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Automation and multiplexing of immunoassays: Improving precision and throughput

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Automation of Assays: Improving Precision and Throughput

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Introduction

- Manual immunoassays, especially functional assays involving living organisms are inherently variable.
  - Assay variability is a consequence of the assay complexity and high sensitivity of live organisms to external factors.
  - Multiple external factors affecting the assay, such as variability of individual techniques between the analysts, are often hard to control.
  - Manual assays usually require extensive technical training of analysts.

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Potential Benefits of Assay Automation

- Numerous potential benefits are considered when the possibility of automation in the laboratory are evaluated, including:
  - improvement of the quality and reliability of test results;
  - increased workplace ergonomics, employee productivity and safety;
  - enhancement of the assay efficiency/throughput;
  - standardization of lab techniques;
- Complete automation presents operational and financial challenges and is not always economically justifiable; partial automation of the process workflow may be beneficial.

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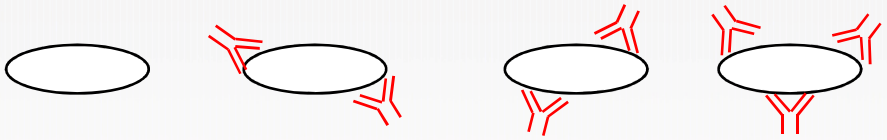
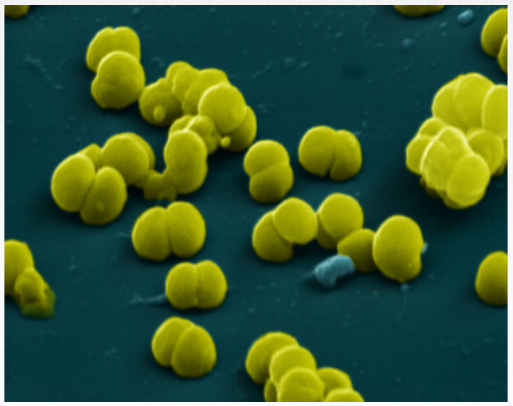
Example 1.
Automation of the Meningococcal Serum Bactericidal Assay (Mn SBA) for Serogroups A, C, Y and W135.

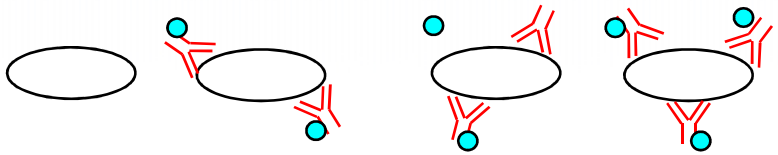
Principle of the Serum Bactericidal Assay

Step 1: Mixing bacteria with test serum

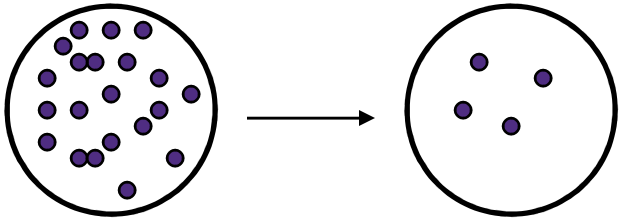




Step 2: Adding complement (●)



Step 3: Complement-mediated killing (defined as reduction in colony counts)



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Manual Mn SBA ACYW135 assay

- Classic Meningococcal Serum Bactericidal Assay.
  - Four individual manual SBAs are performed to measure bactericidal antibody titers to Neisseria meningitidis serogroup A, C, Y and W135.
  - The SBA is a complex assay involving multiple manual dilutions performed by analysts.
- Qualification studies were conducted to objectively evaluate the assay parameters and performance.
  - The statistical analysis revealed that total variability was higher than expected.
- Further analysis demonstrated that among the ruggedness factors, the variability associated with the analyst showed the highest impact on the assay.

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Manual Mn SBA ACYW135 Assay Variability

Serogroup	Variance estimates (%RSD)		
	Analyst to Analyst	Run within Analyst	Total
A	91%	90%	175%
C	15%	74%	100%
W135	29%	39%	65%
Y	19%	34%	74%

- The analysts were producing substantially different titers
  - Within analyst variability changed from analyst to analyst.
  - High variability was observed for both assay controls and test samples.

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Automation of the sample processing for the Mn SBA ACYW135: Hamilton Microlab STAR

Summary of The Mn SBA ACYW135 Automation Effort

- Assay scripts were developed and validated. Scripts were created based on the manual process described in the SOP.
- The control and sample processing was performed by Hamilton.
- The use of the sample master dilution plate enabled simultaneous testing of the four serogroups improving assay throughput.

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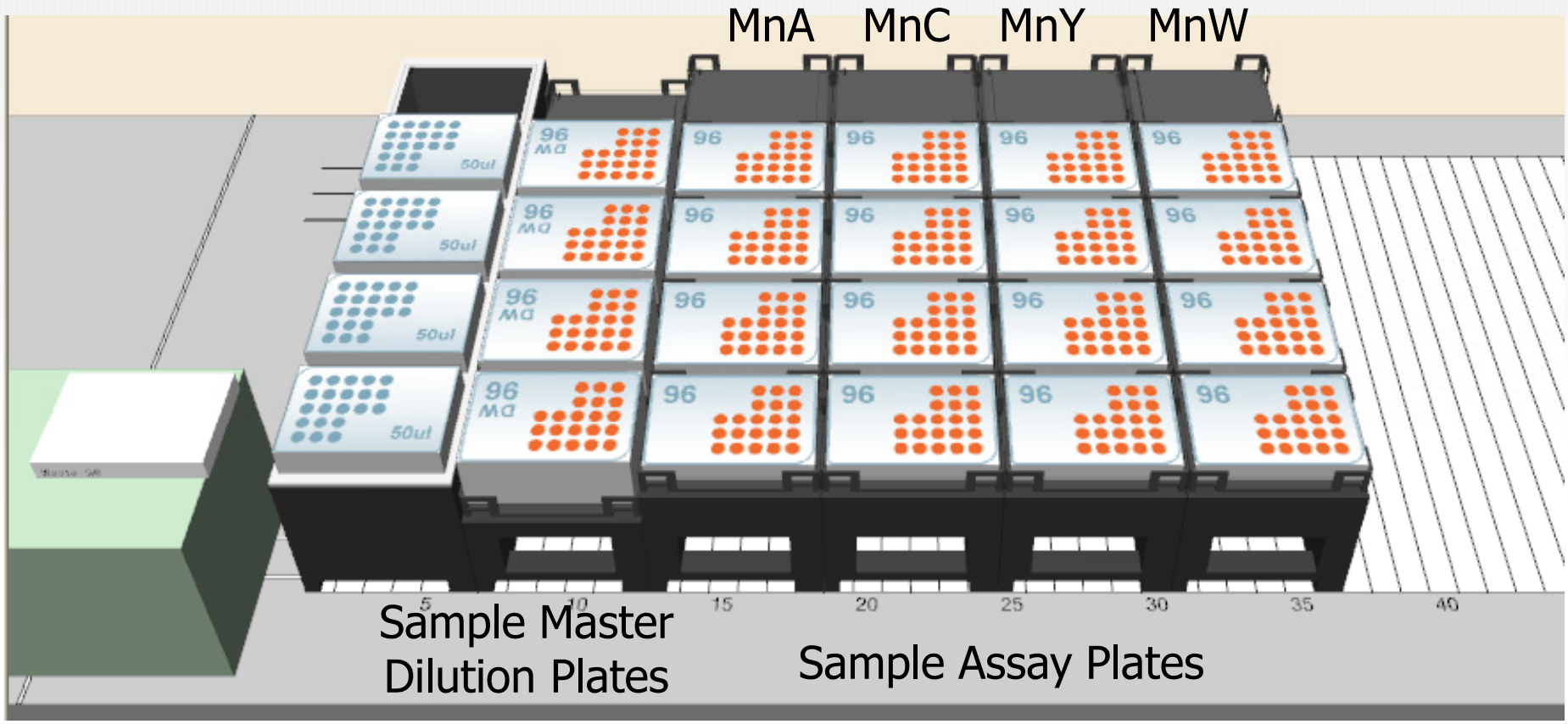
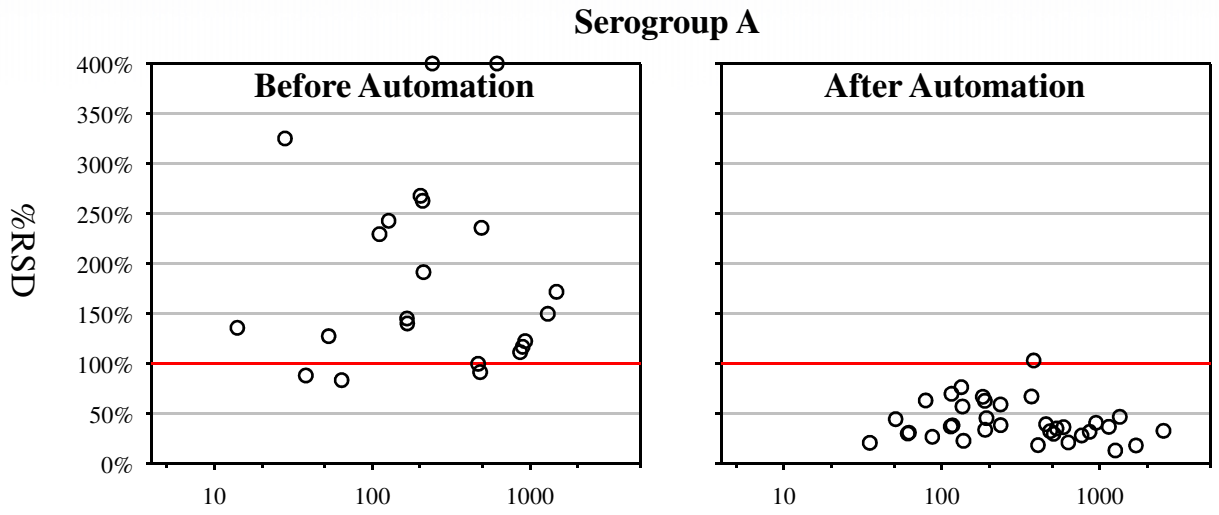
Standardize Sample Processing: Sample Assay Plate Transfer



Deck is reloaded 1-2 times dependent on the number of Master plates.

Improvement of Overall Assay Precision After the Implementation of the Automation Step, per Serogroup

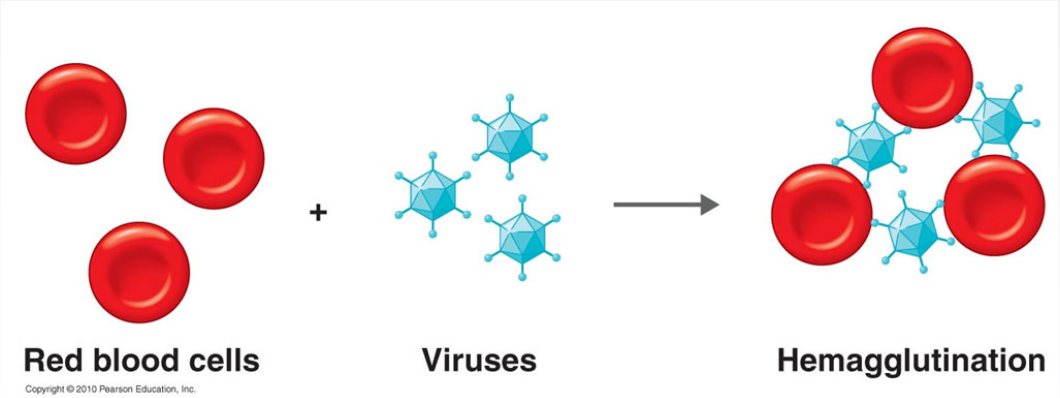
Serogroup	Total Assay Variability (%RSD)	
	Before Automation	After Automation
A	175%	41%
C	100%	36%
Y	65%	27%
W135	74%	35%



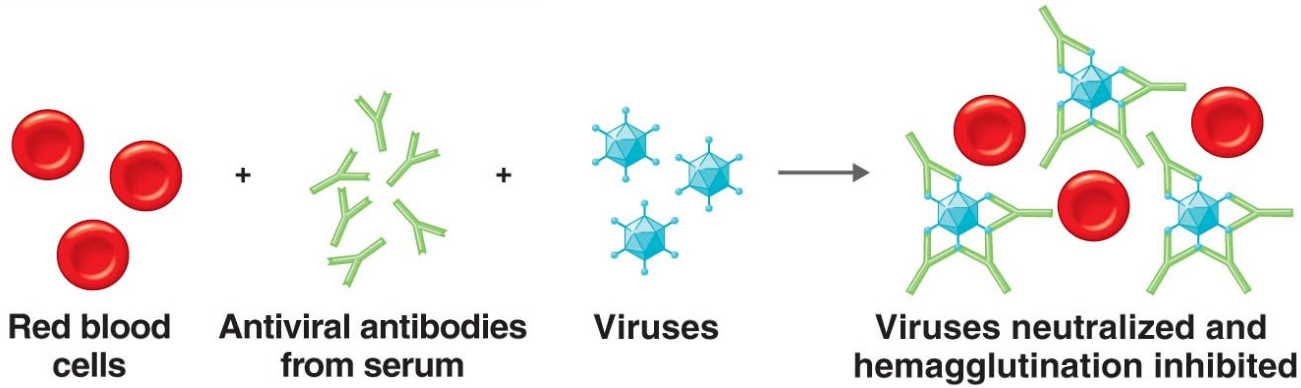
Example 2.
Automation of Seasonal Influenza HAI

Principle of the Hemagglutination Inhibition Assay

Hemagglutination



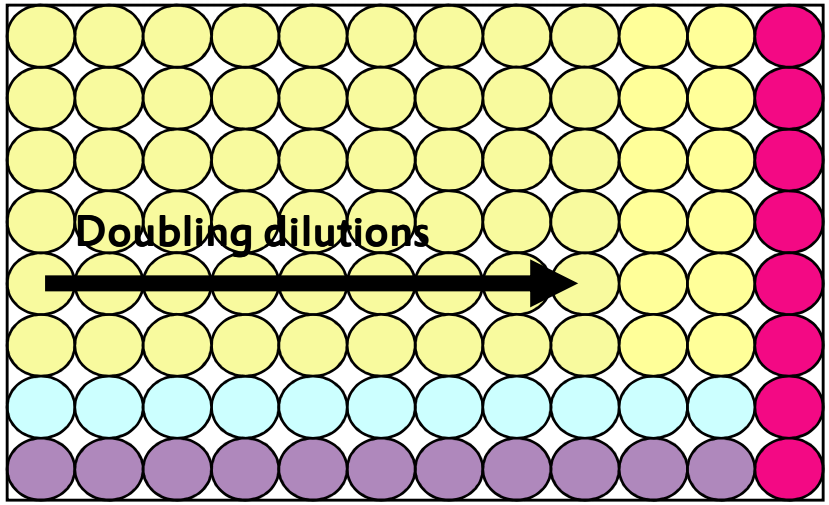
Inhibition of hemagglutination



Seasonal Flu HAI

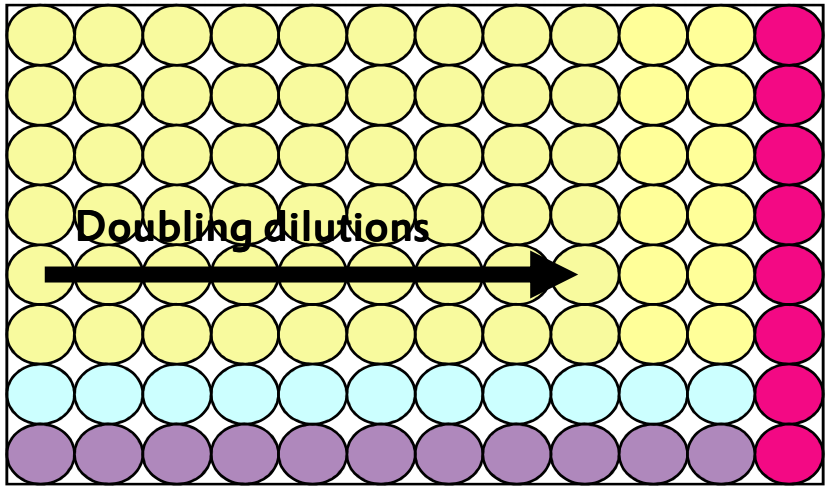
- The HAI assay is used to test various seasonal strains of Influenza virus
- The assay procedure consists of two analyst serially titrating a sample **replicate** with a 2-fold serial dilution for up to 12 microtiter assay plates per analyst, per strain
- Variability is increased because two analysts are generating replicate plates (and independent results)

Analyst 1: Sample Replicate 1



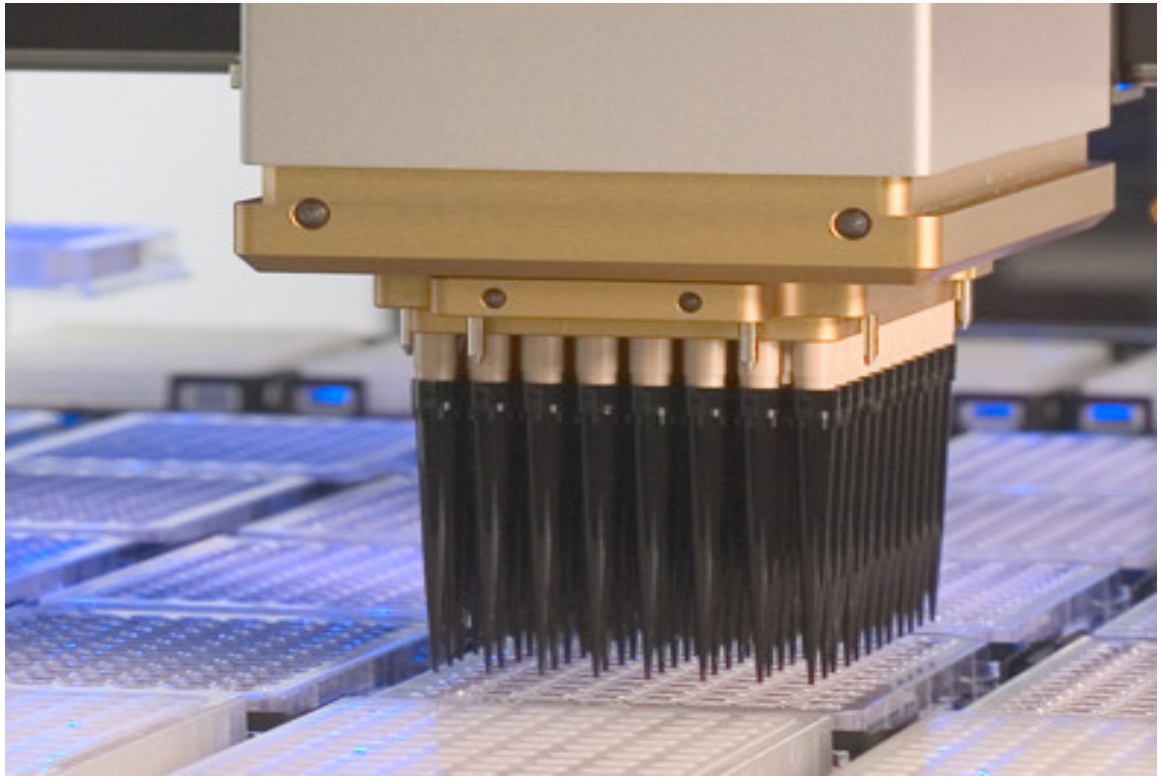
Unk 1
Unk 2
Unk 3
Unk 4
Unk 5
Unk 6
Pos
Neg

Analyst 2: Sample Replicate 2



Seasonal FLU HAI: Sample Processing Applications

- Similar to the MnSBA sample processing standardization
- Hamilton automation methods were designed to facilitate the testing of multiple virus strains against the same sample dilution and reduce sample repeat rates



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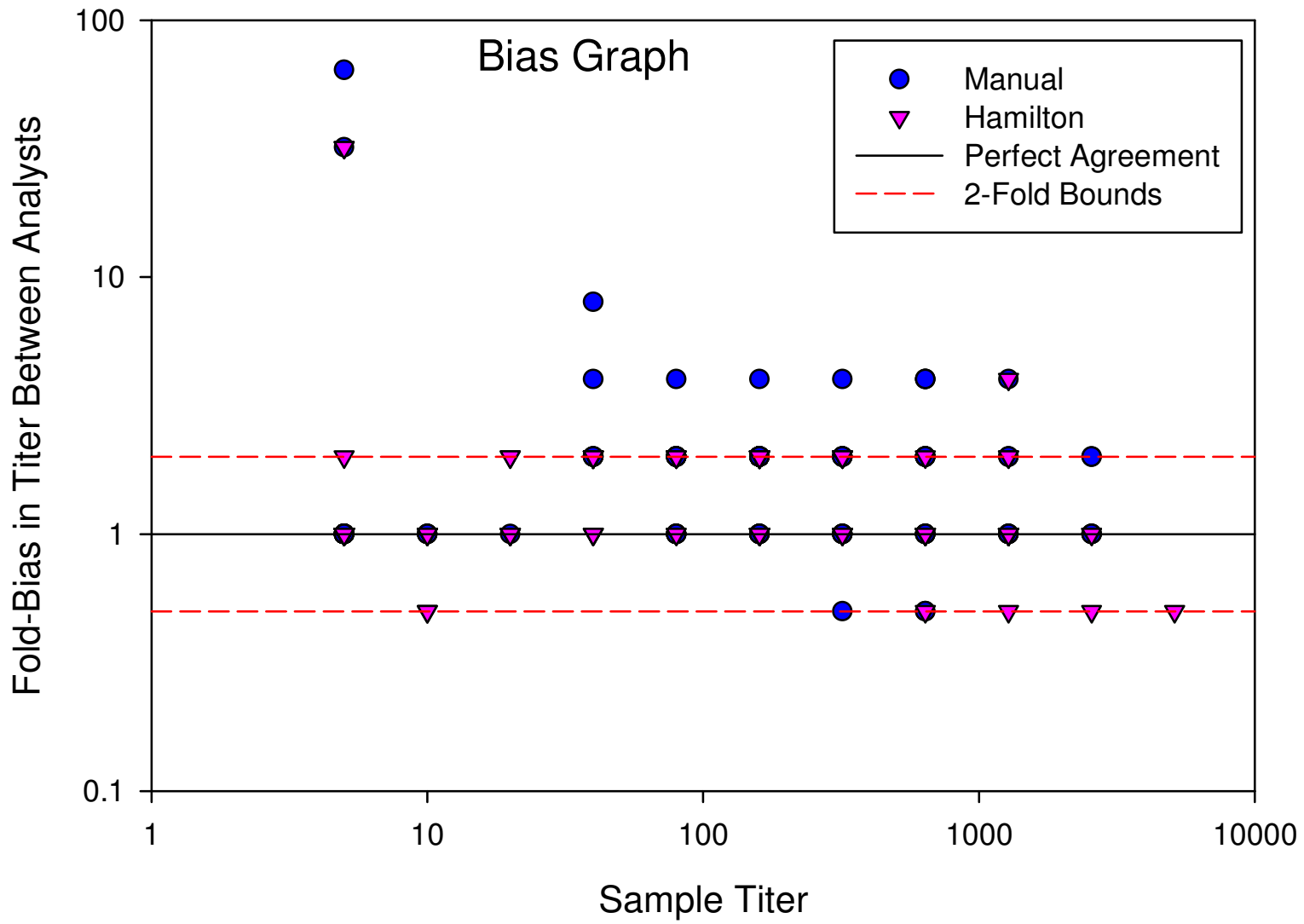
Seasonal Flu HAI: Replicate Repeat Rate Results

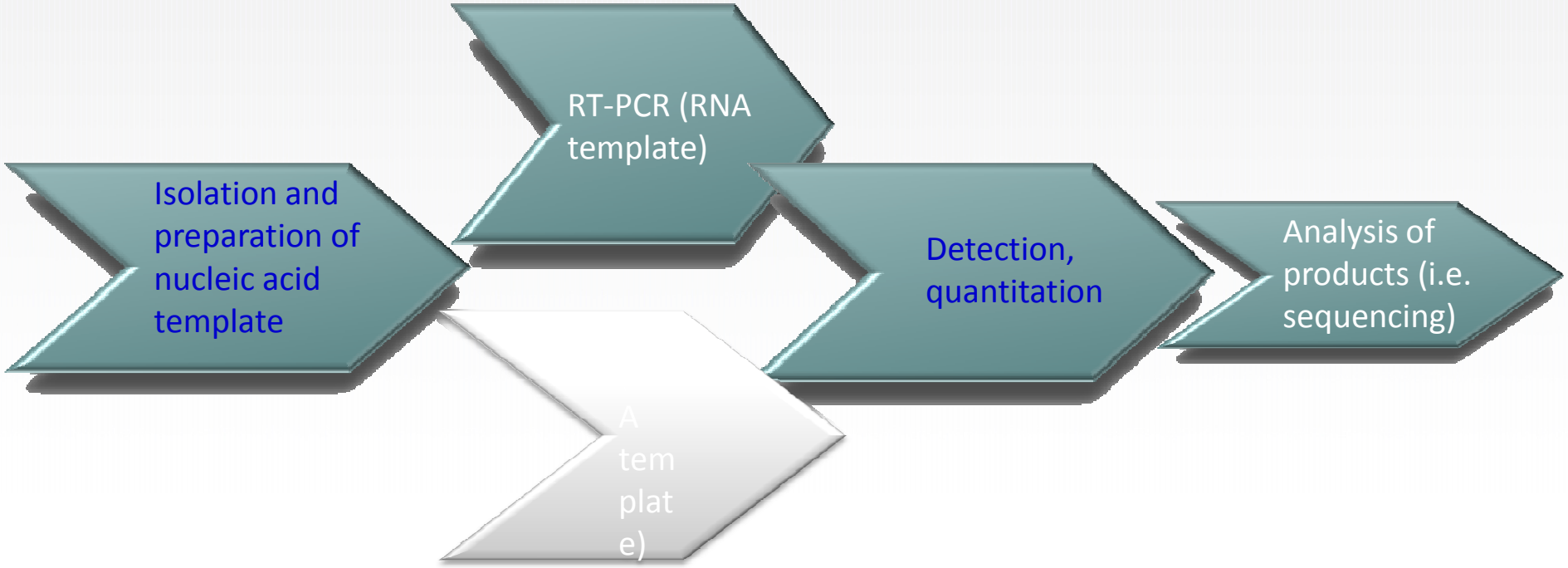


Improved Biofunctional Assay Variability Conclusions

- Utilizing automation for high throughput sample processing applications has improved two of our biofunctional assays by reducing:
  - Variability and sample retest rate for Mn SBA ACYW135
  - Variability and sample retest rate for Seasonal Flu HAI

Example 3. Multiplexing and Automation of PCR Assays.

General PCR Workflow



Each of these steps can be automated

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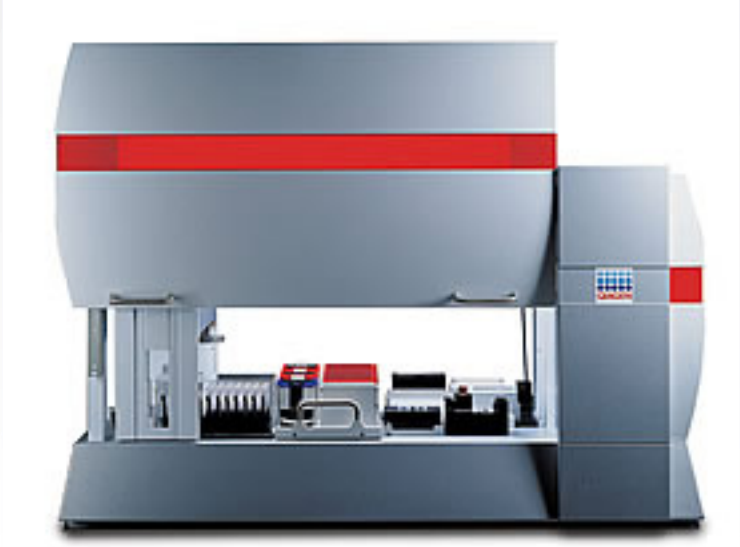
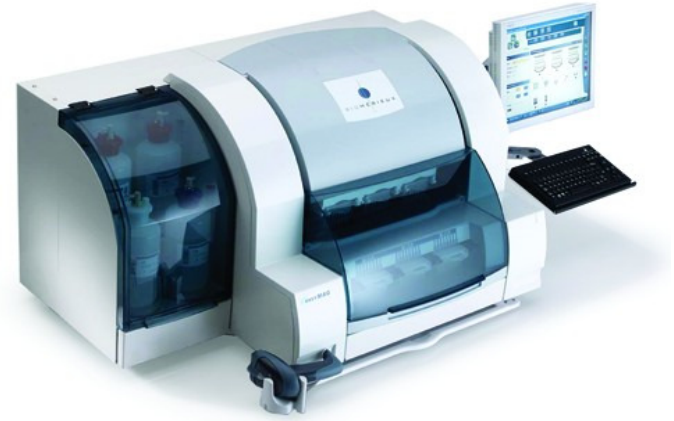
Nucleic Acid Extraction: QIAGEN BioRobot MDx (DNA) and BioMèrieux NucliSens easyMAG (RNA)

- The Qiagen BioRobot MDx Workstation
  - Fully automated
  - High-throughput
  - Accurate liquid handling
    - filtered tips
  - On-board vacuum processing
  - Locked down once program begins



- The NucliSens easyMAG
  - Automated system
  - Magnetic silica beads
  - On-board magnet
  - Requires little hands-on interaction during a run



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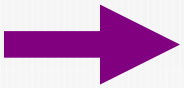
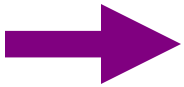
Increased Efficiencies – DNA and RNA Extraction

- Manual DNA Extraction (QIAGEN Blood Kit)
  - 6 hours touch time = 384 samples



- Automated Extraction (Qiagen BioRobot MDx)
  - 3 hours touch time = **1152** samples

- QIAGEN Manual Method
  - 4.5 hours touch time = 72 samples
    - **OR**
- Boom Silica Manual Method
  - 5 hours touch time = 48 samples



- BioMèrieux NucliSens easyMAG
  - 3 hours touch time = **144** samples

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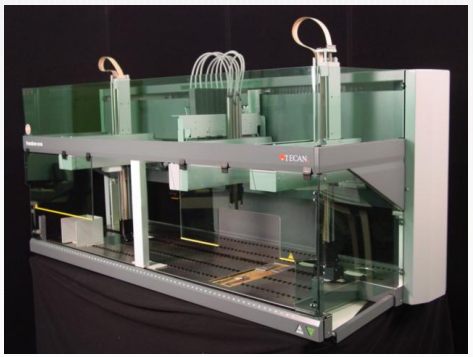
Automation of Sample Processing by TECAN Freedom Evo

- The TECAN Freedom Evo
  - Automated liquid handling system
  - Highly accurate pipetting
  - Decreased cross-contamination
  - Can be customized
    - Different arm configurations
    - Integration of other lab equipment.



- The Molecular Testing Laboratory at PPD VBL utilizes two separate Freedom Evo configurations to maximize the sample preparation potential of our high throughout PCR process.
- TECAN Freedom Evo can process 50 assay plates/FTE/Day with 1.5-2 h of touch time, resulting in a throughput of
  - Up to 200 plates per day
  - Or approximately 6 times the throughput of a single analyst

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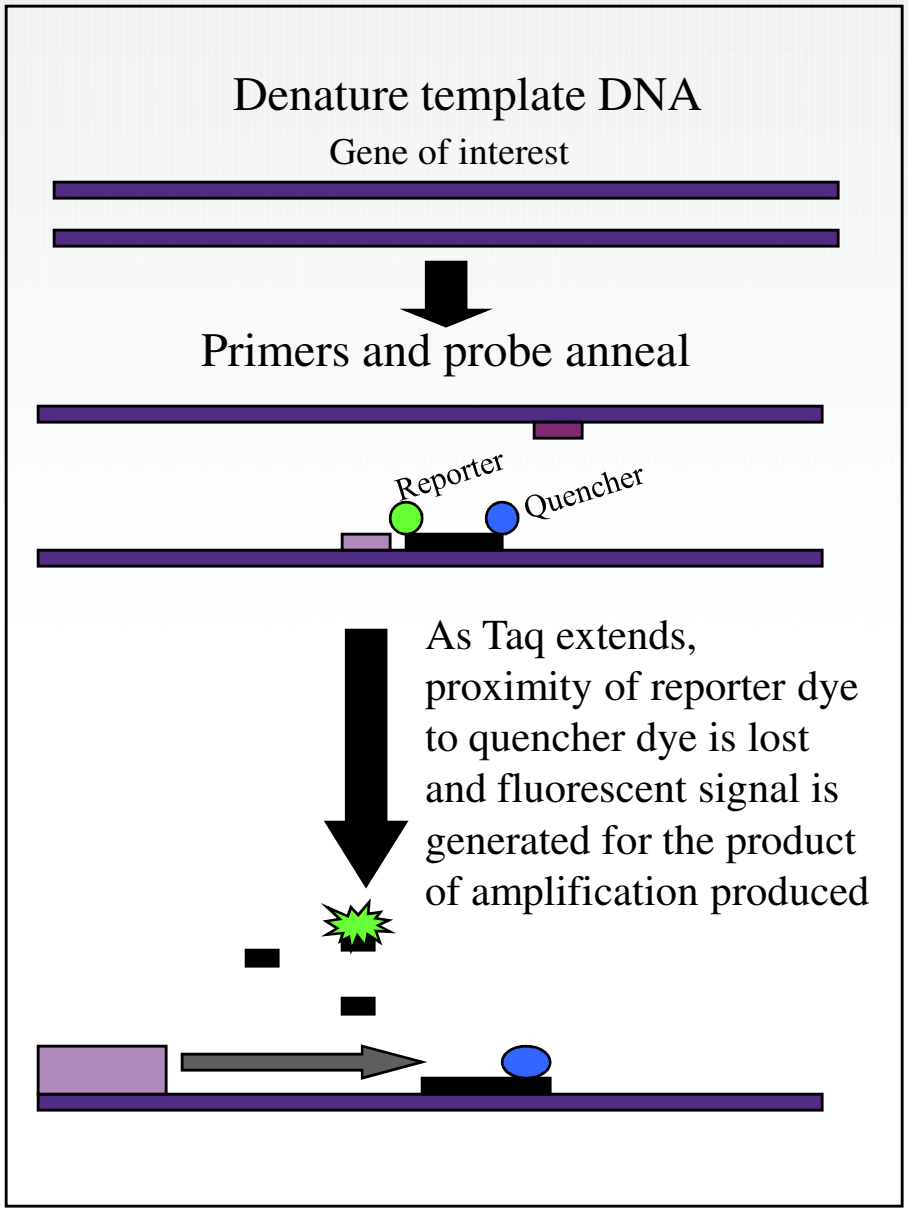
Principle of a Singleplex PCR assay



Throughput calculation,

Example for 90 samples:

- 1 plate
- 15 different strains/organisms
- 3 Open Reading Frames
- 45 Assay Plates
- **1.5 FTE**

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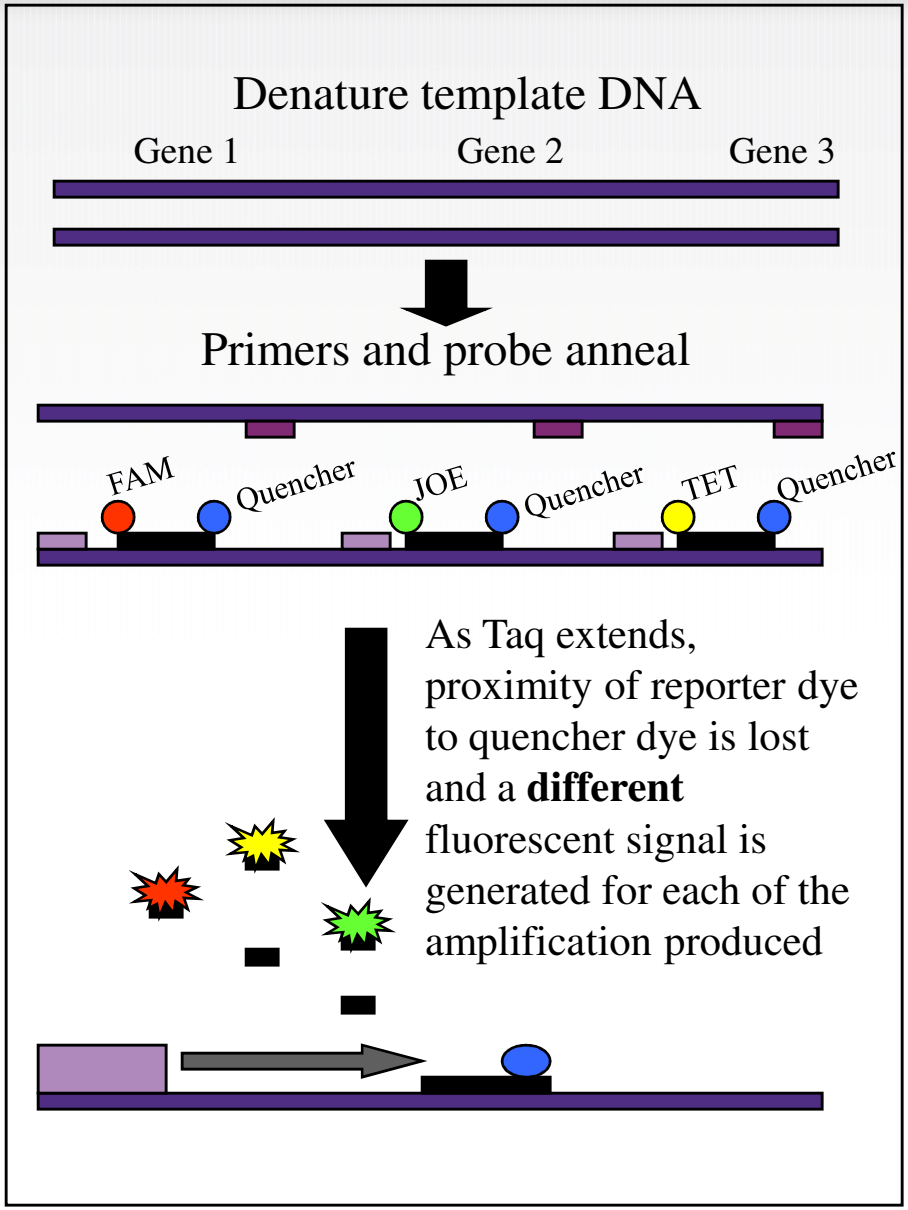
Principle of a Multiplex PCR assay



Throughput calculation

Example for 90 samples:

- 1 plate
- 15 different strains/organisms
- 3 Open Reading Frames measured simultaneously
- 15 Assay Plates
- **0.5 FTE**

- Note: Target genes may come from the same or a different organism.

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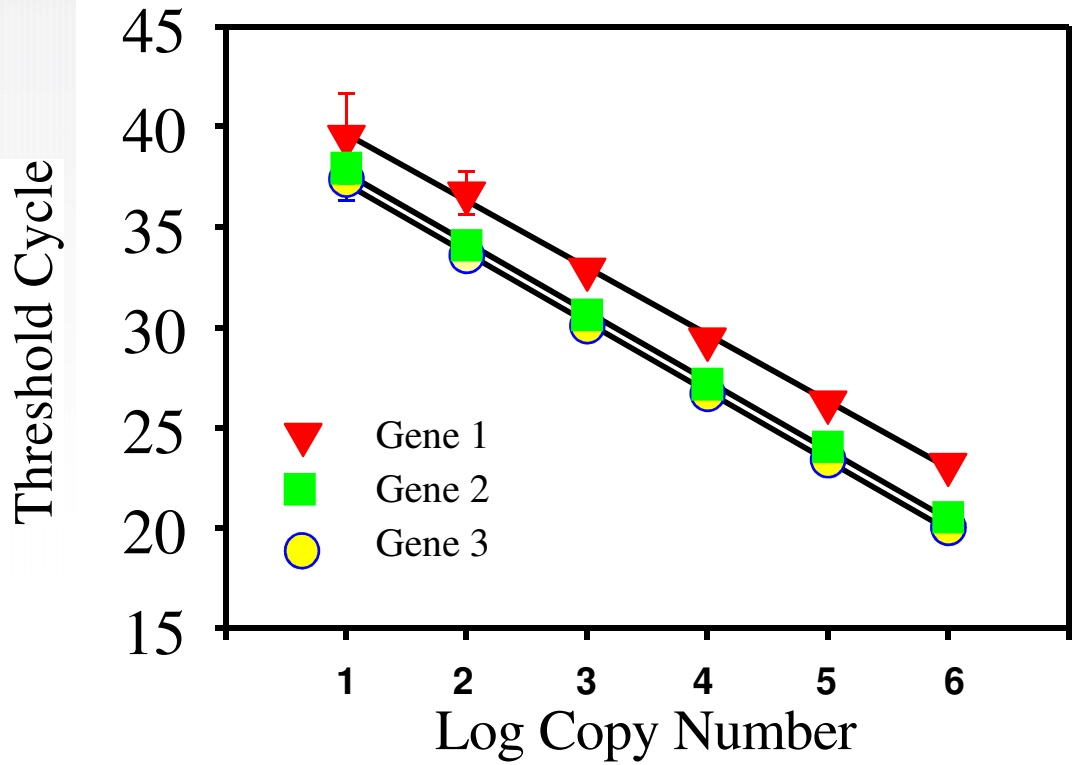
Reaction Efficiency and Linearity of a Multiplexed PCR Assay



- Typical characteristics of the assay:
  - The assay is linear over 6 logarithms of concentration
  - Each target is amplified equivalently
  - Each target has similar sensitivity

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Benefits of Automating and Multiplexing of the Assays

- Increases quality of the data compared to manual assays (reduces error rate, including sample dilutions, placement, cross-contamination, etc.)
- Increases assay throughput
- Increases the assay development flexibility
  - Combination of different targets (i.e. antigens) can be tested simultaneously
- Improves assay parameters
  - Simultaneous detection of different targets to enhance specificity and sensitivity of detection
  - Reduced variability

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- BACKUP

Considerations to be taken into account when automating an assay

- **Why is automation required?** What is the reason for automating a process? What is this going to achieve for the laboratory?
  - What is the testing volume?
  - What are the potential benefits (throughput, retest rate, precision)?
- **How much automation is required?** Is a fully automated system needed or a semi-automated system?
- **What systems are available?**

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Potential drawbacks of automation

- Once that step is taken it is very hard to remove automation from a laboratory. It becomes a part of the validated process.
- Logistical issues. In partial automation (automation of individual steps) balancing the throughput of different steps and eliminating bottlenecks may be challenging.
- Re-training of personnel is needed.
- Significant upfront investment may be required.

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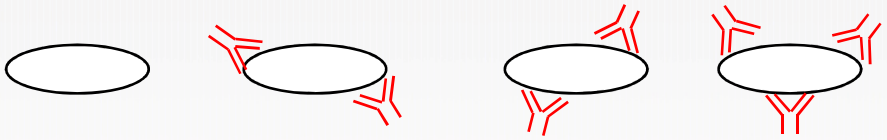
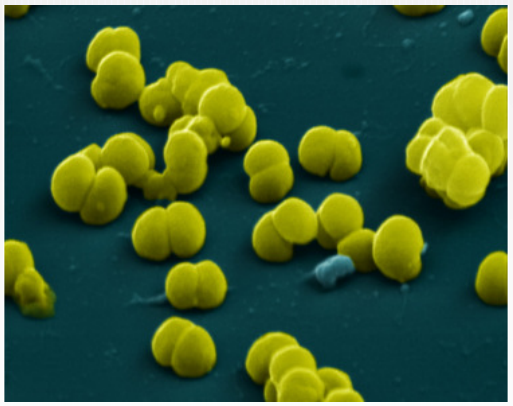
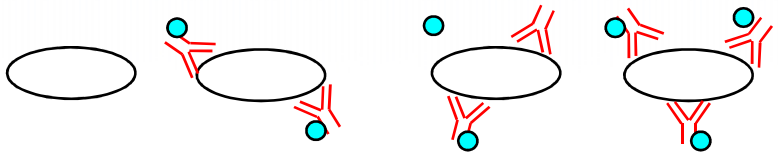
Principle of the Serum Bactericidal Assay

Step 1: Mixing bacteria with test serum

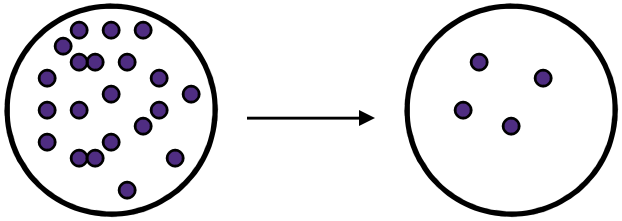




Step 2: Adding complement (●)



Step 3: Complement-mediated killing (defined as reduction in colony counts)



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Variability: Summary for SBAs

		Variance Estimates (%RSD)					
		Before automation			After automation		
Sero group	Analyst	Run Within Analyst	Plate Within Run	Total	Run Within Analyst	Plate Within Run	Total
A	1	10%	0%	44%	0%	5%	31%
C		86%	19%	103%	18%	20%	44%
W		0%	18%	35%	2%	4%	19%
Y		14%	13%	34%	0%	16%	23%
A	2	256%	20%	337%	49%	0%	59%
C		98%	29%	140%	17%	12%	33%
W		78%	9%	97%	0%	11%	20%
Y		41%	28%	83%	0%	0%	16%
A	3	20%	0%	74%	2%	10%	20%
C		6%	19%	41%	9%	13%	29%
W		26%	13%	41%	44%	8%	52%
Y		38%	65%	145%	0%	0%	23%

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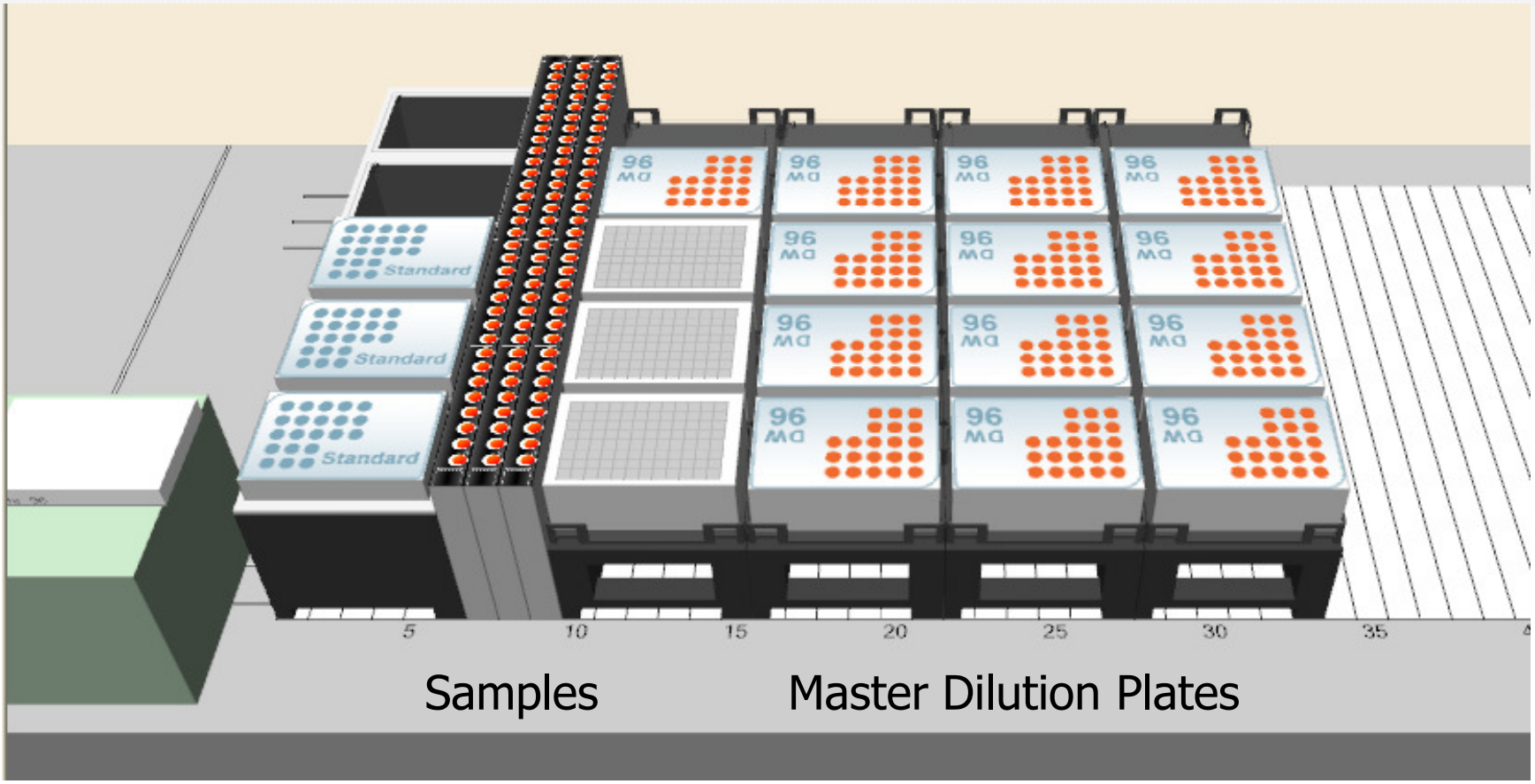
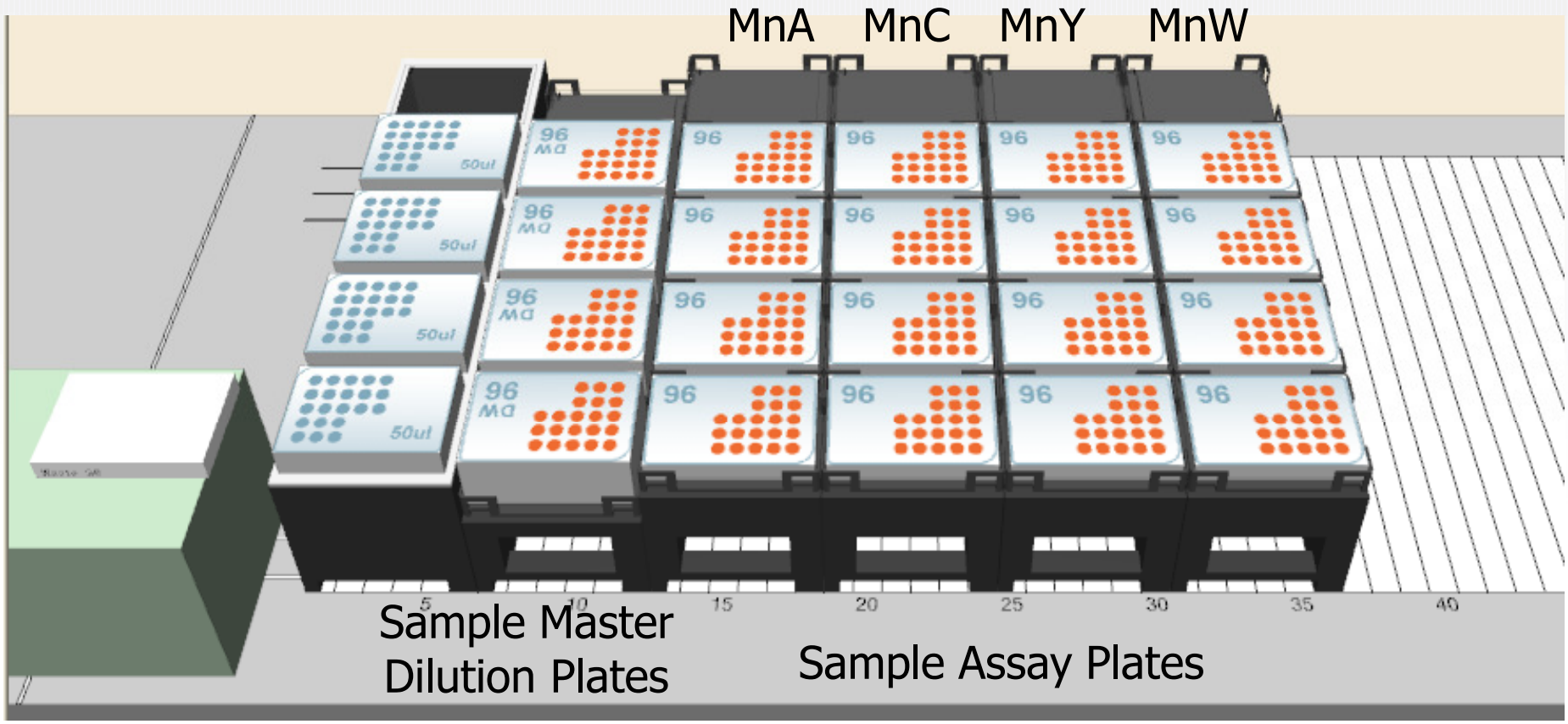
Standardize Sample Processing: Sample Master Dilution Plates

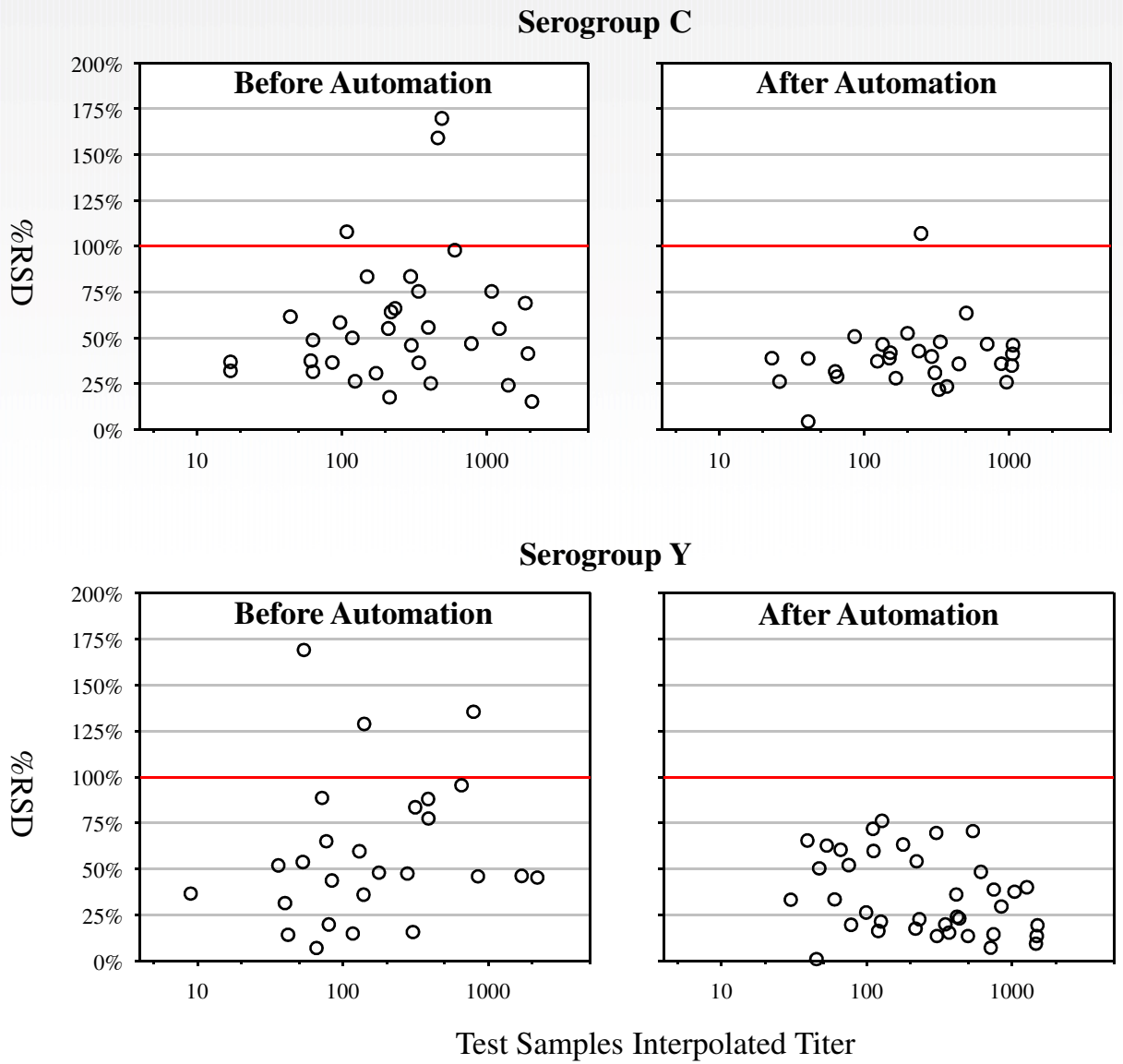


Standardize Sample Processing: Sample Assay Plate Transfer

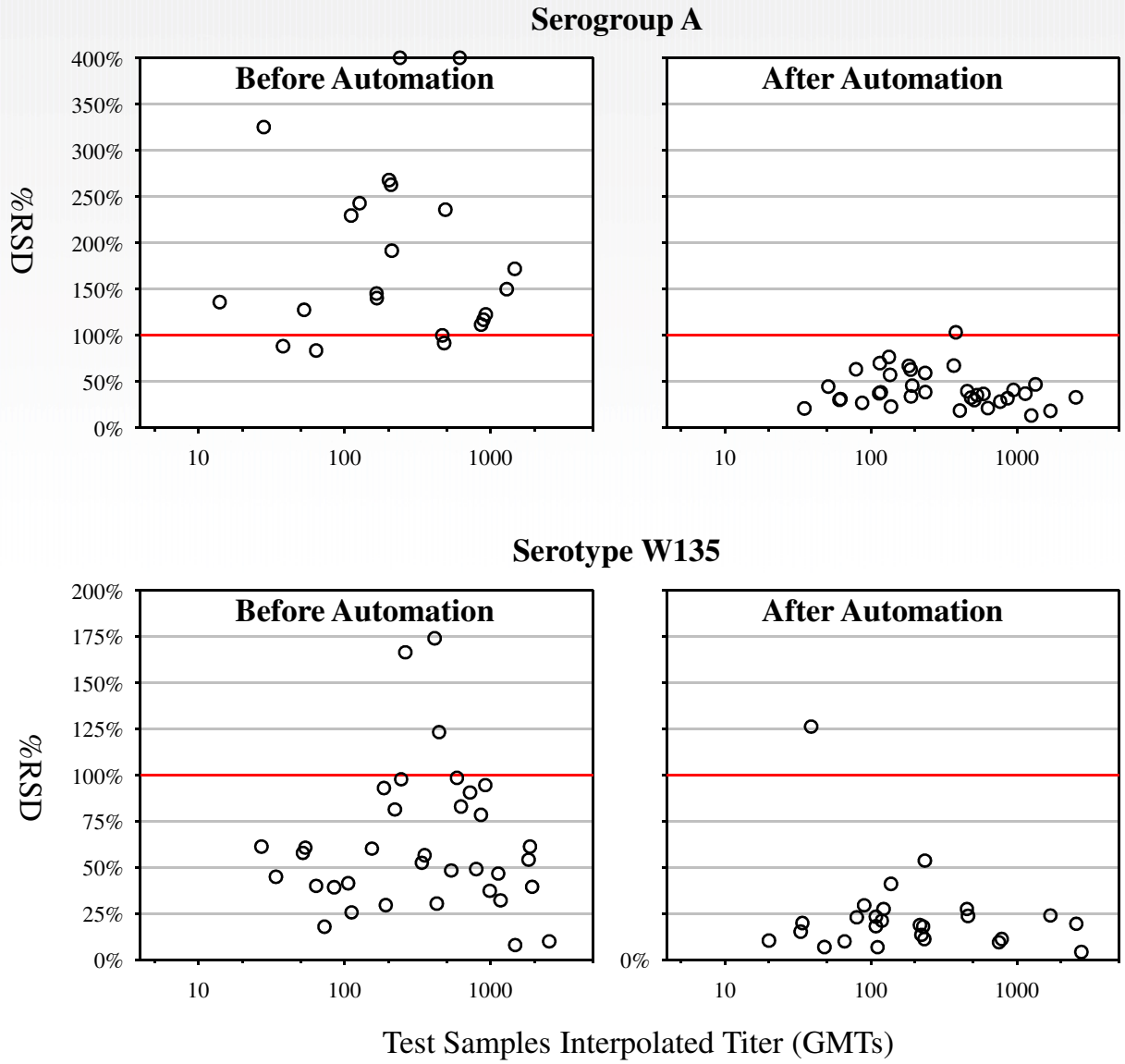


Deck is reloaded 1-2 times dependent on the number of Master plates.

Improvement of Overall Assay Precision After the Implementation of the Automation step (Serogroups C and Y).



Improvement of Overall Assay Precision After the Implementation of the Automation Step



Applied Biosystem 3730xl DNA Analyzer-Sequencing

- The ABI 3730xl DNA Analyzer
  - Fully automated sequence analyzer
  - 16-plate stacker
  - Monitoring software
    - will alert an analyst of any errors

Increased Efficiencies - Sequencing

- GE MegaBACE 1000
  - Assay 1 = 3.0 hour run / 96-well plate
    - Plus 30 minutes touch time / plate
  - Max three 96-well plates / day
    - 288 samples
  - 1 FTE, 1.5 hours touch time
- 1.5 hours touch time = 288 samples
  - Assay 2 = 5.0 hour run / 96-well plate
    - Plus 30 minutes touch time / plate
  - Max two 96-well plates / day
    - 192 samples
  - 1 FTE, 1.0 hours touch time
- 1.0 hours touch time = 192 samples

- ABI 3730xl
  - Weekday
  - Assay 1 and 2 = 2 hour run / 96-well plate
    - Miniscule amount of touch time to load run settings
  - Max 12 plates in 24 hours
    - 1, 152 samples
  - 1 FTE, 30 mins touch time
- 30 minutes touch time = 1, 152 samples
  - Friday Night
  - Max 16 plates / stacker
    - 1, 536 samples
  - 1 FTE, 30 mins touch time
- 30 minutes touch time = 1, 536 samples

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Improvement of Analyst-related Variability After Implementation of the Automation Step

	Variability (%RSD) Attibuted to Analyst	
Serogroup	Before Automation	After Automation
A	91%	5%
C	15%	0%
Y	29%	0%
W135	19%	0%

	Fold-Difference in Titer Between Analysts	
Serogroup	Before Automation	After Automation
A	5.7	1.4
C	2.1	1.3
Y	2.4	1.5
W135	1.5	1.1

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Benefits of Automating Immunoassays

- A contemporary immunoassay used for clinical trials is typically a complex multistep process which is qualified and validated.
- Automation of the most labor intensive and manual steps of immunoassays often improves assay precision and reduces the possibility of costly human errors.
- Automated liquid handling technology should be incorporated in the assay workflow as early as possible, ideally, at the development stage.

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