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Small steps, Big advances

Unleashing the power of miniaturisation and automation for bioanalytical workflows

Mike Wright - EBF Open meeting 2023

Bioanalysis, Immunogenicity and Biomarker Group

Stevenage UK Bioanalysis Hub

Study support:

- Non-GLP discovery
- Studies forming part of Translational Build (research/enabling)
- Ph I > Ph II / Ph III*

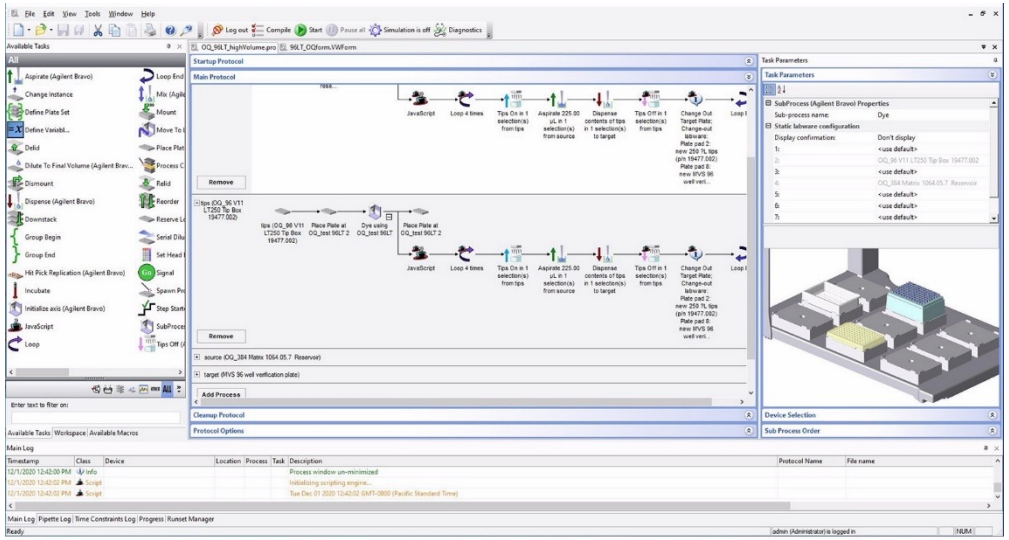
Wide variety of study sizes, platforms & endpoints:

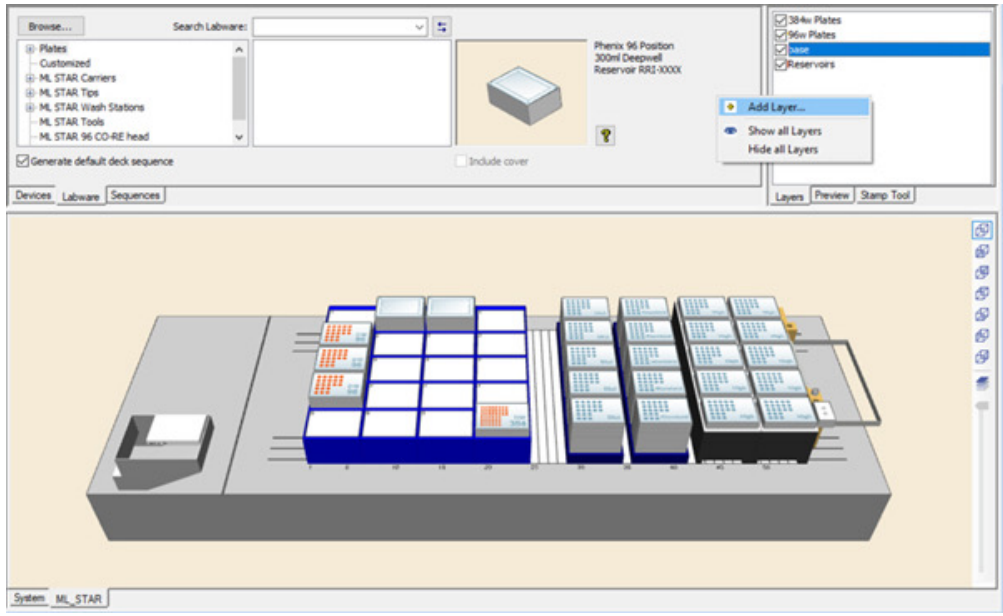
- 100s of samples to 1000s
- LBA (Gyrolab, MSD, ELLA, ELISpot, HD-X, ELISA etc)
- LC-MS
- Flow Cytometry
- PK and/or Biomarkers

Challenges with Historical Platforms

“Walk Away” instruments

- Significant upfront work
  - Complex programming
  - Automation specialist
- Time required for analyst training
- Complicated troubleshooting
  - Not modular
- Large footprint
- Low uptake by lab scientists
  - Fast-turn-around analysis





*Slide courtesy of Rosie Penford from YSS 2023:
“The Evolution of Automation for Ligand Binding Assays”

Changes in EHS Requirements Within GSK

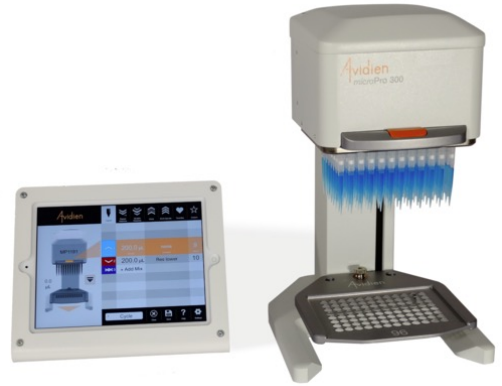
Newer Generation Platforms

Key commonalities

- Small footprint
- Takes seconds to use
- Very simple programming
  - Touchpad
  - Excel Spreadsheet

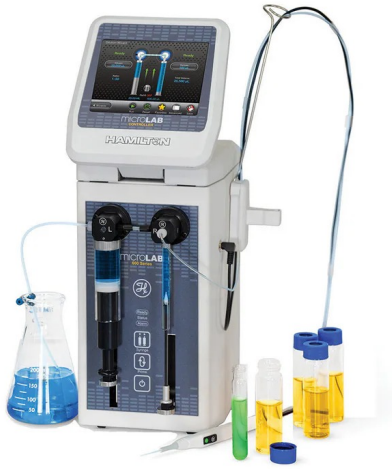
- Non-contact positive displacement, acoustic or digital dispensing
  - no liquid classes
- Enable miniaturisation

Echo



D300e

DragonFly



Is it Time to Let Go of Air-displacement Pipettes?

Dr Warren Gilson & Prof. Henry Lardy

Inventors of the world first adjustable volume air-displacement pipette (1969)

Critical steps?

Aliquoting the sample

Performing MRD/Adding Internal Standard

Type of Dispensing	Range	Inaccuracy % (range)	Imprecision % (best / worst)
Acoustic Droplet Ejection Non-contact	25 nL 250 nL 2.5 µL 10 µL	-0.7 / 2.8 -0.3 / 1.4 -1.0 / 0.9 -2.5 / 1.0	0.7 / 2.4 0.3 / 0.4 0.4 / 0.9 0.8 / 1.1
+ve displacement Non-contact	5 µL (200 nL) 300 µL but larger vol. possible 10 channels	0 / 2.0 -4.4 / -0.7	0.6 / 2.3 0.1 / 2.7
“Digital Dispense” Non-contact	10 nL (11 pL) 50 nL 100 nL 250 nL 500 nL 3 Instruments	0.4 / 6.1 (-8.9) -0.9 / 5.1 (-12.6) -1.0 / 5.2 (-13.1) -0.5 / 5.5 (-12.4) 1.5 / 3.3 (-10.4)	2.0 / 3.2 1.7 / 2.5 1.6 / 3.0 1.3 / 4.0 3.3 / 4.7

Need a way to test nL performance

Artel MVS

Primary use	Set Up / Programming Time	Run Time (96 / 384 well)	Limitations
Sample & QC transfer Cal. Curve Prep MRD/ IS addition MDev (e.g. DoE or chequerboard)	15-20 min (Excel templates)	1 - 2 min / 4 - 10 min (+10 min for 2x centrifuge steps)	Aqueous samples only **10-12uL maximum volume** Heterogeneous samples
MRD / IS addition / Reagent addition MDev (e.g. DoE or chequerboard)	5-10min (Excel templates)	1 min / 2-3 min	10 channel – not suitable for sample transfer
Cal. Curve Prep (Biomarker) Spiking solutions/QCs MDev/Spiking for Validation samples	10-15min (D300e Control)	seconds / 1 min	DMSO or Aqueous with additives only – not for sample transfer

“Pipette- Free” Workflow: Not Enough Time to Walk Away

Sample Reformatting

Add samples, WS and QC to 384 PP using Bravo or MicroPro 300

Spiking of Cal standards

Cal line creation from Working stocks. Transfer to destination plate

QC and sample dispensing

QC & samples transferred to destination plate

Preparation of MRD

Assay buffer added to destination plate to perform MRD

Gyrolab

< 30min

Modified from Biddlecombe *etal* “Sounds Good – Automated high throughput LBA work-flow for Biopharm Discovery Support” EBF Open Symposium 2019

Gyrolab

Default approach (2018)

ELISpot

Stimulations

Sterile aliquoting of cell replicates

ELLA

Assays with MRD ≥ 5

MSD

Large MRD or

384 well plates

SMC-xPRO

384 well plates

LC-MS

Preclinical

Rare Matrices

Ease of Implementation

IP Student Project (Emma Grey) - Automation of LC-MS/MS sample preparation

Week 1

Training and familiarisation with Liquid Handlers and ELLA platform

Week 2

Running testing sets & checking logistics (e.g. Auto-sampler needle depth)

Week 3 & 4

Completed two ELLA assays

(now in use in Phase II studies)

Completed three nGLP LC-MS assays

LBA Workflows

Gyrolab

1:10 MRD (total volume 5000 nL)

- Echo dispense 500 nL sample
- Echo dispense 4500 nL buffer

1:100 MRD (total volume 10000 nL)

- Echo Dispense 100 nL sample
- Dragonfly/ Dispense 9900 nL buffer

ELLA

- Reformat samples to 384 Echo Source plate
- Centrifuge
- Echo: 6µL sample from source plate to 96 well transfer plate
- DragonFly: 54µL diluent
- Mix & centrifuge
- Transfer to ELLA cartridge

Miniaturisation of LC-MS workflows for pre-clinical studies

- **D300e** – into Echo source plate
  - Prepares STDs & ISWS in **human plasma**
  - QCs in rodent plasma

- **Echo**
  - Aliquot **100 nL** rodent samples, STD & QCs
  - Add **125 nL HUPL** to RB and MB
  - Add **125 nL ISWS** to all other wells

- **DragonFly**
  - Add 5 µL of ACN & Vortex
  - Adds 45µL of ACN:H_20 (30:70 v/v)

Vortex, Centrifuge & inject onto LC-MS system

(final matrix component in injection solvent = 0.1 - 0.5%)

GSK

P&As From Miniaturised LC-MS Sample Preparation

STD	Mean	std dev	%CV	Accuracy
100	100	8.16	**8.1**	**100.4**
200	197	5.73	**2.9**	**98.6**
500	507	14.4	**2.8**	**101.4**
2000	1996	15.3	**0.8**	**99.8**
10000	10100	328	**3.2**	**101.0**
50000	48990	682	**1.4**	**98.0**
80000	75230	912	**1.2**	**94.0**
100000	106800	1826	**1.7**	**106.8**

QC	Mean	std dev	%CV	Accuracy
100	94.7	3.31	**3.5**	**94.7**
300	292	11.7	**4**	**97.2**
4000	3737	150	**4.0**	**93.4**
75000	75040	851	**1.1**	**100**

Conservation of Rare Matrices

Applications

Preclinical matrix (3Rs)

Rare clinical matrices

- CSF
- Biopsy (e.g. Core Liver)

Micro-sampling

Volume requirements

- Gyrolab – up to 1µL
- 384well MSD – 10µL following MRD
- LC-MS <1uL for all samples

(up to 0.5% matrix component… so far)

Tasso Case study

Remote sampling study for episodic disease state monitoring

Using the TASSO+

Likely to receive ~100µL of plasma

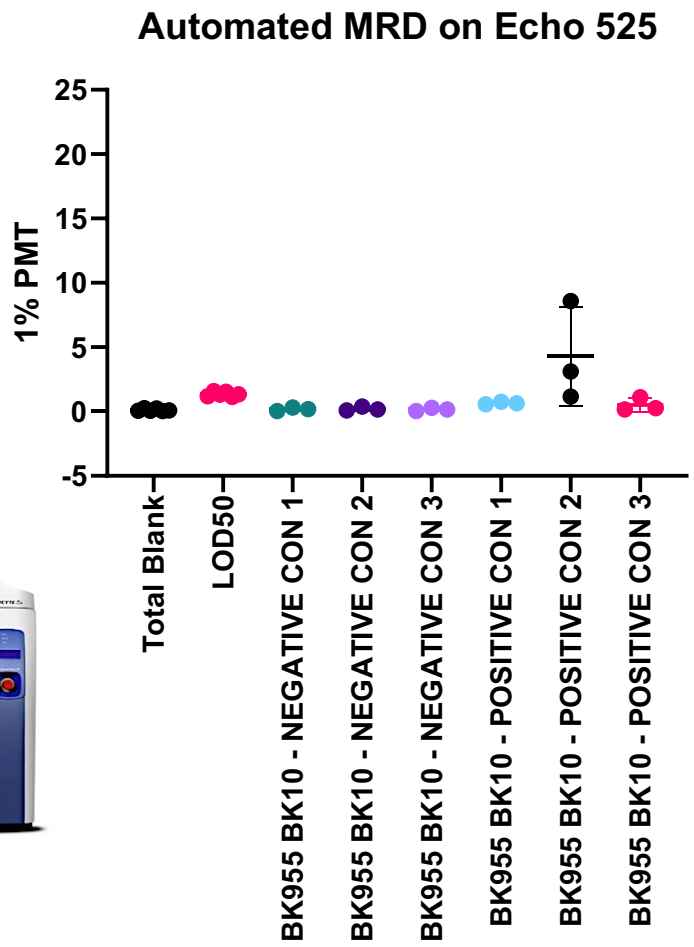
<u>3x Biomarker Assays</u>

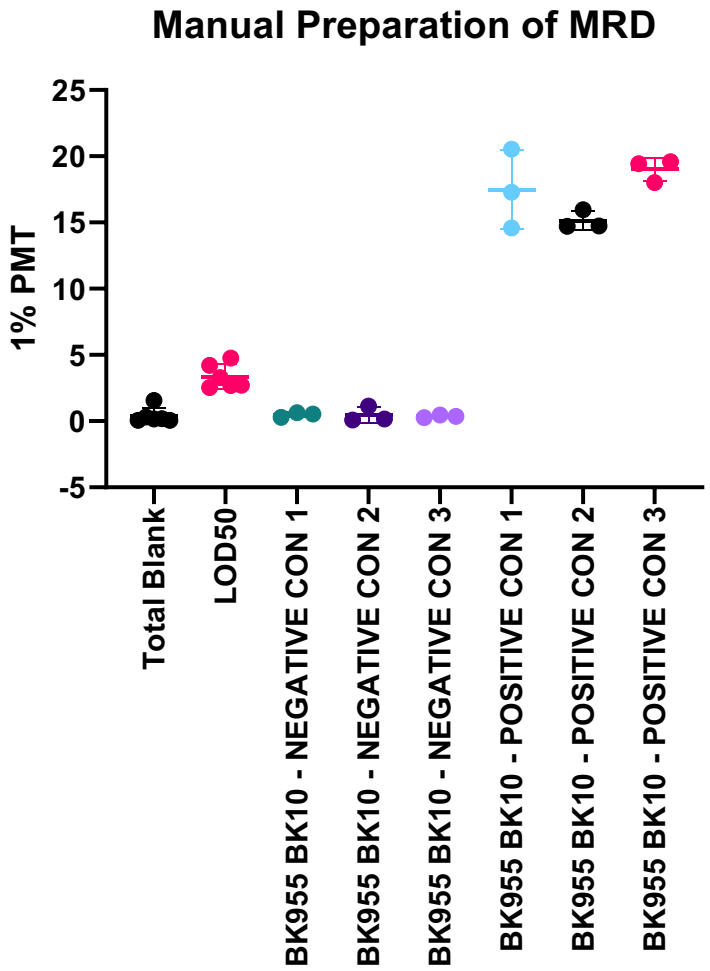
- Assay 1 : 5µL for 384well MSD assay
- Assay 2 : 2.5µL for 384well MSD assay
- Assay 3 : 2.5µL for 96well Gyrolab assay

When doesn't it work?

"Challenges in human tear analysis"
Sarah Childs, EBF Open Meeting 2023

Heterogeneous Samples – e.g. Tears





The knock-on benefits

Expected:

- Precision, accuracy, time and cost efficiency, throughput
- Operate in the nanolitre range when working with rare matrices

Assay step	96 well (µL)	384 well (µL)
Capture Coating	50	10 (1?)
Blocking	150	40
Sample	50 (25 matrix)	10 (5 matrix)
Detector	50	10
Read Buffer	150	40

Additional:

- Standardisation of our bioanalytical workflows (both PK and PD LBA and potential for LC-MS)
- Miniaturised liquid handling steps enabled move to 384well plate format
- Substantial reduction in
    - critical reagent consumption
    - organic solvents
    - biological matrix
    - plastic waste

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