

## Abstract

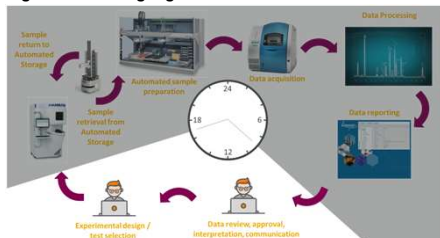
The automation of laboratory and data workflows within AstraZeneca's Analytical Sciences (AS) function of Biopharmaceutical Development (BPD), is becoming ever more critical to support an increasingly demanding pipeline. Investment in this area to automate manual processes has the potential to provide better utilisation of a talented and diverse workforce, bringing significant gains in productivity necessary to deliver the portfolio.

AS have developed a suite of physical and data automation workflows to increase throughput, whilst continuing to deliver the same high quality analytical data to our colleagues across BPD. Here we discuss the implementation of a variety of automation solutions and approximate their value/impact to the business. We also discuss lessons learned and touch on future endeavours to expand our automation toolbox and realise our Automation vision.

## ABC25 Vision

*"Shift the burden of routine analytical workflows from Human to Automation, maximising the contributions of Analytical Sciences by building an Automation Based Culture by 2025"*

Figure 1: Enabling Lights Out Automation



Example of autonomous automation workflow to maximise productivity gains during non-working hours or "lights out".

## Tecan Automated Intact / Subunit MS

This section showcases development of a script for the automated preparation of samples for mass spectrometry (MS) analysis of proteins. The script developed on the Tecan Evo200 liquid handling system can support up to 12 different workflows (Table 1) for up to 196 sample preparations in a single run.

Table 1: Matrix of requirements for Intact/Subunit MS Sample preparation requirements for each of the 12 test conditions, drives script design

Condition	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8	Step 9	Step 10	Step 11	Step 12	Step 13	Step 14	Step 15
Intact	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Red	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Degly	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Red+Degly	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subunit (Intact)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subunit (Red)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subunit (Degly)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subunit (Red+Degly)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subunit (FabR)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Subunit (FabA)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

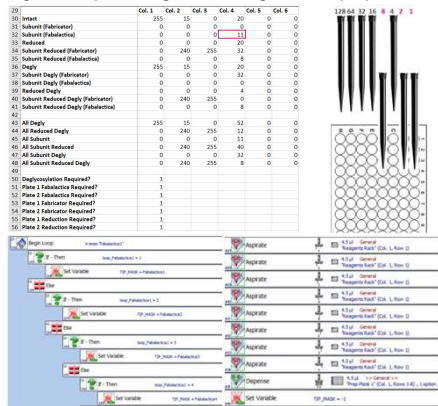
Red: reduced, Degly: deglycosylated, FabR: FabRICATOR®, FabA: FabALACTICA®

Users enter sample details and test requirements into an import file (Fig 2), which drives creation of tip mask variables. Tip mask variables imported into the script are engaged at specific reagent / buffer / sample addition steps, driving targeted sample treatments to specific wells (Fig 3). This enables batching across multiple projects with different preparation requirements, no matter how many or few samples require testing.

Figure 2: Intact/Subunit MS Import File

Well	Project	Sample ID	Subunit	Reduction	Deglycosylation	Condition 1 (Subunit)	Condition 2 (Subunit)	Condition 3 (Subunit & Deglycosylation)
1	A	202	Intact	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
2	A	202	Subunit (Intact)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
3	A	202	Subunit (Red)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
4	A	202	Subunit (Degly)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
5	A	202	Subunit (Red+Degly)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
6	A	202	Subunit (FabR)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
7	A	202	Subunit (FabA)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
8	A	202	Subunit (FabR)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
9	A	202	Subunit (FabA)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
10	A	202	Subunit (FabR)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
11	A	202	Subunit (FabA)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
12	A	202	Subunit (FabR)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
13	A	202	Subunit (FabA)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
14	A	202	Subunit (FabR)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
15	A	202	Subunit (FabA)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
16	A	202	Subunit (FabR)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)
17	A	202	Subunit (FabA)	Red	Degly	Subunit (Intact)	Subunit (Red)	Subunit (Red+Degly)

Figure 3: Tip Masking to drive targeted well preparations



## Script Validation

### Automated Intact / Subunit MS Equivalency

3 molecules were assessed covering all 12 sample conditions with the following criteria.

*"No significant difference in observed masses between manual & automated workflows. Intact < 50pp error. Subunit < 100 ppm error."*

All molecules & conditions produced consistent profiles with good LC/MS resolution and observed masses within the pre-defined limits, passing the acceptance criteria set.

- AZD-1 (Intact) < 43 ppm error
- AZD-2 (FabR subunit) < 43 ppm error
- AZD-3 (FabA subunit) < 38 ppm error

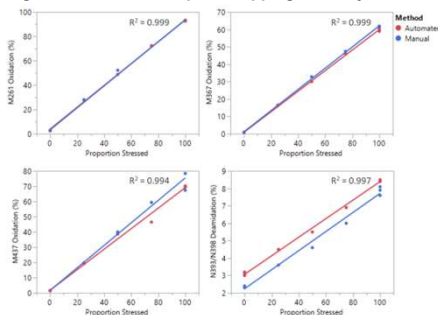
### Automated Peptide Mapping Equivalency

Abundancies of PTMs for AZD-4, AZD-5 and AZD-6 were compared between manual and automated sample preparation. Observed % PTMs (post-translational modifications) were compared against theoretical % for stressed-unstressed sample blends. Accuracy was within ± 20% criteria set (Table 2), with good linearity observed (Fig 4) and robustness, precision & repeatability within the observed variance for manually prepared samples.

Table 2: Automated Peptide Mapping Accuracy Results

	M261 Ox (%)	M367 Ox (%)	M437 Ox (%)	N393/N398 Deam (%)
Theoretical 25% Stress	25.3	15.4	18.6	4.4
Actual 25% Stress	27.7	16.5	19.8	4.5
Accuracy (%)	109.6	106.9	106.6	101.3
Theoretical 50% Stress	47.9	30.2	35.7	5.8
Actual 50% Stress	48.8	30.0	38.7	5.5
Accuracy (%)	101.9	99.4	108.5	95.1
Theoretical 75% Stress	70.5	44.9	52.8	7.1
Actual 75% Stress	72.4	46.1	46.5	6.9
Accuracy (%)	102.7	102.6	88.1	96.8

Figure 4: Automated Peptide Mapping Linearity

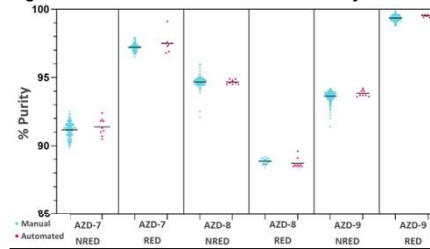


### Automated CE-SDS Equivalency

Purity results for 3 molecules prepared by an automated workflow for both non-reducing and reducing CE-SDS were compared to historical Reference Standard trending data from manually

prepared samples. Accuracy was established by statistical analysis to have a bias of 0.3% which was not statistically significant at the 95% confidence interval.

Figure 5: Automated vs Manual CE-SDS Purity Results



## Capabilities & Impact

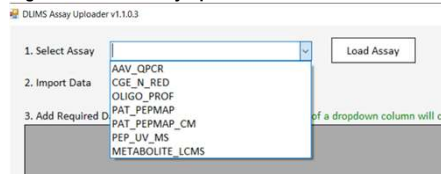
### Automation Scripts

- Normalisation
- Peptide Mapping
- CE-SDS
- Non-reducing Peptide Mapping
- AZD-10 cIEF
- Glycan Profiling
- Intact/Subunit MS
- Phynexus Purification
- GXII Fragmentation
- Trinean A260/A280
- AZD-11 RP-HPLC

### Other Automation Capabilities

In addition to liquid handling scripts, we are building capabilities with automated storage solutions such as Hamilton SAM, Titian Mosaic, and standardising sample tubing using LVL. We are also exploring data automation solutions such as the DLIMS Assay Uploader (Fig 5) and assay barcoding (Fig 6) to automate data transfer between steps in the analytical testing lifecycle.

Figure 5: DLIMS Assay Uploader



Automated upload of assay results to LIMS database, reducing data transcription and checking burden

Figure 6: Assay Barcoding for CE-SDS

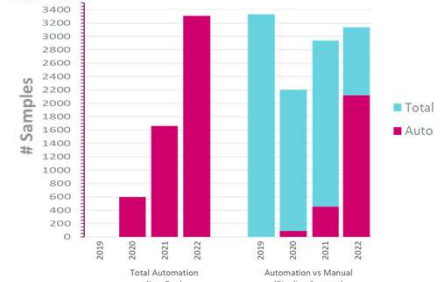


Scanning of barcoded reagent/equipment information into e-lab notebooks, to minimise data transcription and support early error flagging

## Impact

The development of our automation toolbox has enabled a significant increase in automated sample preparation, equating to > 375 FTE hours saved in 2022. We aim to build on this momentum, developing physical & data automation solutions to realise our ABC25 vision.

Figure 7: Automated analytical sample preparation from 2019-2022



## Acknowledgements

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