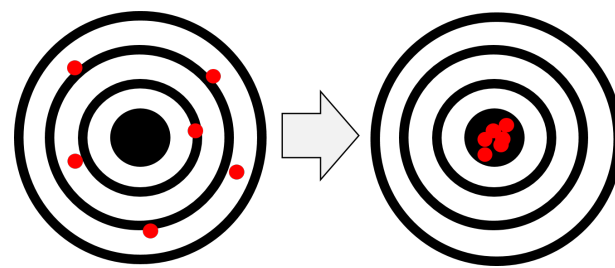
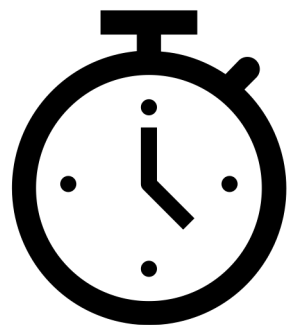
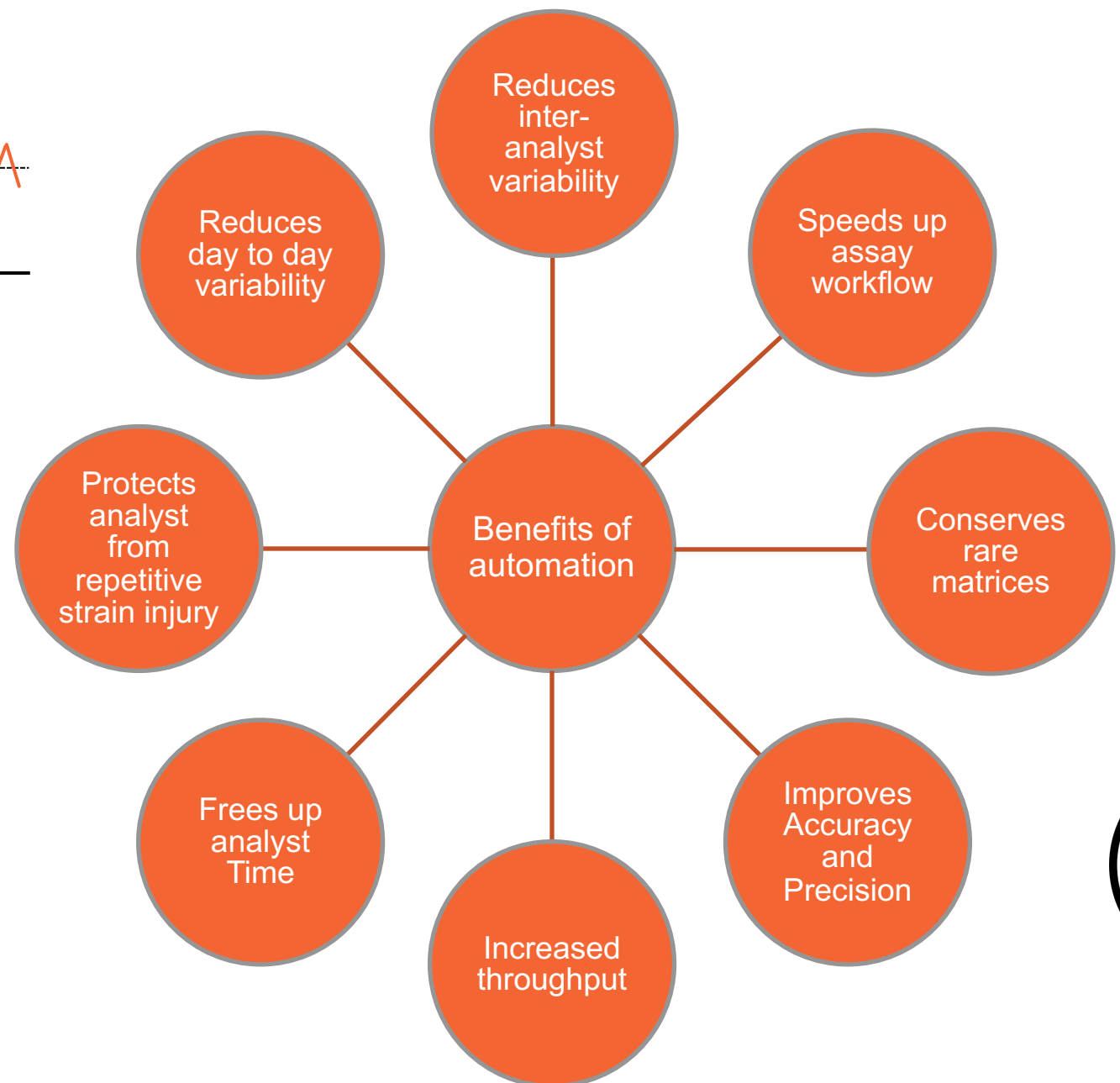
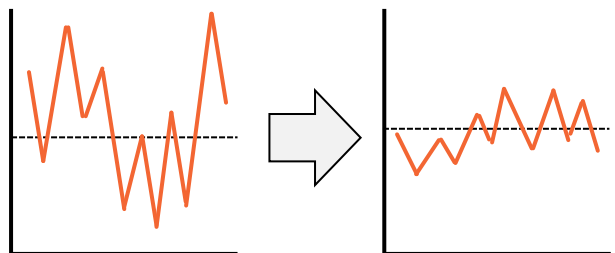




The Evolution of Automation for Ligand Binding Assays

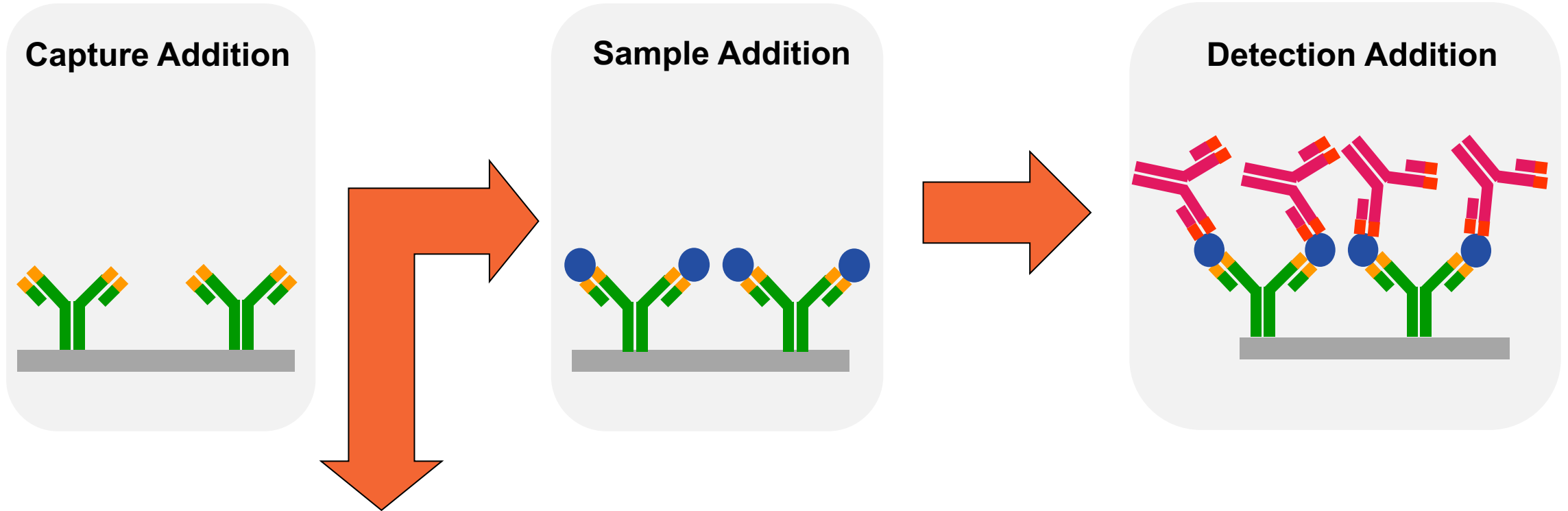
Rosie Penford - GSK

Benefits Of Automation



Automating Ligand Binding Assays

Stages of an Ligand Binding Assay which we can try to automate:



- **Sample preparation (MRD)**
- **Calibration Curve Preparation**
- **Bulk calibrator working stock and QC prep**

Historic Automation Approach

End To End Automation

- Liquid handlers such as the Hamilton Star and Agilent Bravo offer the option of end to end automation
- Multiple steps of an assay performed by one platform

Advantages

- Analyst can “press play and walk away”
- Build in complex protocols
- Can be integrated with barcode readers
- Isolated work station
- High throughput

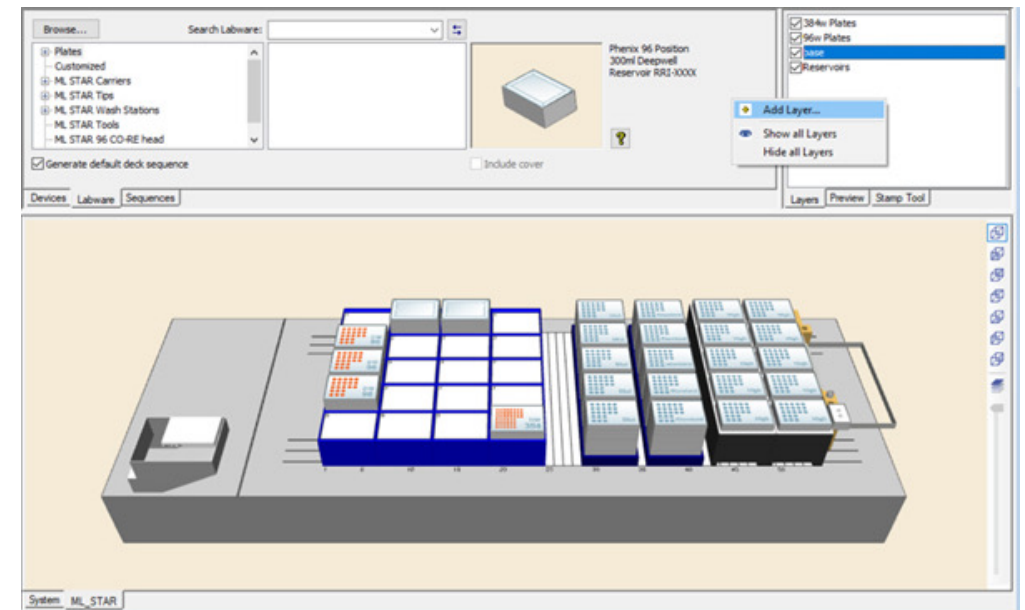
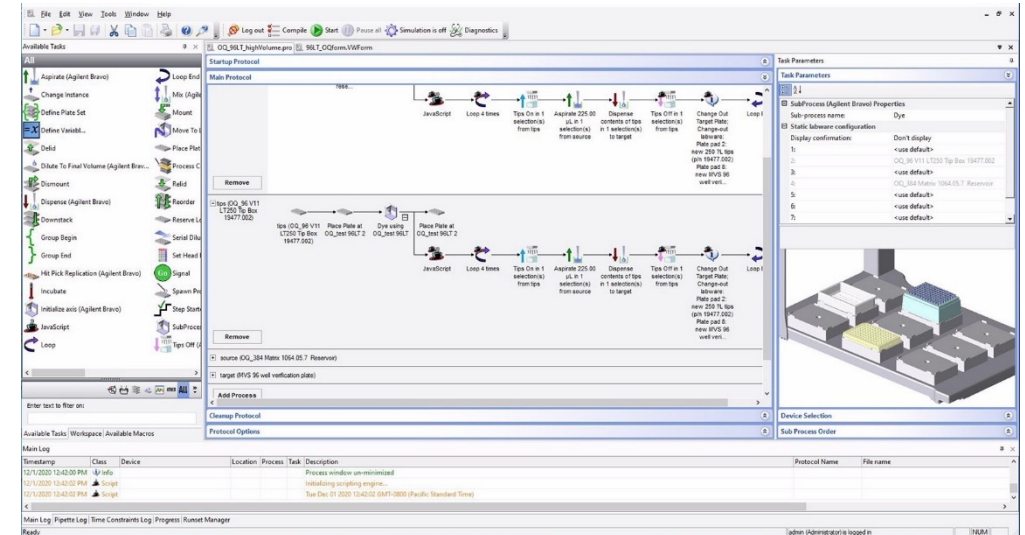


Historic Automation Approach

End To End Automation

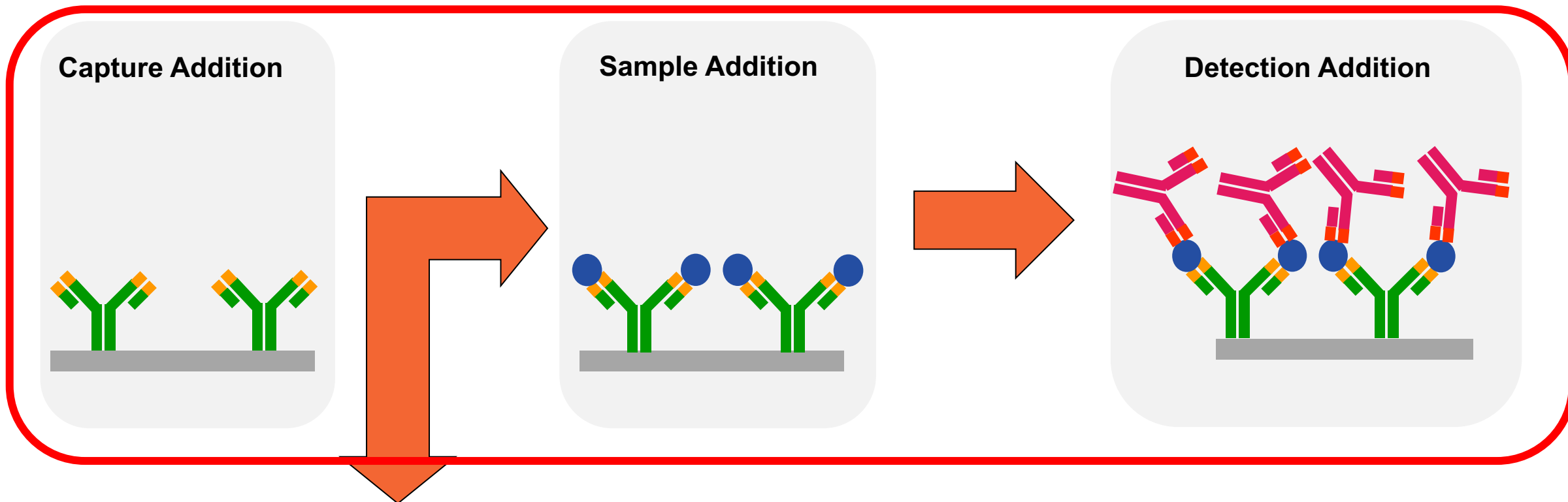
Disadvantages:

- Upfront work
 - **Complex programming**
 - Time consuming
 - Automation specialist often required
- Time required for analyst training
- Complicated troubleshooting
- Whole system cannot be used if one section breaks
- **Air displacement pipetting**



Recent Automation Approach

Isolated automated steps



- **Sample preparation (MRD)**
- **Calibration Curve Preparation**
- **Bulk calibrator working stock and QC prep**

Islands of Automation

- **Scientists move plates not liquids**

Recent Automation Approach

Capture, sample & Detection Addition- Plate based assays

Avidien MicroPro 300

- Benchtop 96 channel pipettor
- Volume range: 5-300 μ L
- 96 well and 384 well capability
- Ideal for plate stamping
- Very user friendly:
 - Minimal upfront programming is required
- **Takes seconds to run**



SPT Labtech Dragonfly

- Multi-channel **non contact** automated dispensing
- Utilises positive air displacement syringes
 - **No liquid classes**
- Volume range: 200nL- 4mL
- Low dead volume requirements
- Very user friendly:
 - Minimal upfront programming is required
- **Takes seconds to run**
- Excellent for method development
 - **DoE compatible**



Recent Automation Approach

Capture, sample & Detection Addition- Automated assay platforms

Ella

- Bench top automated ELISA platform
- 90 Minute run time
- Each well produces triplicate results
- Closed Cartridges:
 - Inbuilt calibration curve
 - only require wash buffer and sample to be added to cartridge



Quanterix HDX

- Fully Automated ultrasensitive immunoassay platform
- Utilizes Simoa Bead Technology
- High throughput with the capability of running 4x 96-well plates in one run



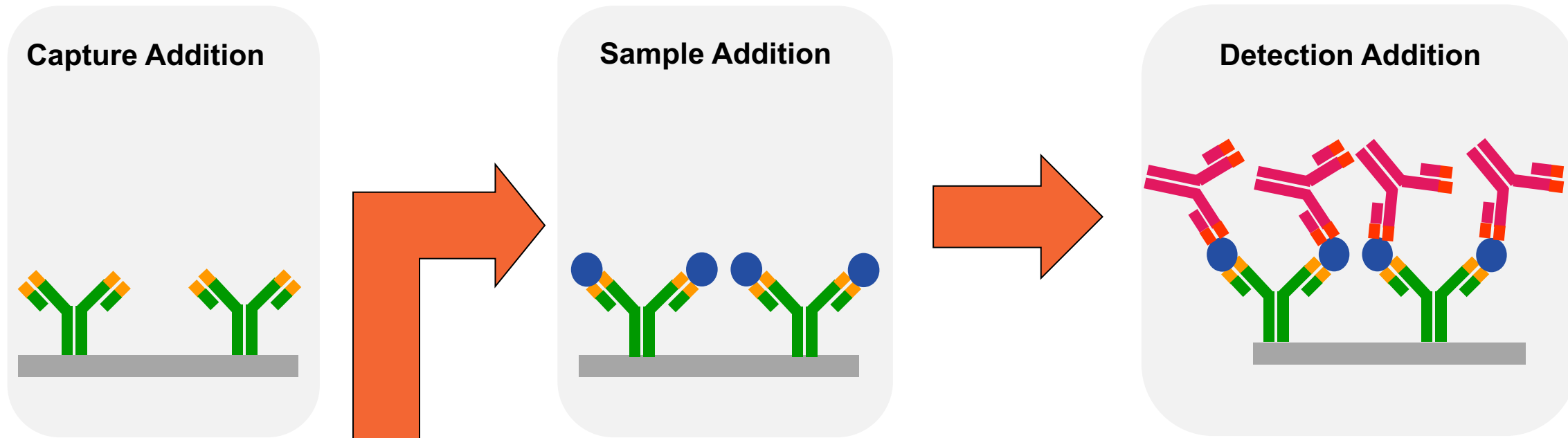
Gyrolab

- Fully Automated CD based immunoassay platform
- Utilises nanolitre microfluidic technology, reducing sample volume
- High throughput
- 5-10 μ L dead volume
 - Rare matrices



Recent Automation Approach

Isolated automated steps



- Sample preparation (MRD)
- Calibration Curve Preparation
- Bulk calibrator working stock and QC prep

Islands of Automation

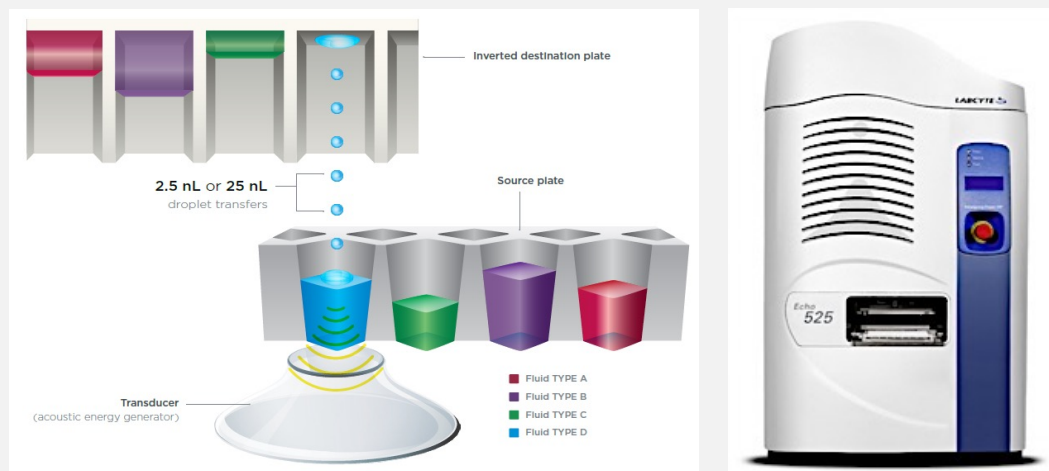
- Scientists move plates not liquids

Recent Automation Approach

Sample Preparation (MRD)

ECHO 525 Acoustic Dispenser

- Acoustic Liquid handler- Uses focused sound energy to eject precise nL droplets into an inverted microplate with excellent precision and accuracy
- Determines fluid composition, fluid height, and the power needed to eject a precise volume of fluid into the destination well
- Transfers drops of 25nL (transfer volume 25nL- 12 μ L)
- **Takes seconds to run**
- **Very user friendly-** Excel file containing volume is loaded into software



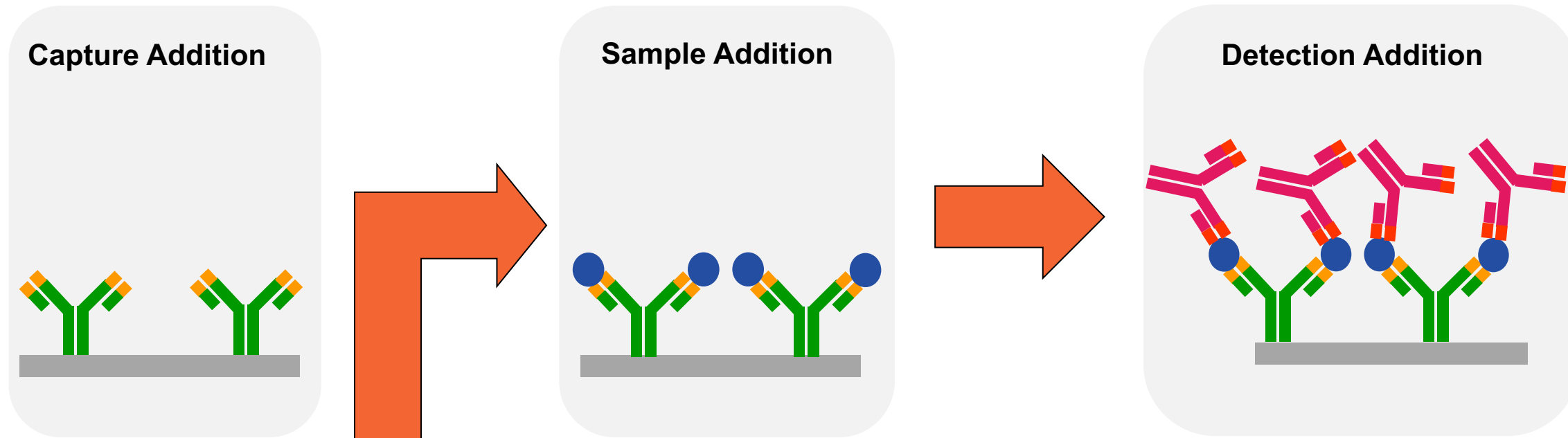
Examples

- E.g. 1:10 MRD (total volume 5000 nL)
 - Echo dispense 500 nL sample
 - Echo dispense 4500 nL buffer
- E.g. 1:100 MRD (total volume 10000 nL)
 - Echo Dispense 1000 nL sample
 - Dragonfly/ micropro Dispense 9000 nL buffer



Recent Automation Approach

Isolated automated steps



- Sample preparation (MRD)
- Calibration Curve Preparation
- Bulk calibrator working stock and QC prep

Islands of Automation

- Scientists move plates not liquids

Recent Automation Approaches

Calibration Curve preparation

Tecan D300e

Benchtop Digital dispenser

Calibration curve can be prepared by programming a titration or entering specific concentrations

Uses very little volume whilst also requiring minimal dead volume

Required minimal training and easy for an analyst to set up

Takes seconds to run

Highly reproducible

ECHO 525 Acoustic Liquid handler

SPT Labtech Dragonfly

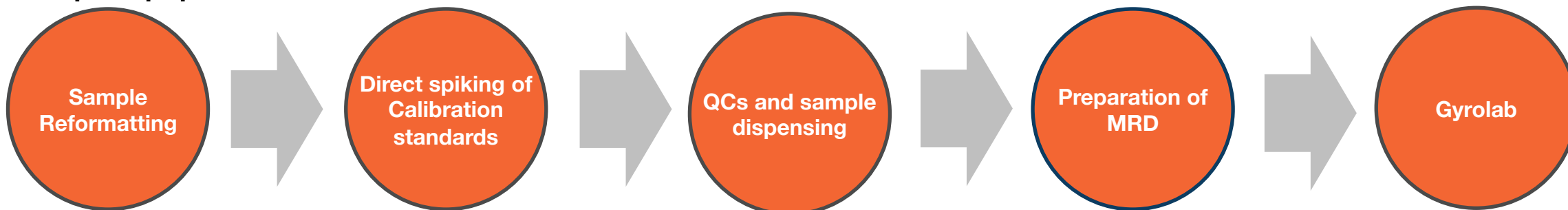
Comparison of Manually prepared calibration curve to calibration curve on the Tecan D300

		Manual	D300
Calibrator	fg/ml	%CV across 3 days	%CV across 3 days
1	69,714	2.40	0.29
2	17,428	7.11	1.10
3	4357	5.88	1.90
4	1089	3.63	5.38
5	272	2.49	3.93
6	68	5.72	2.83
7	17	10.71	13.57
8	0	N/A	N/A

Calibration curve data is taken from an MSD S-plex assay

Recent Automation Approach

Example pipette free workflow



Add samples, WS and QC's to 384 PP or 384 LDV plate using Bravo or MicroPro 300

Working stocks are spiked into matrix and transferred to destination plate (gyrolab plate) to prepare calibration line

QC's and samples are transferred to destination plate (gyrolab plate)

Assay buffer added to destination plate to perform MRD

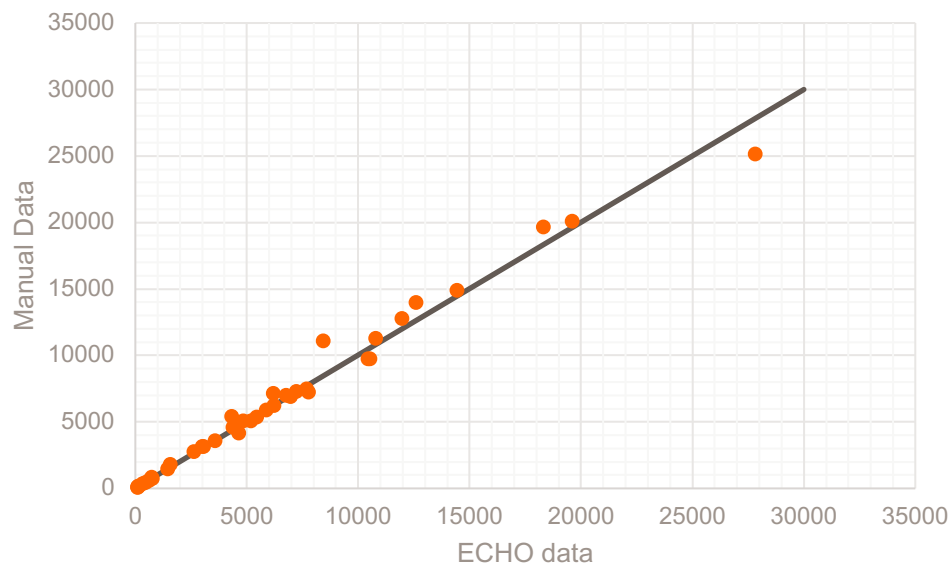


Recent Automation Approach

ECHO vs Manual preparation

nominal Conc (ng/mL)	Actual Conc (ng/mL)	Mean	SD	%CV	%Bias
30	29.2	28.47	1.00	3.50	-2.37
100	97.2	100.26	4.21	4.20	3.15
100	981.7	997.07	25.81	2.59	1.56
8000	7853.8	8087.56	420.67	5.20	2.98
10000	9817.3	10435.44	436.10	4.18	6.30

6 replicates of each validation control were analysed to assess within assay performance



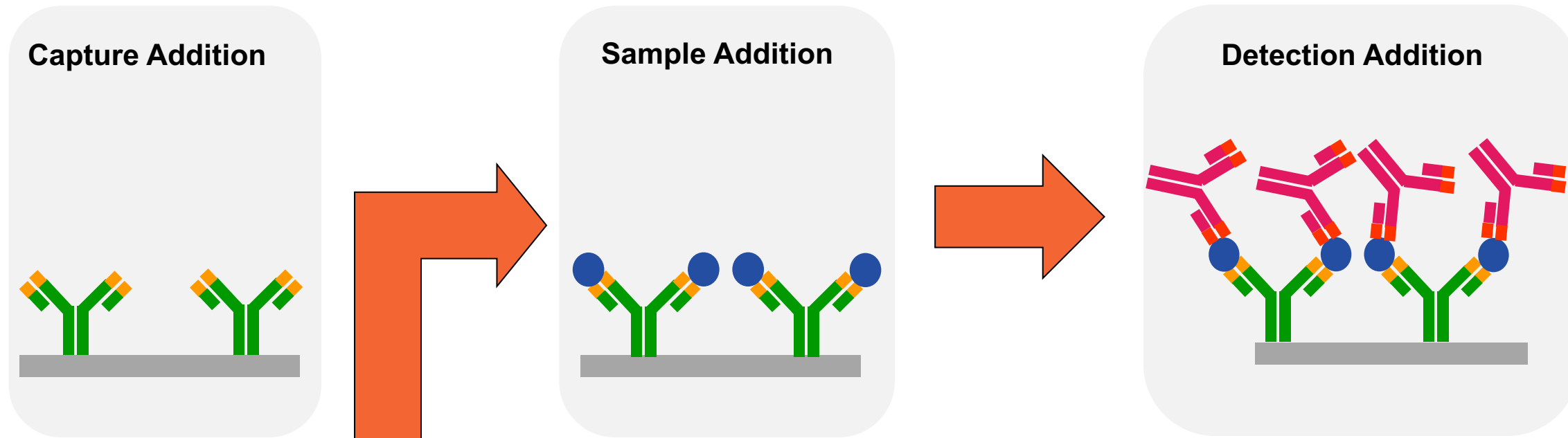
- Excellent correlation between data generated from manually and on the ECHO 525 liquid handler.
- 15 minutes to prepare a plate ready to run on the Gyrolab.
 - two centrifugation steps account for 10 minutes

Future Plans:

- Automate the calculation of the volumes required for each pipetting step directly from the LIMS worklist and import into the ECHO software.

Recent Automation Approach

Isolated automated steps



- Sample preparation (MRD)
- Calibration Curve Preparation
- Bulk calibrator working stock and QC prep

Islands of Automation

- Scientists move plates not liquids

Recent Automation Approaches

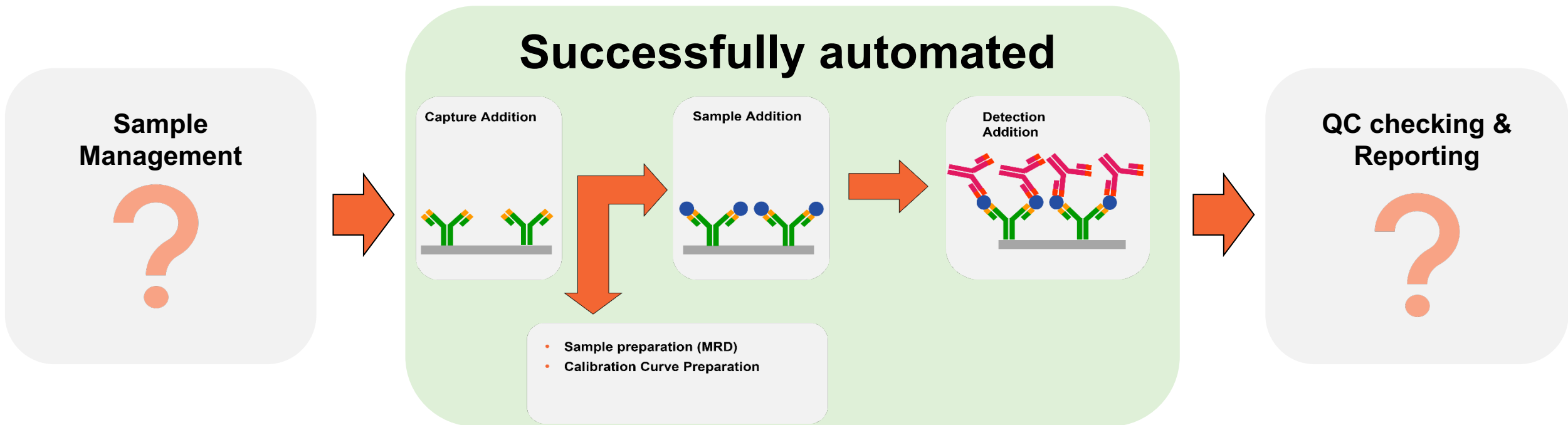
Bulk calibrator working stock and QC prep

Hamilton Microlab 600 Diluter

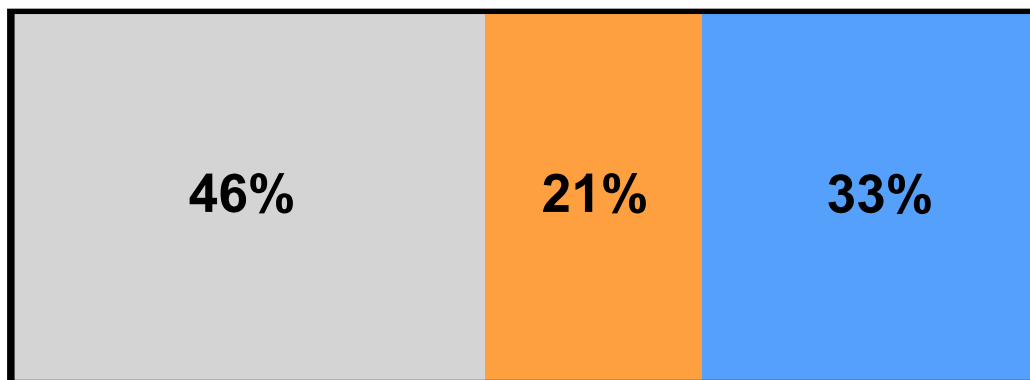


- Syringe pump for diluting and dispensing liquids
- Utilises positive air displacement syringes
 - **No liquid classes**
- Volume range: 10 μ L – 50mL
- Ideal for Bulk prepping Calibration curve working stocks and QCs at the start of a study

Final Considerations:



Resources required (5 people over 5 days)



- Pre-study
- Sample analysis and data processing
- Data Checking and Reporting

Acknowledgements

- Mike Wright
- Bob Biddlecombe
- Sanam Ahmad
- Eve Harding (placement student 2021-2022)

QUESTIONS?

GSK