A modular automated flow cytometry platform for the profiling of novel biologics at room temperature

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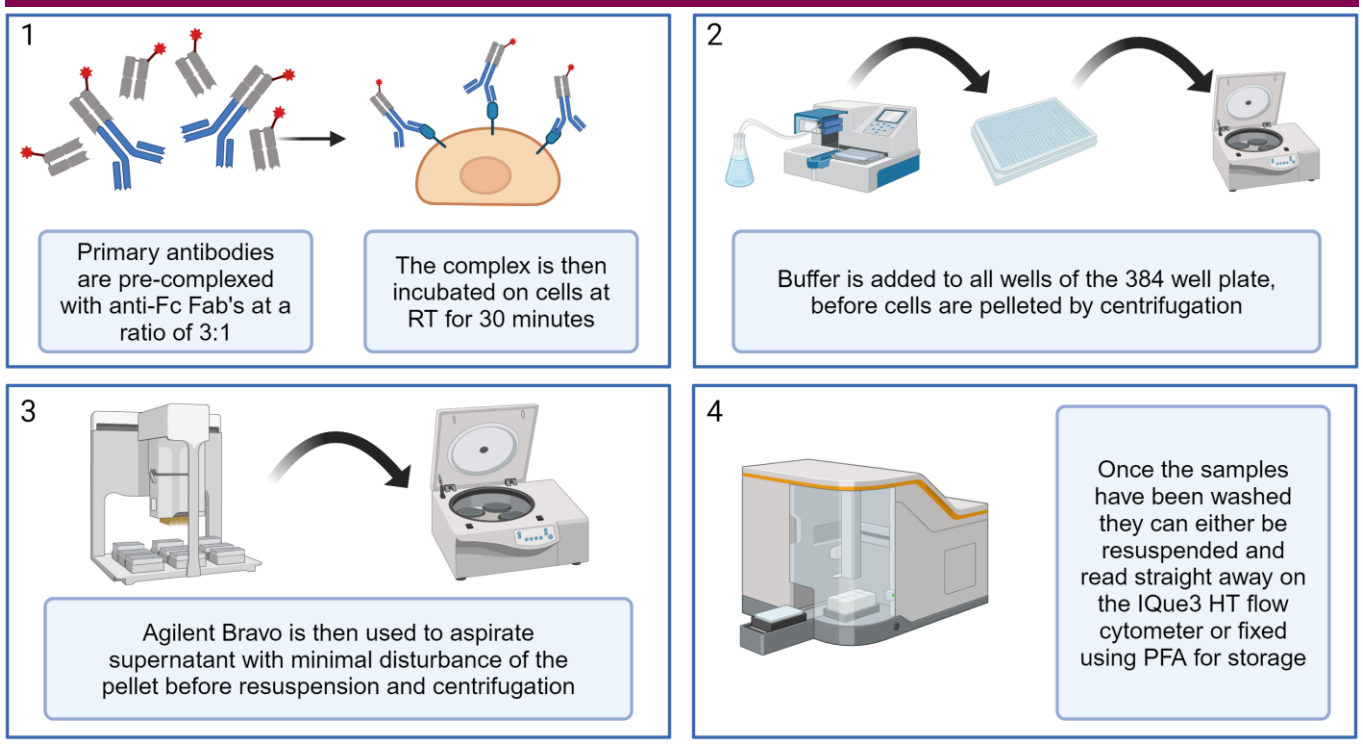
Abstract

A common use of flow cytometry is to profile binding of **biologics** to cell surface expressed antigens. Antibodies are one of the most common biologic modalities and are often specifically selected and engineered to internalise as part of their mechanism of action. This internalisation of the antibody, whether intended or not, can lead to a **loss of signal** at the stage of secondary detection if incubations are not performed at 4ºC to slow the process. This can be a major limiting factor when trying to **automate flow cytometry**, as most liquid handlers sit atop open lab benches at room temperature, meaning that plates cannot practically be cooled.

This poster describes a workflow that allows for automated flow cytometry, whilst not requiring any cooled incubation, utilising user friendly and common lab automation that isn't restricted to one function. **Room temperature** automation was enabled by using fluorophore conjugated anti-Fc Fab's to label the primary antibodies prior to incubation with the target cell, in effect creating a primary conjugate that can be detected even if it has internalised.

The workflow has gone on to be used to profile, in a high-throughput manner, the binding of novel biologics to the antigens expressed on the surface of cells; a vital triaging step in the screening cascades many project rely on

Workflow



Methods

The principal of pre-labelling primary antibodies with fluorescent Fabulight™ Fab anti-Fc secondaries underlies this workflow. Primary antibodies are preincubated at a **3:1 ratio** with the secondary for 30 minutes to form the labelled complex. This complex is then added onto the cell line of interest where it can be incubated at RT in 384 well plates. Washing is performed by a buffer addition using a combi-multidrop to partially dilute the complex. Plates are then centrifuged before the supernatant is removed using an **Agilent Bravo**. A linked Benchcel is used to house the plates, from which they are loaded onto the deck. The Bravo aspirates the supernatant leaving ~10ul in each well, ensuring the pellet is not disturbed. These tips are then washed, ready to aspirate supernatant from subsequent plates, **reducing plastic waste**. A second set of tips are then used to add buffer to all wells to resuspend the pellet and complete the wash, with the tips also washed. A final spin and Bravo aspiration are performed before the plate is resuspended in PFA or buffer and ran on the IQue3.

Results

Figure 1

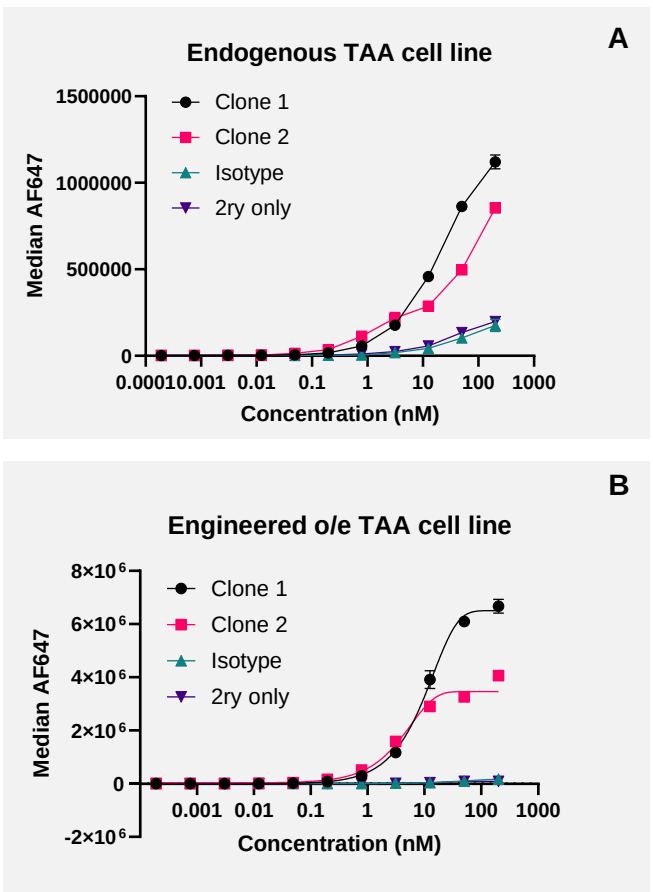


Figure 1 illustrates the ability of the assay to detect, down to **single digit nM**, the binding of primary antibodies to their TAA. This was confirmed in both a high expressing engineered line and in low expressing endogenous expressing line. Statistical analysis gives a **Z' of 0.89**, showing the assay is robust and fit for high-throughput screening.

TAA: Tumour Associated Antigen

Figure 2

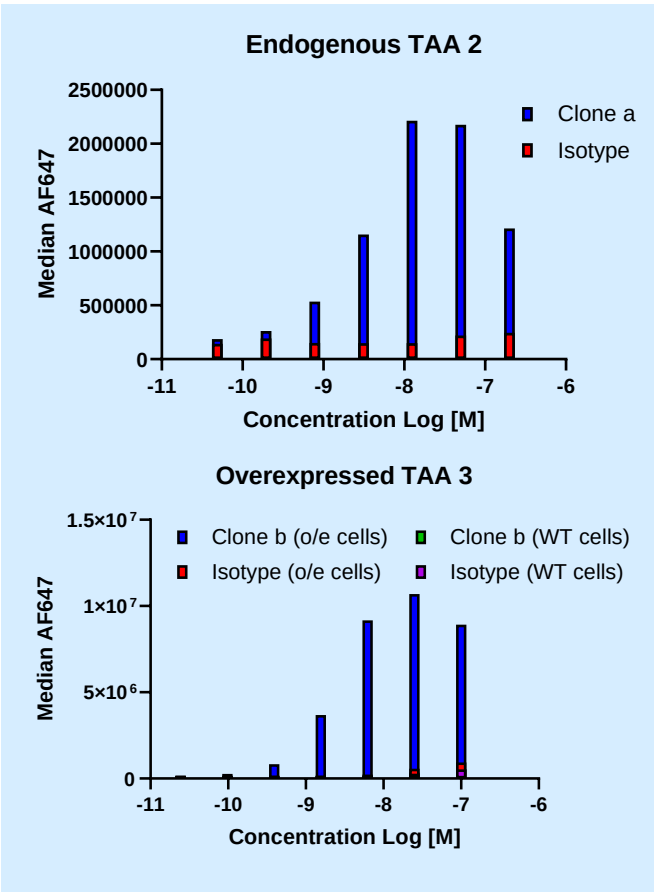


Figure 2. Primary antibodies can be tested at **variable** concentrations, whilst the Fab anti-Fc secondaries are kept at a fixed concentration. This demonstrates the utility of the platform to test novel antibodies expressed at variable concentrations in **HT** discovery campaigns

Conclusions

- Primary Antibodies precomplexed with Fabulight™ Fab Anti-Fc secondaries can detect, in a dose-dependent manner, antigens both overexpressed or at low levels of endogenous expression
- This workflow enables **automation** of the washing process, at room temperature, without the risk that internalisation will result in a loss of signal
- The precomplexed approach allows for the generation of **dose-response** curves with a maintained ratio, or for single point screening of HT expressed primary antibodies at varying concentration with a fixed concentration of secondary
- The implementation of automated washing allows for the use of **384** well flow cytometry, as manual washes of 384 well plates are unreliable

Acknowledgements

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Diagrams Made in BioRender

Graphs plotted in GraphPad Prism

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