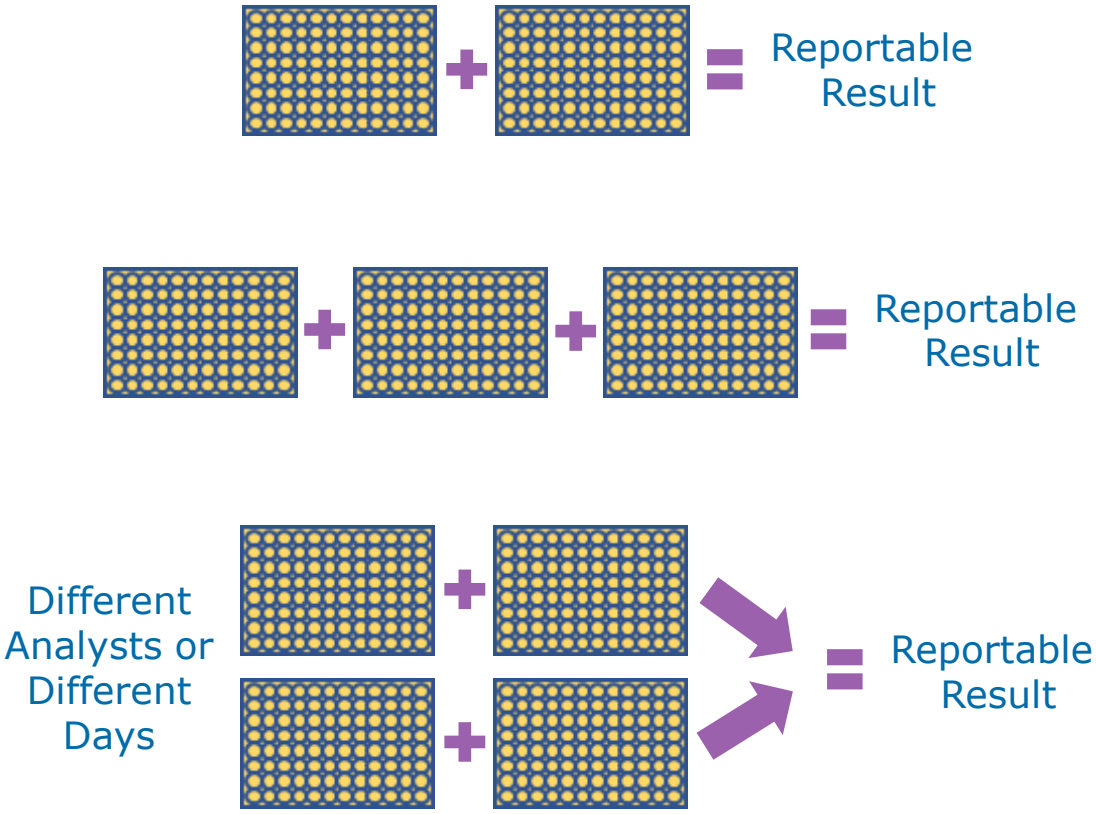
Cell-Based Potency Assays

Biological systems can result in significant variability

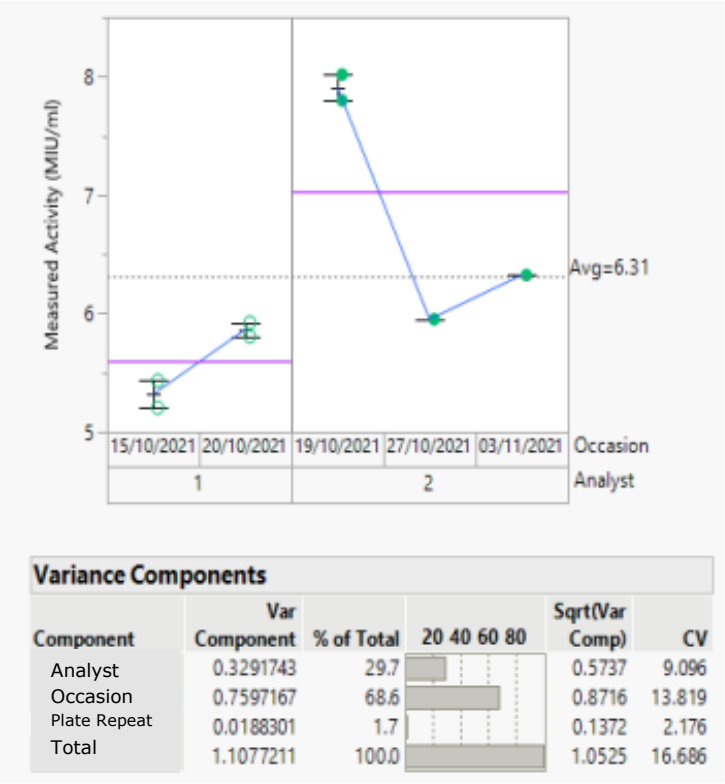
Ways to manage variability

- Properly written method
- Analyst training
- Tight control of cells
- Replication of reference and sample
- Plate replication
- Automation

Plate Replication Examples



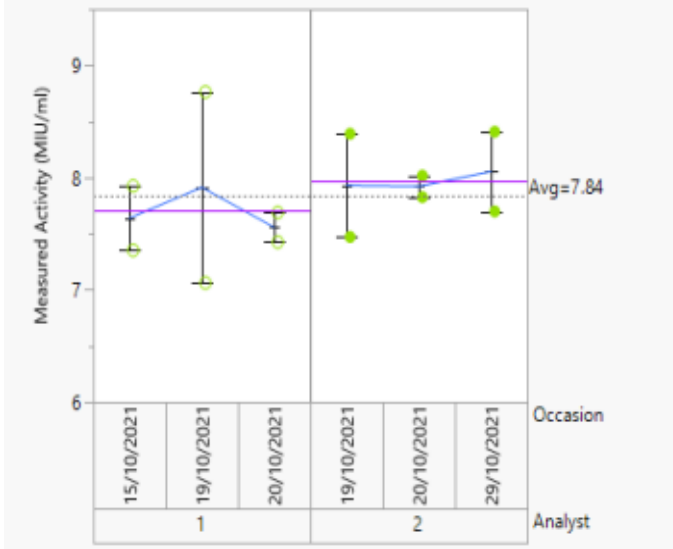
Examples of Variability in Bioassays



Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)	CV
Analyst	0.3291743	29.7		0.5737	9.096
Occasion	0.7597167	68.6		0.8716	13.819
Plate Repeat	0.0188301	1.7		0.1372	2.176
Total	1.1077211	100.0		1.0525	16.686

- Shift in results between analysts
- Variability with test occasion
- Plate replicates within assay are close



Variance Components

Component	Var Component	% of Total	20 40 60 80	Sqrt(Var Comp)	CV
Analyst	0.00000000	0.0		0.00000	0.0000
Occasion	0.00000000	0.0		0.00000	0.0000
Plate Repeat	0.24643440	100.0		0.49642	6.3353
Total	0.24643440	100.0		0.49642	6.3353

- Analysts get similar results
- Test occasion does not change results
- Plate replicates show significant variability

Automation Advantages - Improved Safety and Efficiency

Automation of lab processes makes our workforce more efficient

- Allows analysts to multitask by reducing the time devoted to a single assay
- Hands on time reduced significantly
- Reduced ergonomic risk

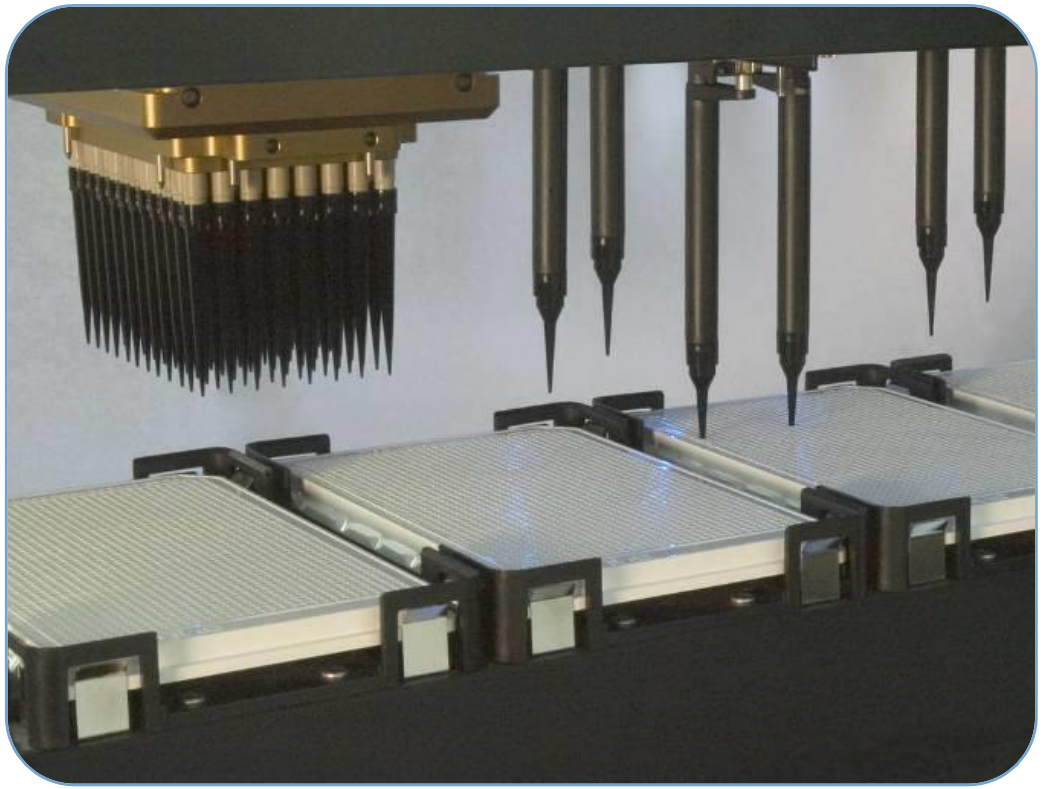
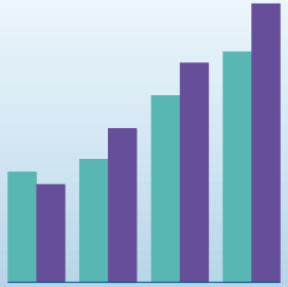


Image used with permission from Hamilton

Automated Advantages - Reliable, Accurate, and Precise

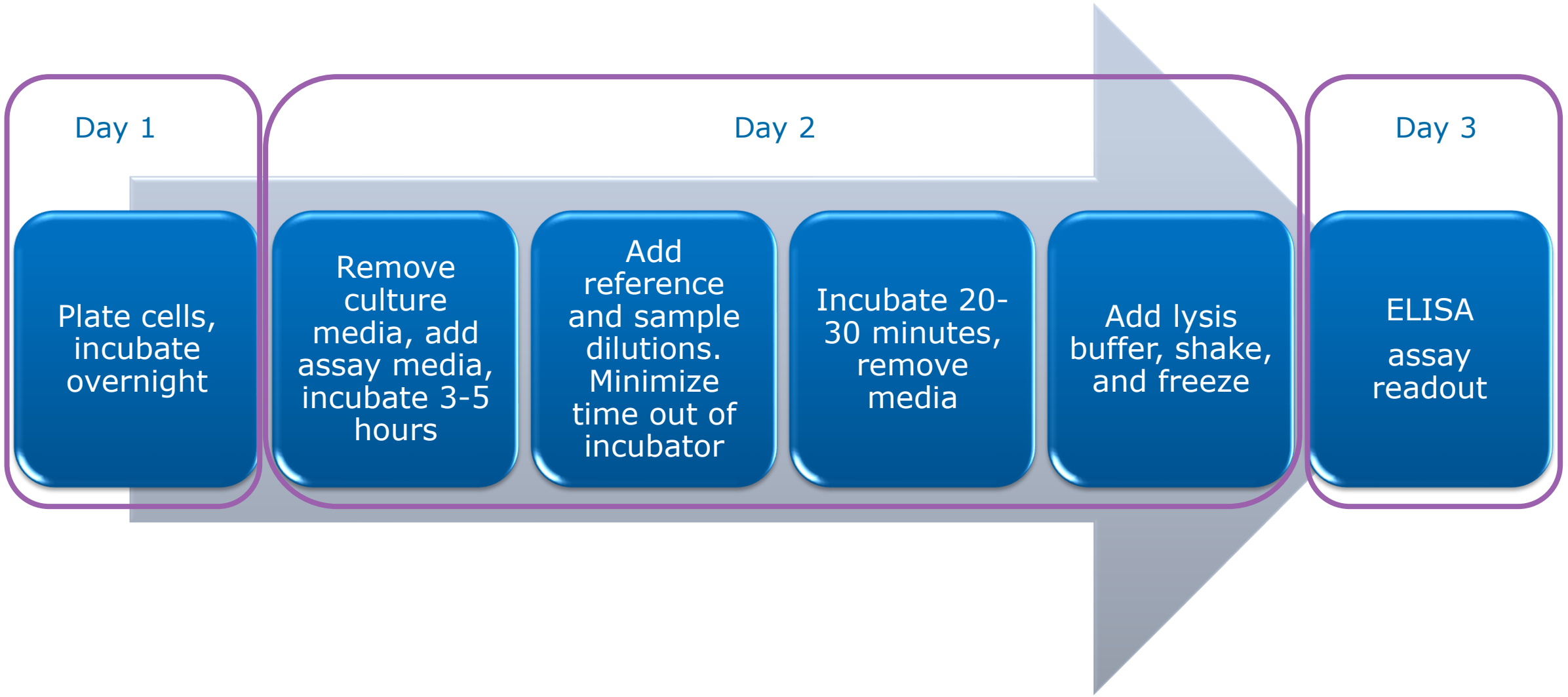


- Removes person-to-person and day-to-day variability
- Does not get fatigued or distracted
- Reduces assay development time
- Faster turnaround time
- Consistent performance
- Increased reproducibility

AGENDA

1. Introduction To Catalent
2. Cell-Based Assays Variability and Automation
3. **HEK293 Potency Assay**
4. Conclusion

HEK293 Cell-Based Assay



HEK293 Cell-Based Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	CNTL	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	CNTL
C	CNTL	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	CNTL
D	CNTL	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	CNTL
E	CNTL	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	CNTL
F	CNTL	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	CNTL
G	CNTL	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	CNTL
H												

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	CNTL	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	CNTL
C	CNTL	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	CNTL
D	CNTL	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	CNTL
E	CNTL	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	CNTL
F	CNTL	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	CNTL
G	CNTL	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	Sam	CNTL
H												

- Two plate assay – 1 sample
- 3 10-point serial dilutions per plate
- Varied sample and reference pattern

Assay Challenges

- Complete media removal twice
- Three additions to all wells
- Speed needed - minimize time out of incubator
- Step with 20-30 minute incubation limits number of plates

HEK293 Cell-based Assay: What Have We Found With This Assay

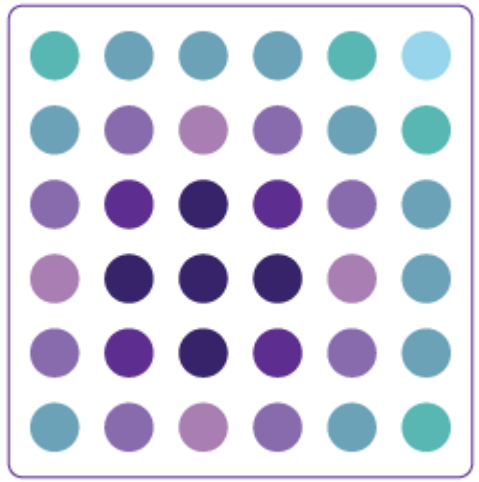
Complex processing and short incubation times limit setup to 4 plates (2 samples)

Multiple aspirate and dispense steps along with semi-adherence of HEK293 cells can lead to variability due to cell loss

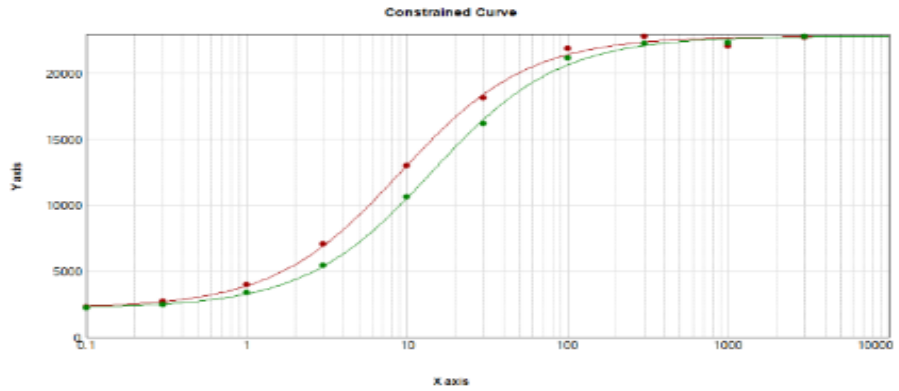
Analysts require significant training to be able to perform

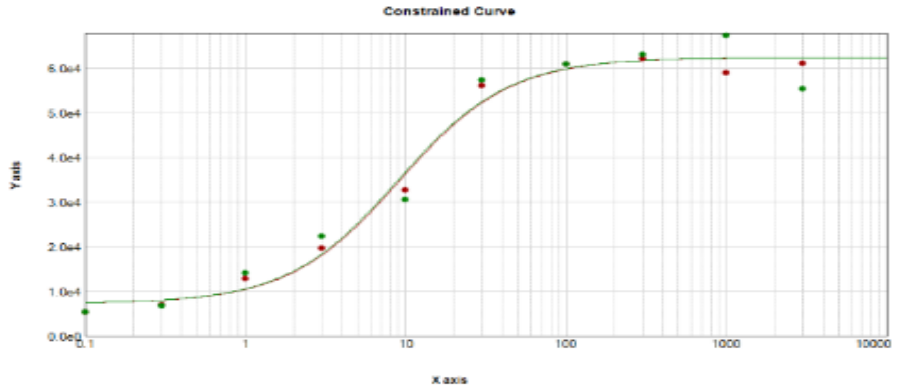
Most common assay failure is high CV between the replicate plates

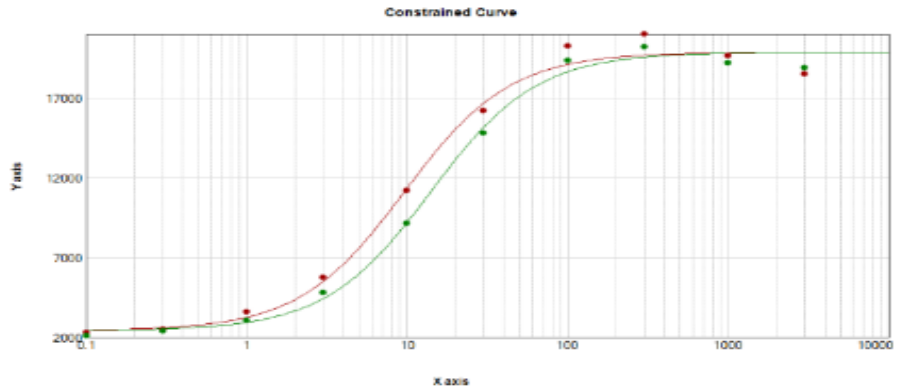
Following cell-based portion analyst performs ELISAs on all plates

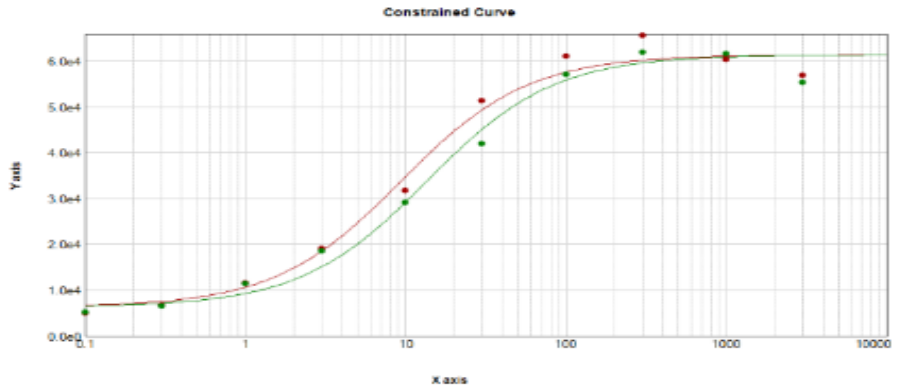


HEK293 Cell-Based Assay









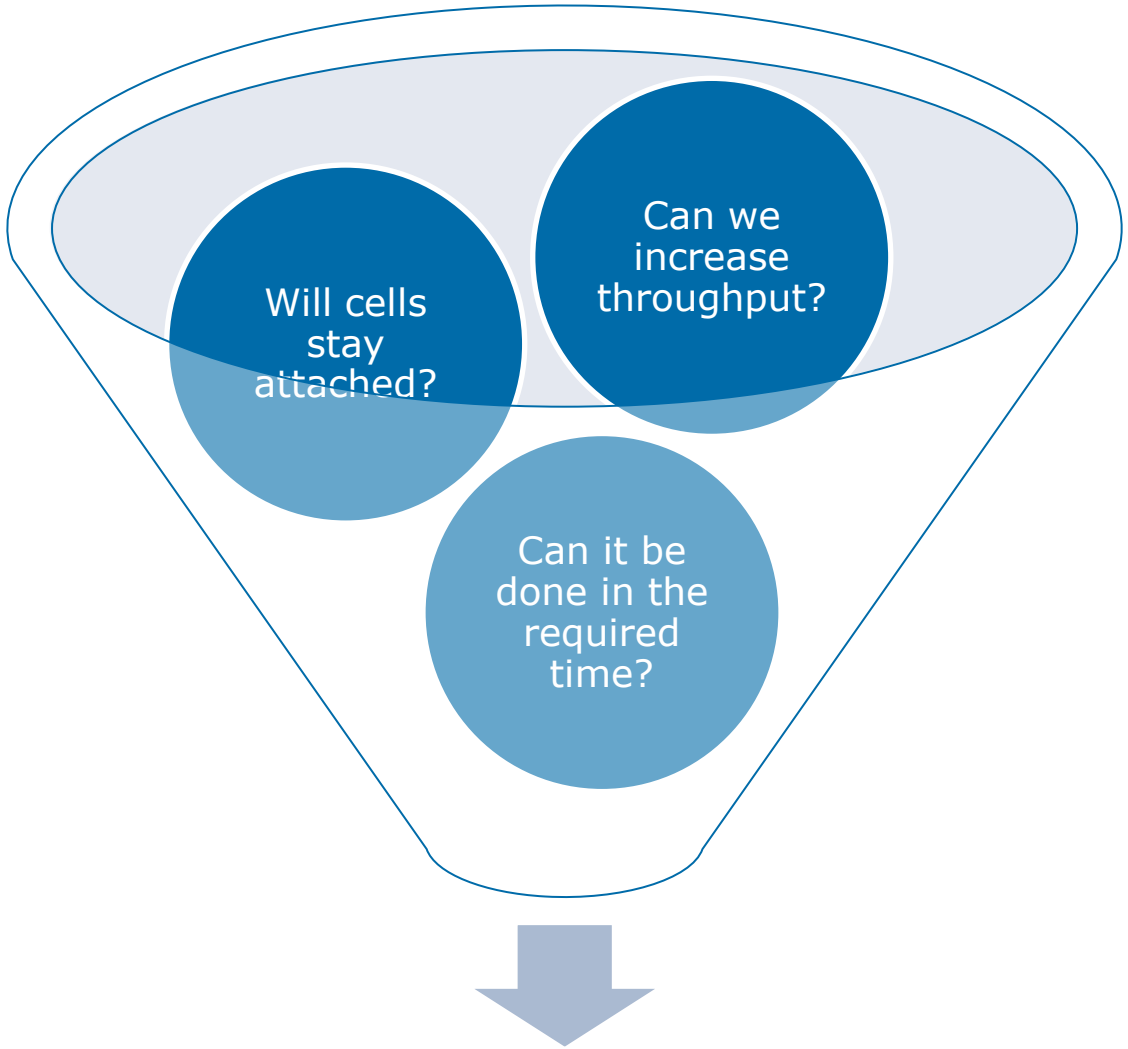
Both plates were run at a nominal potency of 60%. Relative potencies for Plate 1 and Plate 2 were **64.2%** and **68.5%**, respectively.

Reportable value: **66.4**
SD: **3.0**
%CV between RPs: **4.6**

Both plates were run at a nominal potency of 100%. Relative potencies for Plate 1 and Plate 2 were **102.6%** and **69.0%**, respectively.

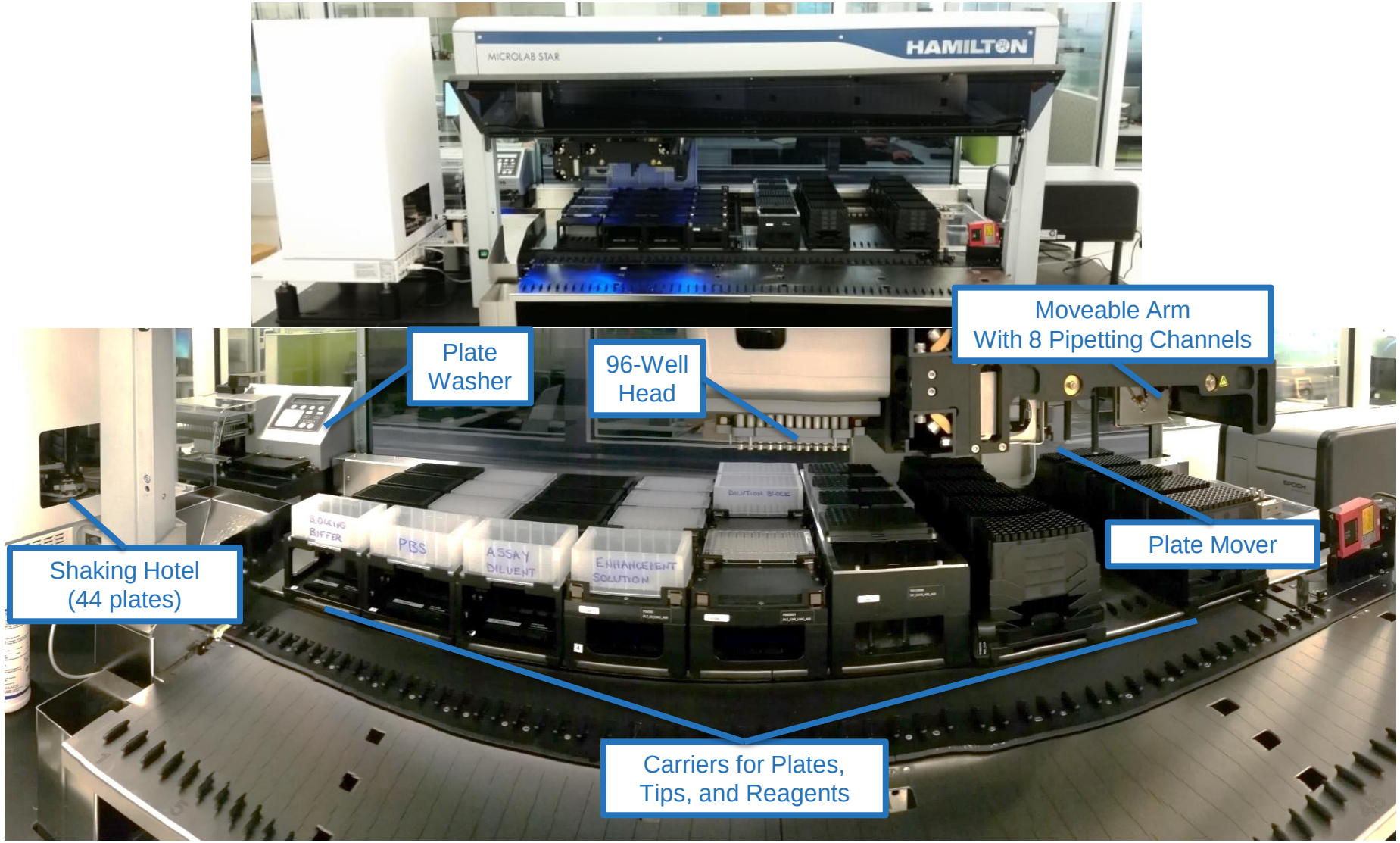
Reportable value: **85.8**
SD: **27.8**
%CV between RPs: **27.7**

Can We Automate This Assay?



Passing Results

The Bioassay Robot – HAMILTON® Microlab STAR™

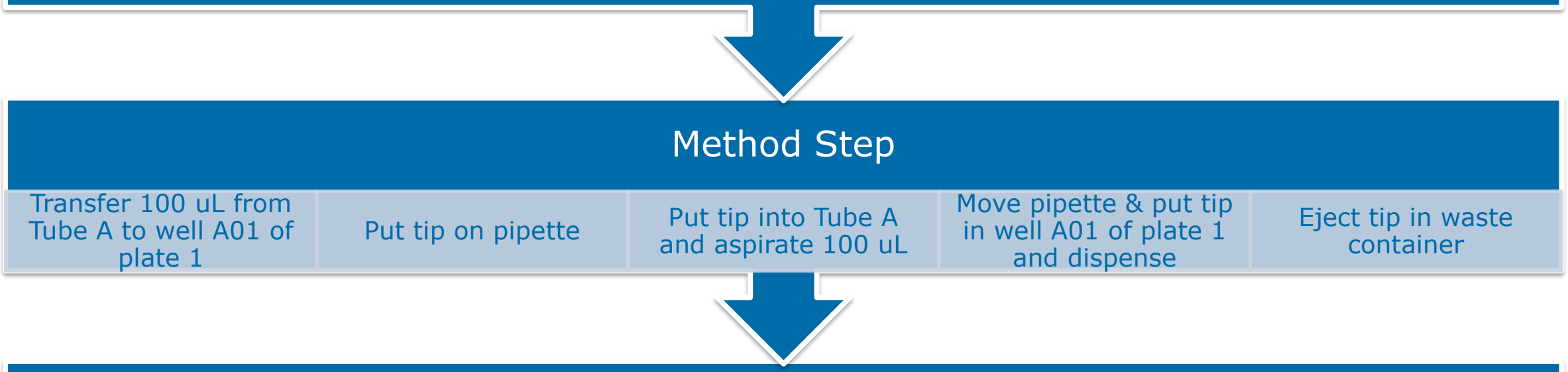


Automating the Assay – Build the Deck

Labware **Carriers** **Location**

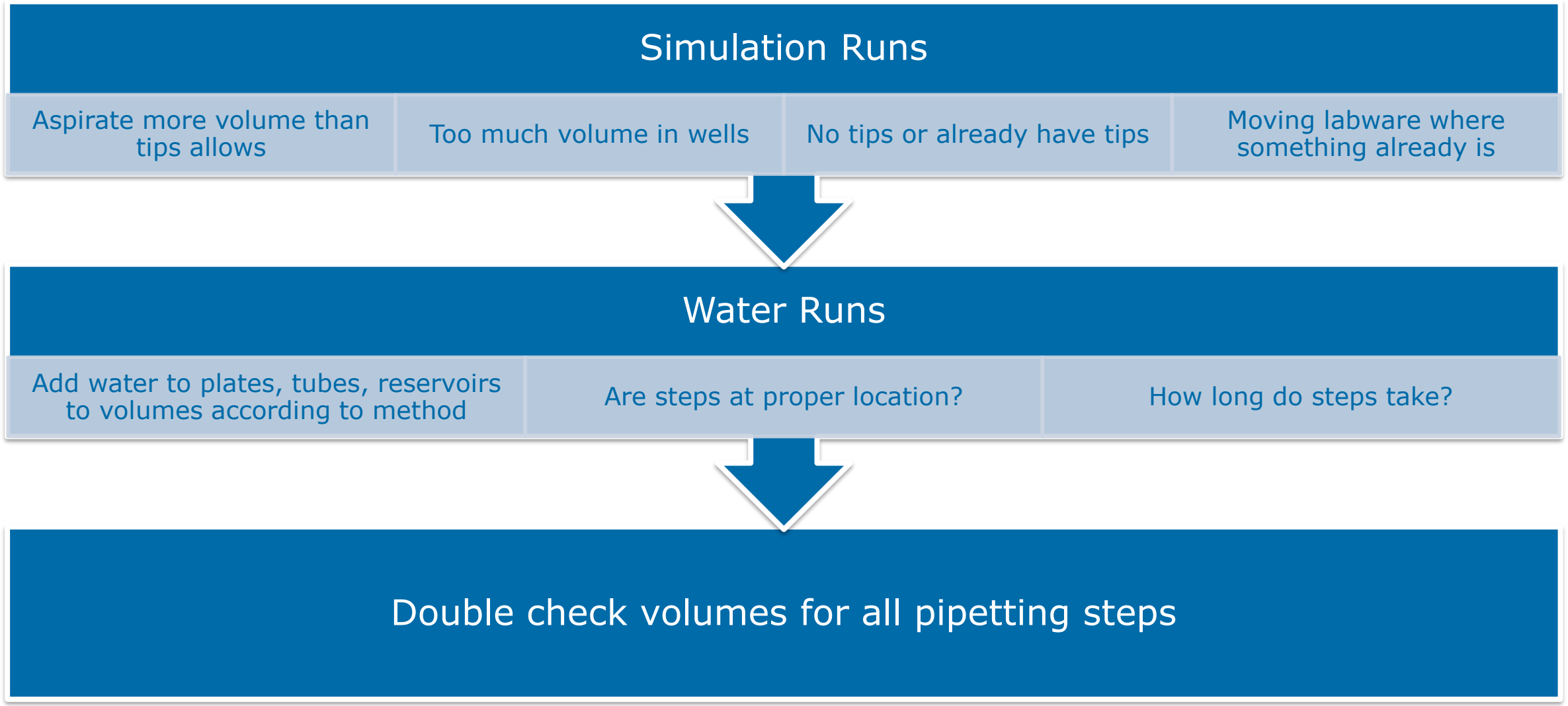
Automating the Assay – Setup the Steps

Same process as running yourself; you just don't think about many of the steps anymore

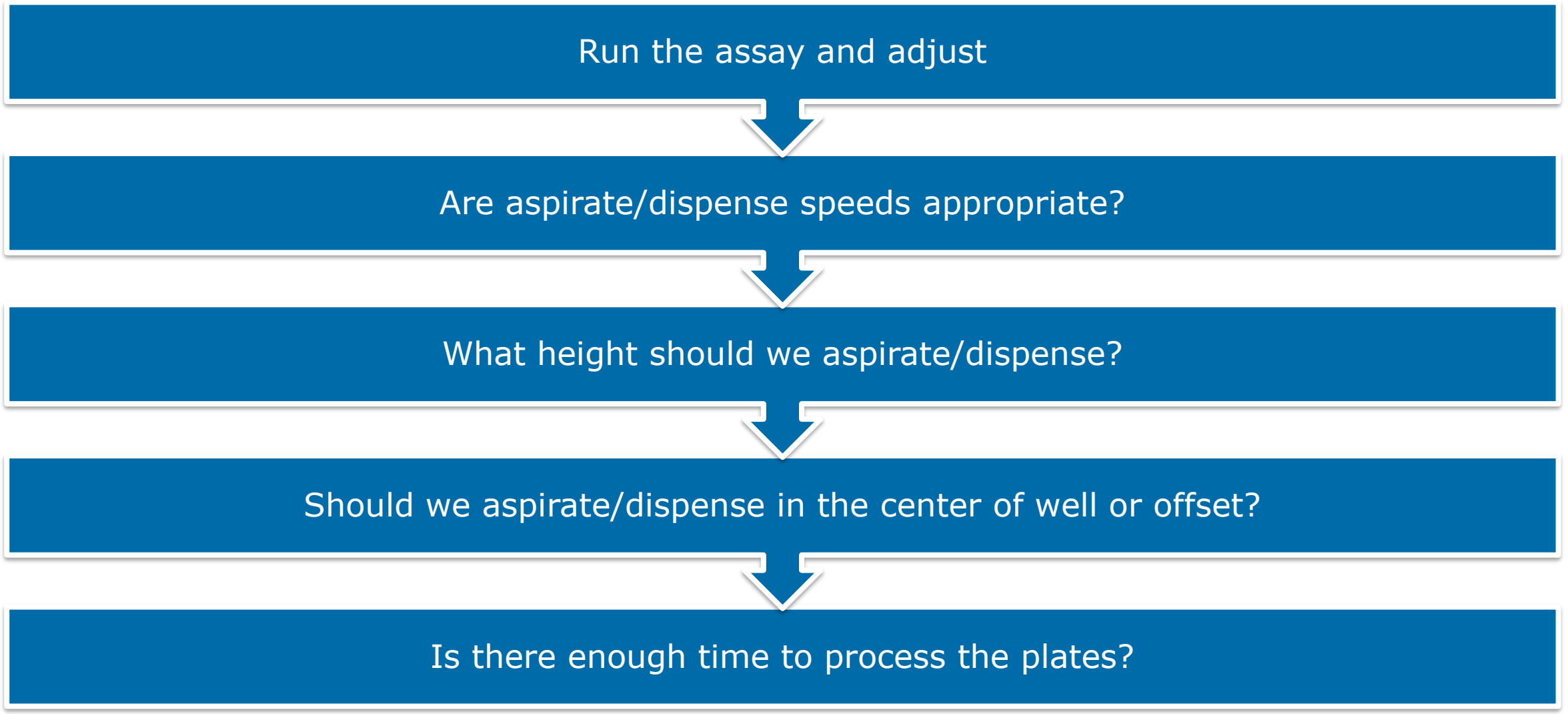


Transfers, dilutions, mix, move plates, loops, timers, etc.

Automating the Assay – Simulations and Water Runs



Automating the Assay – Assay



Automating the Assay – Lessons Learned

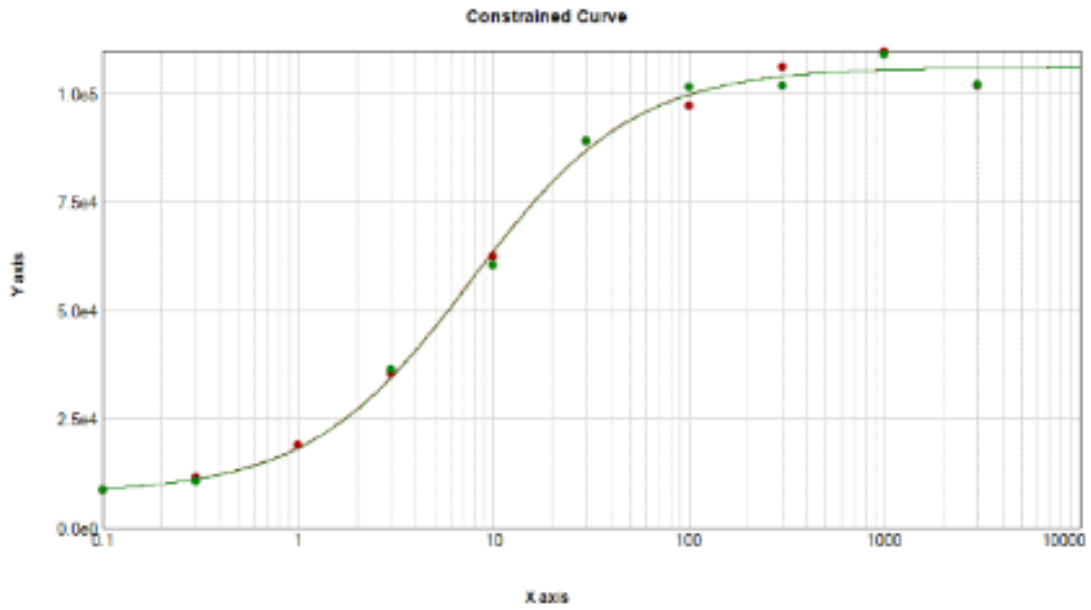
1. **Initial media change manually and start incubation**

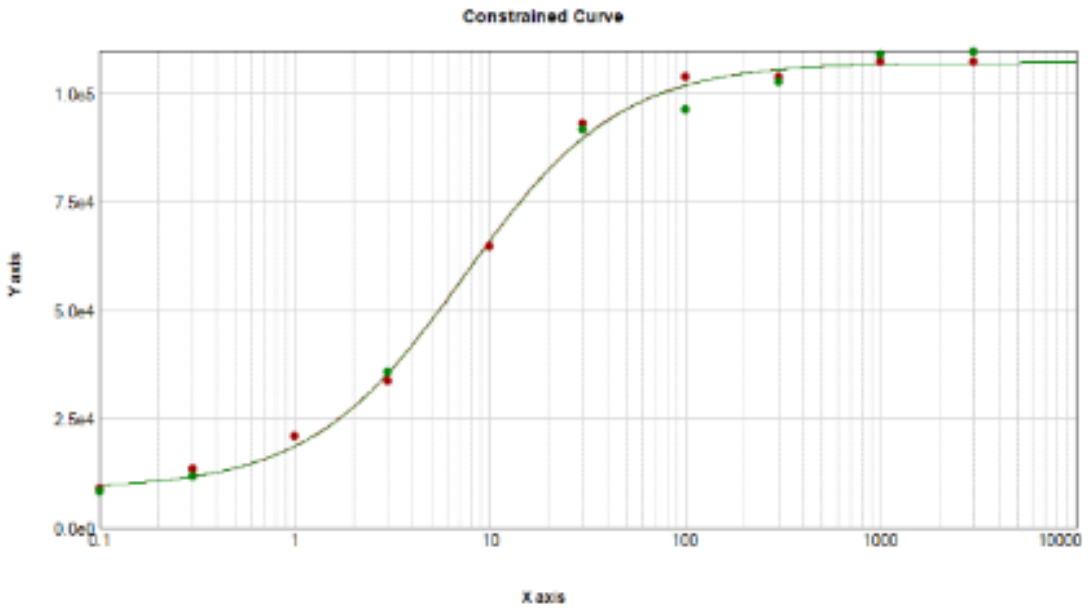
2. **Reference and Sample dilutions**
   - Formulation has some viscosity - reduce aspirate/dispense speeds, minimize depth of tips in liquid, follow liquid level

3. **Cell plates**
   - Disruption of cells – reduce aspirate/dispense speeds, moved pipetting close to edge of wells

4. **Throughput**
   - Set up 8 plates and start 3-5 hour step, after 3 hours process 4 and then process second 4 plates

HAMILTON® Microlab STAR™ Assay Run





Both plates were run at a nominal potency of 100%. Relative potencies for Plate 1 and Plate 2 were **98.6%** and **98.8%**, respectively.

Reportable value: **98.7**
SD: **0.1**
%CV between RPs: **0.1**

AGENDA

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Bioassay Automation Summary

Overview

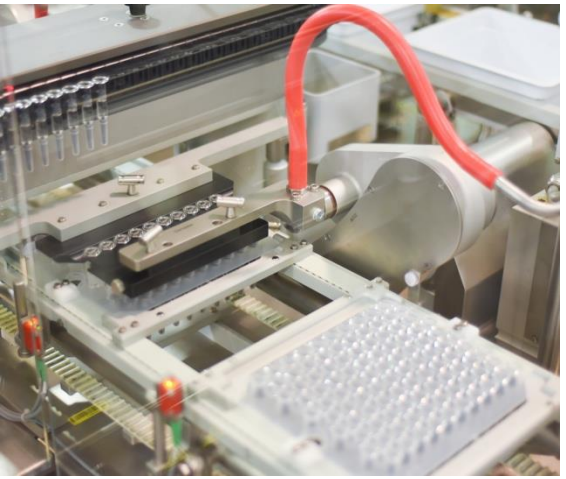
- Advantages: safety, performance, quality

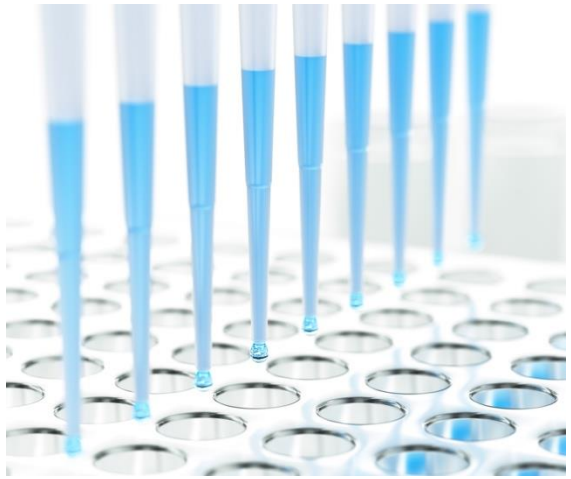
Comparisons

- Manual vs. automation

Adapting to automated

- Complex steps possible





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BIOLOGICS

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