Optimisation of robust reliable BlueWasher washing methods for High Throughput Screening

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AstraZeneca's UK Centre for Lead Discovery (UKC4LD) contains high quality equipment to facilitate testing of libraries of up to two million compounds, employing a range of high throughput screening technologies and capabilities to deliver high quality hit identification at scale. AZ has invested in HighRes Biosolutions CoLAB automation systems, comprising modular CoLAB Flex carts that are docked onto larger Microstar platforms. Currently four BlueCatBio centrifugal microtiter plate washers are automated on CoLAB Flex carts, and three are for manual use offline. Within AstraZeneca the BlueCatBio Bluewasher (Fig. 1) is routinely used during assay development, HTS and secondary screening. A non-contact centrifugal plate washer was selected over traditional vacuum and aspirate manifold washers based on improved assay data[1] (higher Z' and RZ'), quality (gentle cell monolayer washing ability), the ability to process 1536 well plates and the speed of plate processing, as fewer wash cycles are required.

Fig.1 Centrifugal washing
- Uniform action across plate
- Adjustable rotational speed
- Complete or partial evacuation

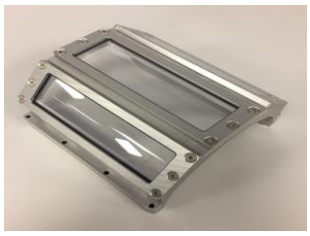

Fig.2 Clear rotor shield
- Incorporates 'viewing windows'
- Bullet proof acrylic & polycarbonate used for safety
- Used up to 1400 rpm

The BlueWasher is supplied with a wide range of factory pre-set wash cycles, either utilising the wash head or just evacuation alone. These typically vary from 1000 rpm – 3000 rpm and utilise small pauses for evacuation of the residual plate contents from the wash drum. These methods have been used successfully to achieve good quality plate washing data, however some reliability issues have been encountered when processing large numbers of microtitre plates, with plates being 'dirty' and 'frothy' after washing.

This was believed to be due to build up of foam during multiple plate washes, caused by HTS reagents containing mixtures of proteins and detergents. In order to resolve this foaming problem (the "washing machine" effect) it was important to see inside the washer to visualise what was occurring during plate washing.

AZ worked collaboratively with BlueCatBio to redesign their centrifugal washer internal rotor cover to incorporate 'viewing windows' (Fig. 2). A prototype version was used to optimise protocols, varying the rotor speeds, direction of spin, and waste pump durations. The viewing window meant that it was possible to directly see the impact of each change on the level of foaming (Fig.3A & B).

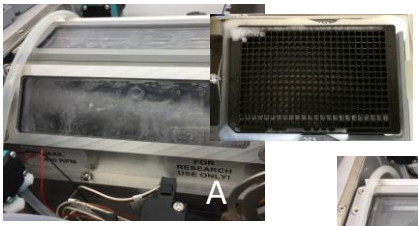

Fig.3A Pre-method optimisation
- Foaming within drum
- Foam on microtitre plate

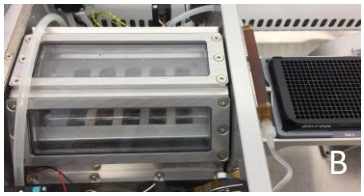

Fig.3B Post-method optimisation
- No foam within drum
- No foam on microtitre plate

Washing methods have been optimised to include the following steps:

- Use slow initial spin (300 – 600 rpm) - dumps bulk of plate well contents into the drum, allows time to evacuate to drain
- Longer & faster spin (1000 – 1800 rpm) - removes remaining liquid, washes plate consistently
- Spinning both clockwise & counter clockwise also has some benefits to improving washing & reproducibility

Angled dispensing onto the side of the well, instead of directly onto a cell monolayer has also shown benefits, this can be further improved by adding a small volume carefully to create a protective layer of wash solution prior to a faster dispense of the remaining wash solution.

AZ have also developed in house cleaning regimes to clean & decontaminate the internal Bluewasher mechanism before and after use:

- Pipette 100 mL hot water, then 50 mL 70% ethanol into the rotor drum through the open door
- Run wash protocol spinning 180 rpm without evacuation (coating and washing the interior of the drum)
- Wait for 1 minute and then evacuate to waste for 20 seconds with suction turned on
- To avoid washer waste container also filling with foam, add a drop of AntiFoam Y-30 emulsion (Sigma-Aldrich)
- When processing multiple plates the BlueWasher waste filter should be removed & washed with water on a daily basis. This removes debris which also helps to reduce the build-up of foam

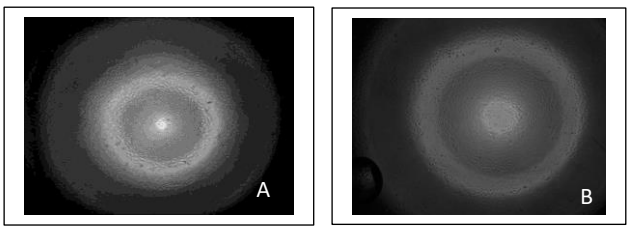

Fig.4 U2OS cells 4X brightfield images of plates A) before processing and B) post-process, comprising three optimised BlueWasher wash steps and four additions

Summary

Since delivery of the prototype clear rotor shield it is now possible to see exactly what is happening during the plate washing steps and therefore modify methods to improve the plate washing, whilst maintaining or improving the data quality (Fig. 4A & B). Reducing levels of residual foam and liquid has improved the overall robustness and reliability of the plate washing processing. These improvements have raised the quality of cell based HTS assays run within the UKCLD.

[1]Knight *et al* SLAS Discovery **2017** 22 732-742