20 TIPS TO OPTIMIZE YOUR AUTOMATED ASSAY

Slides originally presented by Thomas E. Strader, MS-Biotech at the Midwest LRIG 2018 annual conference on May 17, 2018 at the Madison Marriott West in Middleton, WI, USA.
This talk – 20 tips

20 tips to consider to help speed up and ease the optimization process
Tip 1 – Look carefully at the assay workflow.

Assay components (buffers, compounds, cells)
Experimental design
Dispensing steps (volumes, #, time-critical)
Plate format
Timing
Mixing

Take a careful look through the manual assay protocol. It is useful to have a clear understanding of the chemistry, what occurs at each step and what end-result is expected.
Tip 2 – Perform the assay manually first.

First-hand experience
How it “should work” in the larger format
Timing
Dispense volumes
Qualities of the liquids
Watch/learn from the assay developers

Reading the protocol is one thing; running the assay is another! Try to perform the assay manually before automating to find out where the time-consuming steps are and which reagents are tricky to dispense.
Tip 3 – Get to know the assay reagents.

Qualities
Color
Precipitate
Temperature
Total volume supplied
Evaporative

Take time to play with reagents before creating an automated method. Quickly you will have a better sense of how each reagent flows as well as learn which components may be more tricky to dispense.
Tip 4 – Pick the best-suited robot(s).

- Expected throughput
- 8-, 96-, 384-channel dispenser
- Accuracy/precision
- Low volumes
- Plate movement
- Pricey components
- Temperature control
- Tip type
- Flexibility
- Software
- Speed
- Ease of use

Robot selection will depend on availability. Try to use the robot(s) that best-fit the majority of your assay dispensing needs.
**Tip 5 – Seek out suitable labware.**

Tips  
Microplates  
Troughs  
Tubes  
Compound storage vessels  

Get creative! Look online or ask your colleagues for novel plates and reservoirs that might better fit to your assay workflow and minimize dead volumes.
Tip 6 – Use plates with clear-bottoms to visualize cells.

Select a plate that provides you with the most return. Clear-bottom plates enable you to visibly check for changes, such as in cell-based assays with treatments.
Tip 7 – Simplify the dispensing steps.

Combine addition steps
Remove mix steps
Reuse tips
Remove unnecessary washes
Use existing robotic software tools (loops, worklist, titration)
Use sample tracking

Talk with the assay developers to learn if multiple assay components could be combined together to simplify the robotic method but not affect the chemistry. This can save assay time and simplify the assay overall.
Tip 8 – Stay within the recommended working volumes in assay plates.

96-well (75-200µl or LV 25-100µl)
384-well (20-80µl or LV 5-20µl)
1536-well (8µl)
3456-well (2.5µl)

Stick to the recommended working volumes for each assay plate. Crosstalk and carryover of liquid from neighboring wells can be prevented.
Tip 9 – Directly scaling volumes is not always best.

¼ volume in 96-well → 384-well

¼ volume in 384-well → 1536-well

Adjust based on individual reagents

Directly scaling down volumes per addition when using a higher density assay plate does not always work equally. Assay components may respond or act differently at lower volumes.
Tip 10 – Reuse or adapt existing methods.

Time savings

Different workflow approaches

Predefined labware or liquids

There is no need to reinvent the wheel. Look back at methods already created and “Save as” to create a new method.
Tip 11 – *Use robotic dispensing tricks and tools.*

Tip touch (well side, bottom)
Blow out
Liquid detection
Air gaps

Incorporate a tip touch to the side of plate walls or troughs to avoid drips which can lead to contamination and poor Z’-factors with inaccurate volumes.
Tip 12 – Reuse tips.

Cost savings
Conserve deck space
Wash tips
Pre-wetted
Simply method workflow

If the robot allows, consider reusing tips in the same method to perform subsequent dispensing steps.
Tip 13 – Perform practice runs (dry and wet).

Define labware accurately

Time the automated protocol

Check robot movements

Make corrections

Practice makes perfect, right? Test the automated method you have created first using empty labware. This will allow you to check and correct movement issues, dispensing heights, and make improvements to the methods without wasting precious assay reagents.
Tip 14 – Check that liquid properties are defined correctly.

Liquid class settings
Pipetting speeds
Sterility
Viscous
Foamy
Temperature
Density

Stick to the same volumes and temperatures used in the assay when characterizing the liquid properties on the robot.
**Tip 15 – Confirm dispensing locations in assay wells.**

Pause at each step of the method
Assess static in wells or on tips
Adjust labware definitions

During testing, consider introducing color/dye into your reagents to better visualize the exact location at dispensing. Often reagents stick to the sides of wells or tips!
Tip 16 – Assess effectiveness of mixing steps.

Plate shaker (orbital, linear)
Tip mix
Plate centrifugation
Larger volume additions
Bubbles

Pause the automated method between reagent additions to peek into wells to observe how well or poor components are mixing. High frequency shakers can be used for 384-well plate formats.
Tip 17 – Repeat the assay on another robot.

Easier/harder to perform

Different dispensing hardware

Software suitability

Customer needs

If time permits, create and test the assay protocol on another robot. You may be surprised to find performance differences between the instruments based on dispensing accuracy or workflow differences.
Tip 18 – Repeat the assay on another day.

Temperature
Humidity
Ozone
Smog
Coworkers
Time
Instrument components
Tip 19 – Maintain your instruments.

Clean tips and tubing
Run diagnostic checks
Clear used labware
Clean deck surfaces

Maintain your robots on a weekly basis. They are expensive and time-consuming to repair.
Tip 20 – Show your colleagues.

Suggestions
Improvements
Corrections
Different design
Feedback

Share what you have learned and created with your coworkers. Having a few eyes on the task can help provide insight and improvements that you did not think of. Even if they do not seem too interested or “automation saavy”, warm them up to the world of automation!
**Final thoughts**

Think about your own jobs

Take time to plan before automating

Look for new tools to help ease your assay workflow

Talk with others about automation issues you have
We can't solve problems by using the same kind of thinking we used when we created them.
Thank you!

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