Automation and multiplexing of immunoassays: Improving precision and throughput

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PPD

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Automation of Assays: Improving Precision and Throughput

Ilia I. Tikhonov, Director of Vaccines & Biologics Lab, PPD
23 May 2012
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.
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.
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)
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.
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.
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.
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

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Before Automation</th>
<th>After Automation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>175%</td>
<td>41%</td>
</tr>
<tr>
<td>C</td>
<td>100%</td>
<td>36%</td>
</tr>
<tr>
<td>Y</td>
<td>65%</td>
<td>27%</td>
</tr>
<tr>
<td>W135</td>
<td>74%</td>
<td>35%</td>
</tr>
</tbody>
</table>

Total Assay Variability (%RSD)

Before Automation

<table>
<thead>
<tr>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
</tr>
<tr>
<td>50%</td>
</tr>
<tr>
<td>100%</td>
</tr>
<tr>
<td>150%</td>
</tr>
<tr>
<td>200%</td>
</tr>
<tr>
<td>250%</td>
</tr>
<tr>
<td>300%</td>
</tr>
<tr>
<td>350%</td>
</tr>
</tbody>
</table>

After Automation

<table>
<thead>
<tr>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
</tr>
<tr>
<td>50%</td>
</tr>
<tr>
<td>100%</td>
</tr>
<tr>
<td>150%</td>
</tr>
<tr>
<td>200%</td>
</tr>
<tr>
<td>250%</td>
</tr>
<tr>
<td>300%</td>
</tr>
<tr>
<td>350%</td>
</tr>
</tbody>
</table>

Serogroup A

Graphs showing the distribution of %RSD before and after automation for Serogroup A.
Example 2.
Automation of Seasonal Influenza HAI
**Principle of the Hemagglutination Inhibition Assay**

**Hemagglutination**

Red blood cells + Viruses → Hemagglutination

**Inhibition of hemagglutination**

Red blood cells + Antiviral antibodies from serum + Viruses → Viruses neutralized and hemagglutination inhibited
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

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
Seasonal Flu HAI: Replicate Repeat Rate Results

Bias Graph

Sample Titer

Fold-Bias in Titer Between Analysts

Manual
Hamilton
Perfect Agreement
2-Fold Bounds
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
General PCR Workflow

- Isolation and preparation of nucleic acid template
- RT-PCR (RNA template)
- Detection, quantitation
- Analysis of products (i.e., sequencing)

Each of these steps can be automated
Nucleic Acid Extraction: QIAGEN BioRobot MDx (DNA) and BioMérieux 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
### Increased Efficiencies – DNA and RNA Extraction

<table>
<thead>
<tr>
<th>Method</th>
<th>Touch Time</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual DNA Extraction (QIAGEN Blood Kit)</td>
<td>6 hours</td>
<td>384</td>
</tr>
<tr>
<td>Automated Extraction (Qiagen BioRobot MDx)</td>
<td>3 hours</td>
<td>1152</td>
</tr>
<tr>
<td>QIAGEN Manual Method</td>
<td>4.5 hours</td>
<td>72</td>
</tr>
<tr>
<td>Boom Silica Manual Method</td>
<td>5 hours</td>
<td>48</td>
</tr>
<tr>
<td>BioMérieux NucliSens easyMAG</td>
<td>3 hours</td>
<td>144</td>
</tr>
</tbody>
</table>
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 throughput 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
**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**

**Diagram:**
- Denature template DNA
- Gene of interest
- Primers and probe anneal
- Reporter
- Quencher
- As Taq extends, proximity of reporter dye to quencher dye is lost and fluorescent signal is generated for the product of amplification produced
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.
Typical characteristics of the assay:
- The assay is linear over 6 logarithms of concentration
- Each target is amplified equivalently
- Each target has similar sensitivity
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
• 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?**
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.
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)
Variability: Summary for SBAs

<table>
<thead>
<tr>
<th>Sero group</th>
<th>Analyst</th>
<th>Before automation</th>
<th>After automation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Run Within Analyst</td>
<td>Plate Within Run</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>86%</td>
<td>19%</td>
</tr>
<tr>
<td>W</td>
<td></td>
<td>0%</td>
<td>18%</td>
</tr>
<tr>
<td>Y</td>
<td></td>
<td>14%</td>
<td>13%</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>256%</td>
<td>20%</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>98%</td>
<td>29%</td>
</tr>
<tr>
<td>W</td>
<td></td>
<td>78%</td>
<td>9%</td>
</tr>
<tr>
<td>Y</td>
<td></td>
<td>41%</td>
<td>28%</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>20%</td>
<td>0%</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>6%</td>
<td>19%</td>
</tr>
<tr>
<td>W</td>
<td></td>
<td>26%</td>
<td>13%</td>
</tr>
<tr>
<td>Y</td>
<td></td>
<td>38%</td>
<td>65%</td>
</tr>
</tbody>
</table>
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).

Serogroup C

Before Automation

After Automation

Serogroup Y

Before Automation

After Automation

Test Samples Interpolated Titer
Improvement of Overall Assay Precision After the Implementation of the Automation Step

Serogroup A

Before Automation

After Automation

Serotype W135

Before Automation

After Automation

Test Samples Interpolated Titer (GMTs)
• The ABI 3730xl DNA Analyzer
  - Fully automated sequence analyzer
  - 16-plate stacker
  - Monitoring software
    • will alert an analyst of any errors
### Increased Efficiencies - Sequencing

| Instrument          | Assay 1 Duration                  | Max Plates per Day | FTE, Touch Time
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GE MegaBACE 1000</strong></td>
<td>Assay 1 = 3.0 hour run / 96-well plate</td>
<td>Max three 96-well plates / day</td>
<td>1 FTE, 1.5 hours touch time</td>
</tr>
<tr>
<td></td>
<td>Plus 30 minutes touch time / plate</td>
<td>288 samples</td>
<td>1.5 hours touch time = 288 samples</td>
</tr>
<tr>
<td></td>
<td>Assay 2 = 5.0 hour run / 96-well plate</td>
<td>Max two 96-well plates / day</td>
<td>1 FTE, 1.0 hours touch time</td>
</tr>
<tr>
<td></td>
<td>Plus 30 minutes touch time / plate</td>
<td>192 samples</td>
<td>1.0 hours touch time = 192 samples</td>
</tr>
<tr>
<td><strong>ABI 3730xl</strong></td>
<td>Weekday Assay 1 and 2 = 2 hour run / 96-well plate</td>
<td>Max 12 plates in 24 hours</td>
<td>1 FTE, 30 mins touch time</td>
</tr>
<tr>
<td></td>
<td>Miniscule amount of touch time to load run settings</td>
<td>1, 152 samples</td>
<td>30 minutes touch time = 1, 152 samples</td>
</tr>
<tr>
<td></td>
<td>Friday Night Assay 2 = 5.0 hour run / 96-well plate</td>
<td>Max 16 plates / stacker</td>
<td>1 FTE, 30 mins touch time</td>
</tr>
<tr>
<td></td>
<td>Plus 30 minutes touch time / plate</td>
<td>1, 536 samples</td>
<td>30 minutes touch time = 1, 536 samples</td>
</tr>
</tbody>
</table>
## Improvement of Analyst-related Variability After Implementation of the Automation Step

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Variability (%RSD) Attributed to Analyst</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Automation</td>
</tr>
<tr>
<td>A</td>
<td>91%</td>
</tr>
<tr>
<td>C</td>
<td>15%</td>
</tr>
<tr>
<td>Y</td>
<td>29%</td>
</tr>
<tr>
<td>W135</td>
<td>19%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Fold-Difference in Titer Between Analysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Automation</td>
</tr>
<tr>
<td>A</td>
<td>5.7</td>
</tr>
<tr>
<td>C</td>
<td>2.1</td>
</tr>
<tr>
<td>Y</td>
<td>2.4</td>
</tr>
<tr>
<td>W135</td>
<td>1.5</td>
</tr>
</tbody>
</table>
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.