Automating Sample Prep to Eliminate the Disconnect Between Processes and People

Paul J. Jannetto, PhD, DABCC, FACB, MT(ASCP)
Mayo Clinic
Director, Toxicology & Drug Monitoring Laboratory
Director, Metals Laboratory
Financial Disclosures

• None
Objectives

After this session, the attendee will be able to:

• Identify manual sample preparation steps in the laboratory which could be easily automated.

• Describe the process of automating sample preparation from start to finish

• Evaluate the advantages and disadvantages of automating sample preparation

• Justify task-targeted sample prep automation
Outline

• Discuss the process of automating sample preparation
  • Identify challenges
  • Needs
  • Solutions

• Define the advantages/disadvantages of sample prep automation

• Share examples of past and on-going automation projects in the Toxicology and Drug Monitoring Lab at Mayo Clinic
  • Lamotrigine & Levetiracetam (serum)
  • Tacrolimus, Sirolimus, Cyclosporin A, and Everolimus (whole blood)
  • Drug of abuse screens (meconium)
  • Opioid, Benzodiazepine, Amphetamine confirmations (urine)
Automated Liquid Handling & Robotics

• A lot of vendor options and configurations available
  • 1,000’s of possible configurations

Where do you start???
Identification of Applications for Automation

• What applications can automation do?
  • Nucleic acid purification
  • PCR setup
  • Sequencing
  • Microarray sample prep
  • Cloning
  • Protein crystallization
  • In-gel digestion
  • MALDI TOF spotting
  • Protein precipitation
  • Colony picking
  • ADMET
  • Solubility assays
  • Compound handling
  • Solid phase extraction
  • Liquid-liquid extraction
  • ELISA processing
  • Blood grouping
  • Pooling
  • Mixing
  • Combinational Chemistry
### Why Consider Automation?

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Consistency</td>
<td>• Cost/Affordability $$$</td>
</tr>
<tr>
<td>• Precision</td>
<td>• Limited lab space</td>
</tr>
<tr>
<td>• Accuracy</td>
<td>• Application/sample type may not be amendable to automation</td>
</tr>
<tr>
<td>• Personnel Safety</td>
<td></td>
</tr>
<tr>
<td>• Reduction in repetitive motion injury's</td>
<td></td>
</tr>
<tr>
<td>• ↑ throughput/efficiency</td>
<td></td>
</tr>
<tr>
<td>• ↓ TAT</td>
<td></td>
</tr>
<tr>
<td>• Financial Savings</td>
<td></td>
</tr>
<tr>
<td>• Labor</td>
<td></td>
</tr>
<tr>
<td>• Supplies</td>
<td></td>
</tr>
</tbody>
</table>
Selecting the Right Automation Solution

- **Degree of automation needed**
  - Full (walk-away)
  - Partial

- **What tasks/steps do you want to automate?**
  - Pipetting
  - Plate sealing/unsealing
  - Washing
  - Incubation
  - Centrifugation
  - SPE/LLE
  - Mixing
  - Transferring

- **Scalability**
  - Current and future volume

- **Required throughput**
  - # of samples/plates/assays
Selecting the Right Automation Solution

• Integration
  • Data handling
  • Sample tracking
  • LIS/HIS system

• Financial restrictions

• Physical restrictions
  • Space
The Automation Process

1. Establish Lab Needs/Challenges
2. Define Automation Goals
3. RFP Identify Solutions
4. Project Realization/Build
5. Installation/Validation
6. Go Live & Post Live Support
Example #1: Serum Sample Prep
(Mayo Clinic Toxicology and Drug Monitoring Lab)

• Assays: Lamotrigine/Levetiracetam (anti-epileptic meds)
• Challenges:
  • High volume LC/MS/MS assays
  • TAT is clinically important
  • Completely manual extraction/sample preparation
• Needs/Goals:
  • Fully automate sample prep process
  • Simplify sample clean-up (dilute-n-shoot)
  • Improve assay performance
  • Achieve some labor/cost savings
  • Improve assay TAT
Solution: Hamilton Microlab Starlet

- Preliminary Outcome (Development Stage of New Assay):
  - Fully automated sample prep (dilution)
  - Injected diluted samples onto LC/MS/MS using TLX-4 with TurboFlow columns
  - Worked great under developmental conditions
  - Under high-volume clinical conditions it wasn’t robust without additional sample prep (protein crash)

- Final Outcomes:
  - Partially automated assay:
    - Automatically pipet calibrators, QC, patient samples (50 µL) into DW96 plates
    - Hamilton then adds internal standard (450 µL)
    - Manually cover/vortex DW96 plate stack
    - Uncover/place on positive pressure manifold
    - Cover and analyze collection plate by LC/MS/MS

Only partial automation, was this a success or failure?
Mayo’s Solution: Continued

- Final Outcomes:
  - Improved TAT/throughput
Mayo’s Solution: Continued

- Final Outcomes:
  - Improved Precision/Assay performance:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Lamotrigine CV Before Automation</th>
<th>Lamotrigine CV After Automation</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC Level 1 (~5 µg/mL)</td>
<td>8.7%</td>
<td>3.5%</td>
</tr>
<tr>
<td>QC Level 2 (~20 µg/mL)</td>
<td>8.9%</td>
<td>2.9%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Levetiracetam CV Before Automation</th>
<th>Levetiracem CV After Automation</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC Level 1 (~5 µg/mL)</td>
<td>5.7%</td>
<td>2.8%</td>
</tr>
<tr>
<td>QC Level 2 (~40 µg/mL)</td>
<td>5.7%</td>
<td>4%</td>
</tr>
</tbody>
</table>
Mayo’s Solution: Continued

• Final Outcomes:
  • Financial savings:
    • $1.73/sample (> $135,000/year)

End Result
Example #2: Whole Blood Sample Prep

- Assays: Everolimus, Sirolimus, Tacrolimus, and Cyclosporin A
- Challenges:
  - High volume LC/MS/MS assays
  - TAT is clinically important
  - Completely manual extraction/sample preparation
  - Whole blood samples
- Needs/Goals:
  - Automate sample prep process (lysis/protein precipitation)
  - Improve assay performance
  - Achieve some labor/cost savings
  - Improve assay TAT
  - Use same automation solution/platform from example #1
Immunosuppressant Workflow

- **Steps:**
  1. Sort samples into two separate batches:
     - Siro/CsA
     - Tacro/Evero
  2. Mix (rock) samples 5 min
  3. Pipet sample into deep well plate
  4. Add internal standard
  5. Mix thoroughly
  6. Centrifuge
  7. Transfer supernatant into a new deep well plate
  8. Analyze on LC/MS/MS
Immunosuppressant Layout

- Sample Tips
- Sample Racks
- 2 mL Extraction Plate
- 2 mL Plate after Centrifugation
- 1.5 mL Final Plate
- EVERO / TACRO Internal Standard Boats
- Samples 1-24
- Samples 73-96 – Not used – Do Not Run batches larger than 63
- SIRO / CYC Internal Standard Boats
- Samples 25-48
- Samples 49-72
Example #2 continued

• Final Outcomes:
  • Partially automated assay
  • Improved TAT
    • Consistently >95%
  • Improved assay performance
    • %CV
  • Improved efficiency/labor savings
    • 0.5 FTE
Example #3: Meconium Drug of Abuse Screen

• Assays: Amphetamine/Methamphetamine, Cocaine(BE), Opiates, PCP, THC-COOH

• Challenges:
  • Meconium is a difficult sample; sticky, non-homogenous
  • TAT/implications of results is clinically important
  • Completely manual sample preparation/analysis

• Needs/Goals:
  • Automate sample analysis
  • Improve assay performance
  • Achieve some labor/cost savings
  • Improve assay TAT
Meconium Drug Screen Workflow

• Steps:
  1. Weigh out 0.25 g meconium
  2. Add specimen diluent, make slurry
  3. Vortex 5 min
  4. Centrifuge
  5. Transfer 1 mL supernatant to labeled 13 x 75 tubes, use barcode comparator
  6. Pipet calibrators, QC, patient samples into appropriately coated plates (Amp, Methamp, Cocaine, THC, PCP, and Opiate)
  7. Add pre-buffer (PCP, Amp only)
  8. Add enzyme conjugate
  9. Incubate 30-60 min (assay dependent) at RT in dark
  10. Wash plate 6X
  11. Add substrate
  12. Incubate 30 min at RT in dark
  13. Add stop solution
  14. Read plates
What Steps Are Most Amendable to Automation?

- Steps:
  1. Weigh out 0.25 g meconium
  2. Add specimen diluent, make slurry
  3. Vortex 5 min
  4. Centrifuge
  5. Transfer 1 mL supernatant to labeled 13 x 75 tubes, use barcode comparator
  6. Pipet calibrators, QC, patient samples into appropriately coated plates (Amp, Methamp, Cocaine, THC, PCP, and Opiate)
  7. Add pre-buffer (PCP, Amp only)
  8. Add enzyme conjugate
  9. Incubate 30-60 min (assay dependent) at RT in dark
  10. Wash plate 6X
  11. Add substrate
  12. Incubate 30 min at RT in dark
  13. Add stop solution
  14. Read plates
Meconium Deck Setup on Starlet

- Incubators
- Plate washer
- Plate reader
- Reagents
- 1000µL Tips
- Plates
- Samples
- 300µL Tips
Example #3: continued

- Final Outcomes:
  - Partially automated assay
  - Meconium sample prep not amendable to automation, so remains a manual step

- Improved TAT
  - Consistently >90%

- Improved assay performance
  - %CV
  - Decreased assay failure rate

- Improved efficiency/labor savings
  - 0.5 FTE
Example #4: Urine Opioid, Benzodiazepine, Amphetamine Confirmations

• Challenges:
  • High volume urine assays which are being converted from GC/MS to LC/MS/MS
  • TAT is important (competition offers 2 day TAT)
  • Completely manual SPE and sample preparation
  • Tech time better spent reviewing GC/MS or LC/MS/MS data vs. pipetting and performing extractions
  • Cost/test is high (need to be marketable)
Example #4: continued

• Needs:
  • Ability to fully automate sample prep process
  • Improve assay TAT/throughput
  • Improve assay performance
  • Achieve some labor/cost savings (↓ cost/test)
  • System must be able to accommodate:
    • Pipetting from 12 x 75 mm tubes to deep well plates; plate-to-plate transfers
  • Tasks Included:
    • Barcoding/tracking of samples/plates
    • Pipetting
    • Mixing
    • Plate Sealing/Unsealing
    • Incubations (RT and 50°C)
    • SPE
    • Centrifugation
  • Flexible scheduling
  • Scalable
Typical Urine Confirmation Assay Workflow

• Steps:
  1. Pipet calibrators, QC, patient samples into 96-deep well plates from 13 x 75 mm barcoded tubes
  2. Add internal standard to each well
  3. Add enzyme (β-glucuronidase) to each well
  4. Add buffer to each well
  5. Mix thoroughly
  6. Cover plate
  7. Incubate 2 hours at 50°C
  8. Centrifuge plate
  9. Uncover plate
  10. Transfer 100 µL supernatant to filter plate stacked on collection plate
  11. Add 0.5 mL deionized water to filter plate
  12. Use positive pressure/vacuum to pull sample through filter plate
  13. Cover collection plate
  14. Transfer collection plate to LC/MS/MS for analysis

Not applicable to all assays
Example #4: continued

- Options (alphabetical order):
  1. Agilent:
  2. Beckman Coulter
  3. Hamilton
  4. Tecan
  5. Others…
Additional 3rd Party Components/Options

- Hotels/Incubators

- Storage: Key questions:
  1. What is your current volume/workload and anticipated future volume/workload?
  2. What is your desired throughput?
  3. What is the rate limiting step?
  4. What is your budget constraints?

- Centrifuges:

- Plate sealers/peelers
Example #4: continued

- Solution:

To Be Determined
Summary

• Liquid handling robotics can be used successfully to automate sample preparation and analysis

• Key to success is to identify which steps are most amendable to automation and what steps could/should be left to manual intervention

• Sample prep automation is scalable, which also makes it more affordable since you can usually add components at a later time as your volumes/needs grow
The Conclusion

• Automated sample preparation can lead to:
  • Increased efficiency/throughput
  • Financial and labor savings
  • Improved safety for lab personnel
  • Decreased TAT
  • Improved assay performance