

Evaluation of the Use of Cold Plasma for Microtiter Plate Cleaning to Reduce Plastic Biohazard Waste

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Abstract

Plastic pollution is the accumulation of plastic objects in the Earth's environment and is a global problem of increasing importance. The laboratory and health care industries contribute to this problem by the widely accepted single use of plastics, including microtiter plates used for compound testing. At AstraZeneca, we predict the use of more than 45,000 384-well and more than 11,000 1536-well microtiter plates per year. IonField Systems has developed a microplate cleaning system (MCS) powered by PlasmaKnife technology that uses cold plasma to clean microtiter plates. AstraZeneca proposed the use of this system for standard ANSI (<https://slas.org/resources/information/industry-standards/>) microtiter plate regeneration. Here we present the results of an evaluation using a model system involving the cleaning of plates following an enzyme-based biochemical assay, as well as the software and hardware enhancements that were incorporated into the production PlasmaKnife MCS. The method involved determining the level of inhibition achieved by residual compound following different cleaning protocols and showed that cleaning achieved in about 2 min was sufficient to remove trace compound contamination. Future work will focus on assessing the number of regeneration cycles that can be reliably achieved.

Keywords

cold plasma, microtiter plate cleaning, microplate, plastic waste, reuse

Introduction

Plastic pollution is an increasingly serious problem for today's society, with most of Earth's environments affected by the presence of plastic, from micro- to macro-scale particles.^{1–4} About 8.3 billion tons of plastic has been produced since the 1950s, and only about 9% of this plastic has been recycled, 12% has been burned, and the remaining 79% has ended up in a landfill or the environment.⁵ Within the pharmaceutical industry, there has been a steady rise in the amount of plastic biohazard waste produced, as operations within laboratories are largely built on the accepted use of disposable products. Every day, vast amounts of plastic, including pipette tips, microplates, cell flasks, and other items, are used and thrown away after a single use, only to be incinerated or sent to a landfill. This adversely contributes to the high levels of plastic pollution with a negative impact on the environment. In 2014, it was estimated that 5.5 million tons of laboratory plastic waste was generated by some 20,500 research institutions worldwide.⁶

As the problem is becoming increasingly recognized within the industry, scientists are trying to address the issue

by applying the 3 Rs strategy—Reduce, Reuse, Recycle—to develop solutions aimed at reducing the amount of plastic waste generated within laboratories. These actions include adapting protocols to use less labware, universities setting up recycling programs preventing plastics from going to landfill, and novel solutions offered by the companies that manufacture labware.

Within AstraZeneca, we have been exploring practical solutions to help us reduce our plastic footprint. Currently, our high-throughput screening (HTS) center performs about

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20 HTS campaigns per year, testing up to 2 million compounds per campaign, and using more than 40,000 plastic microtiter plates in the process. Process efficiency and cost, as well as the impact on the environment around us, have led us to continually explore the best technological advances to progress the screening field, using a wide range of commercial equipment for screening purposes,^{7,8} including reagent dispensers, plate washers,⁹ and multiparameter plate readers.¹⁰ In this report, we have worked with IonField Systems (IFS; Moorestown, NJ) to evaluate and develop technology to clean plastic microtiter plates so that they can be reused multiple times. IFS has developed a microplate cleaning system (MCS) powered by PlasmaKnife technology that uses a combination of liquid wash and cold-plasma exposure¹¹ to clean used assay plates. For microtiter plates to be reused, they must be functionally indistinguishable from new plates when in repeated use within and between assays. Here we present data demonstrating success in cleaning microtiter plates used in biochemical assays, allowing them to be reused in subsequent HTS campaigns. Optimization and integration of this methodology will help us to take a significant step toward operating in a more environmentally conscious manner while continuing to produce reliable, high-quality data.

Materials and Methods

Biochemical Absorbance Assay

Enzyme activity for human recombinant arginase (His-TEV full-length arginase 1 protein in 50 mM HEPES, pH 8.0, 100 mM KCl, 100 μ M MnCl₂, 2 mM TCEP, 5% glycerol, custom synthesis supplied by Viva Biotech, Shanghai) generated a thiol group from the substrate thioarginine; this was detected by Ellman's reagent (5,5'-dithiobis-[2-nitrobenzoic acid] or DTNB [D218200]; Sigma-Aldrich, St. Louis, MO), and product was measured by absorbance at 405 nm. Briefly, compounds in DMSO (100 % v/v) were acoustically dispensed in 10-point concentration format (each compound was tested at 18.3 nM to 200 μ M in a single well for each concentration) using an Echo 555 (Labcyte, San Jose, CA) into 1536-well polystyrene clear microtiter plates (Greiner Bio-One, Kremsmünster, Austria). A Multidrop Combi (Thermo Scientific, Waltham, MA) was used to add 3 μ L/well enzyme/DTNB, and plates were then incubated at ambient temperature for 30 min. Thioarginine (custom synthesized by Pharmaron, Shanghai, China), 3 μ L/well, was then added and plates incubated for a further 50 min at ambient temperature before measuring absorbance at 405 nm using a PHERAstar (BMG, Ortenberg, Germany) plate reader. Final buffer conditions were 50 mM HEPES (H3375, Sigma-Aldrich), pH 7.5, 0.0005% Brij (B4184, Sigma-Aldrich), 100 μ M MnCl₂ (M3634, Sigma-Aldrich). The

final enzyme concentration was 5 nM, and the final concentrations of thioarginine and DTNB were 0.72 mM and 0.4 mM, respectively. Plates that had been used in the assay were then cleaned using the PlasmaKnife MCS. To test the efficiency of this process, cleaned plates were retested in the biochemical absorbance assay by adding enzyme, DTNB, and thioarginine, followed by measuring absorbance.

PlasmaKnife MCS Components

The PlasmaKnife MCS consists of six hardware components. The three main components are the rinse station, plasma station, and the human machine interface (HMI) with an integrated touchscreen (**Fig. 1A**). Cleaning protocols (setting parameters such as fluid volumes, addition times, centrifugal force) are programmed using the HMI touch screen. When processing microplates, the rinse station is the first step in the cleaning process (**Fig. 1B**). Pairs of microtiter plates are placed into a robot-friendly holder, which moves them into the station where the previous assay material is spun out centrifugally (at 254g) and the fluid is expelled out from the wells and collected as solvent waste by the rinse module. This is followed by a high-pressure wash using an ethanol/water mix, which is again collected as solvent waste by the rinse module. Again, the microplate is centrifuged, this time almost to dryness. Robotically or manually, the microtiter plates are moved one at a time into the plasma station plate holder, which then moves the plate under a source of atmospheric pressure cold plasma that is blown over the upper surface of the plate in a sufficient quantity to satisfy a minimum threshold amount of plasma in each well. Plasma is generated by dielectric barrier discharge, and as it is blown onto the surface of the microplate, it displaces the air in the wells and fills the space around the microplate for the entire process. The atmospheric pressure cold plasma is a concentrated source of energetic and highly reactive free radicals, which rapidly react to destroy surface species, such as the substrate and its water of solvation. The process for generating plasma employed by the IonField instrument is very similar to that described by Kogelschatz et al.¹² Many factors affect the rate of evaporation (including concentration, pressure, surface area, temperature, and intermolecular forces), and the rate at which this occurs will be dependent on the individual conditions of the experiment and so is difficult to quantify and has not been studied.

The secondary components include an enclosure for the rinse station's high-pressure pump, a TipCharger controller used to generate plasma, and an optional automated ethanol/water mixing system. The PlasmaKnife system includes two software modules. The first module is the programmable operating system running on the control module. The second module is the application programming interface

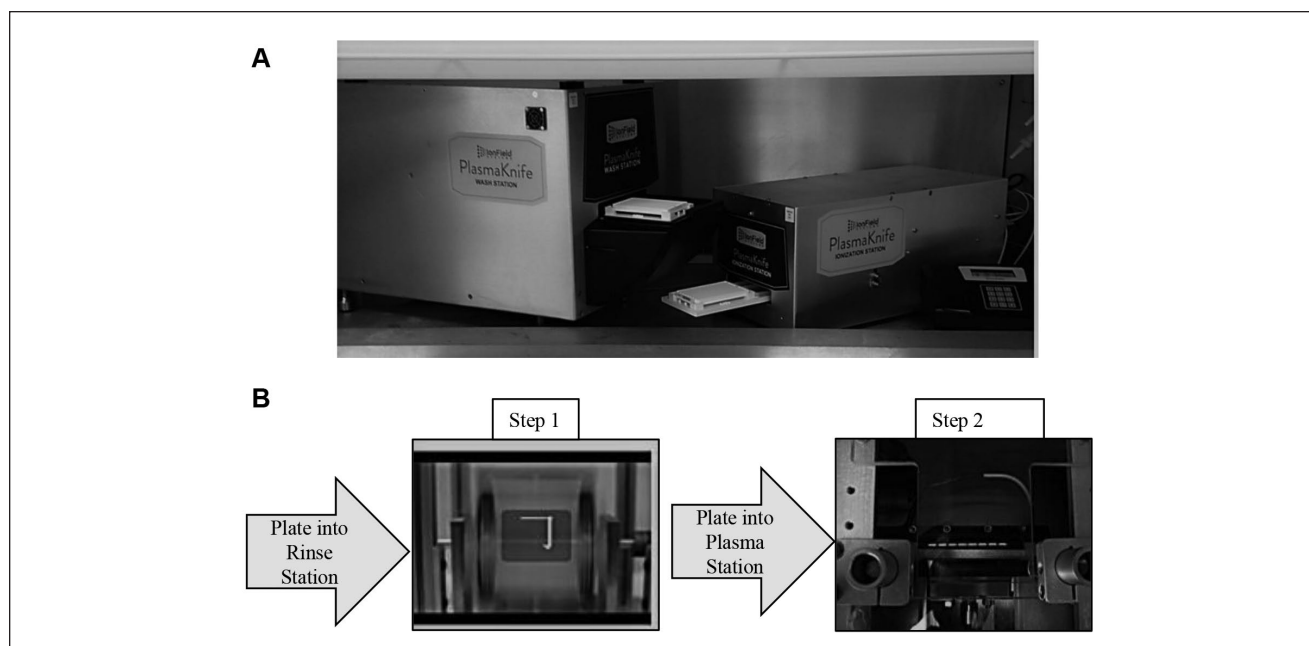


Figure 1. The PlasmaKnife microplate cleaning system. **(A)** Prototype PlasmaKnife microplate cleaning system (MCS). The three main components are the rinse station (wash station in image), the plasma station (ionization station in image), and the human machine interface with an integrated touchscreen. **(B)** Workflow using prototype MCS for microplate cleaning. Step 1: rinse station removes assay well contents from the microtiter plate using an integrated centrifuge, followed by a wash with 14% ethanol and quick spin. This step removes most of the well contents, typically reducing the concentration by 99.99% and leaving the wells “damp” for plasma treatment. Step 1 takes ~45 s for two plates. Step 2: the plate moves to the plasma station and is treated with cold plasma for up to 60 s. All organic material is ionized. (The organics are not liquids but are molecules in the liquid matrix that are converted to ions, typically by removing one or more electrons, which happens by energy transfer from the plasma gas. It is also likely that electrons may jump to higher energy levels during this process). Thus, a “like new” surface is restored.

needed for robotic integration. The hardware for the application programming interface communications is integrated in the control module.

Standard Plate-Cleaning Process Using Prototype PlasmaKnife MCS

Used assay plates were cleaned using prototype rinse and plasma stations supplied by IFS. Following a 5 s spin by the rinse station to centrifugally empty the well assay contents, plates were washed using 14% ethanol by passing under the water knife for 30 s. The water knife sprays a thin (2/1000th inch) layer of wash fluid over the upper surface of the microplate. The pressure used is up to 150 PSI (or 10.34 Bar). The volume dispensed is user programmable, with a typical volume per microplate of 0.3 to 0.4 L. The water knife is positioned across the breadth of the microplate at 6.5 mm above the top surface. Following the wash step, plates were centrifuged at 1500 rpm (relative centrifugal force = 254g) for 5 s before being treated with plasma for up to 60 s. The prototype unit used an eight-channel plasma-generation head that spans the breadth of the microplate and

evenly blows the plasma over the surface. Plasma-treated plates were then left to air dry before being retested in the biochemical absorbance assay as empty cleaned plates (with no further compound additions).

Data Analysis

All assay plates (1536-well format) included control wells. Negative control wells (0% inhibition; 18 wells in total) contained a matched concentration of DMSO (1% v/v) to compound wells. Positive control wells (100% inhibition; 18 wells in total) contained an assay-specific control compound. All screening data were analyzed using Genedata Screener (Basel, Switzerland). The activity of each test compound was normalized to the median values of negative control wells. IC_{50} values were calculated by nonlinear regression fitting to a four-parameter logistic model using the automated curve-fitting technology in Genedata Screener. GraphPad Prism (GraphPad Software, San Diego, CA) was used to analyze the percentage inhibition data exported from Genedata Screener to compare the effect of modifications to the plate cleaning process.

Results and Discussion

Initial Data Using Prototype PlasmaKnife MCS

The biochemical absorbance assay described in the Materials and Methods section was used for screening chemical inhibitors of a target enzyme, ARG1, and using this experimental model, we generated a set of used assay microtiter plates that tested a set of 78 active known pharmacology compounds, with a pIC_{50} of 3.7 to 7.5. Using a 10-point concentration format, 780 (plus 36 control wells) of 1536 wells were used per plate for all experiments, as can be seen in the heat map (**Fig. 2A**) showing the percentage signal decrease data from the original assay plate. For illustrative purposes, a visual representation of all 78 IC_{50} curves from the Genedata Screener automated curve-fitting analysis is shown in **Figure 3A**, illustrating the range of compound activities. The used assay plate was cleaned (using the standard cleaning process with the prototype PlasmaKnife MCS as described in the Materials and Methods section) and then retested as cleaned plates (with no further compound additions) in the biochemical absorbance assay. Data from the cleaned plates were analyzed using Genedata Screener automated curve fitting to determine whether any inhibition of the assay signal could be detected (**Fig. 3B**), presumed to be due to incomplete removal of the compound during plate cleaning. Data showed that the standard cleaning process removed most of the compounds, including the most potent ones, with a pIC_{50} of ~ 7.5 , as no IC_{50} curve could be fitted to the data generated from the cleaned plate. However, 2 of the 78 compounds were not completely removed, because inhibition of the assay signal could be detected in wells that had contained the highest compound concentrations in the original assay (**Fig. 3B**). Structural information and associated molecular weight, solubility, and cLogP data for the compound that showed the greater amount of residual activity on the cleaned plate (compound X) is shown in **Figure 3C**, and **Figure 3D** shows the percentage inhibition data for this compound on the original assay plate and after the plate had been cleaned. Data are also shown for a representative compound (**Fig. 3E**) that had been successfully cleaned (compound Y), as no inhibition was detected after MCS treatment (**Fig. 3F**). Compound X is more hydrophobic and less soluble than compound Y, which could contribute to its being harder to completely clean from the microtiter plate. Repeat experiments using assay plates testing the 78 compounds in the ARG1 assay, in which the compounds were arrayed in a different layout, showed that incomplete cleaning of compound X was not due to a positional effect arising from the sample location on the microtiter plate.

Effect of Including Water Prewash and a More Focused Cold Plasma Source

Following these initial results, we worked with IFS to improve the prototype instrument to deliver a more robust

and reliable plate-cleaning process. Hardware improvements included the use of more hydrophobic materials to reduce the wetness of the components, such as the rinse station plate holder after processing plates through the device. Parts were also upgraded to focus the plasma more directly onto the microtiter plate, by increasing the pumping pressure of the plasma gas from 90 to 150 PSI, through a narrow slot (the bottom gap of the holder is 0.060 inches) to accelerate the flow rate and minimize the dilution of the energy intensity (the electron density is in the range of 10^{14} to 10^{15} cm^{-3} using factory settings) by the surrounding room air. The gap size provides the appropriate flow speed based on the pressure of the air feed into the plasma generator for optimal plasma generation. Plasma exposure is not determined by time under the gap, and the microtiter plate movement involves multiple passes under the plasma generator. Both the speed of the plate movement and the number of repeat passes have been optimized to yield the maximum cleaning effectiveness and is independent of plate format for standard-height microplates.

The rinse station centrifuge spin speed was reduced to 1000 rpm (relative centrifugal force = 113g) to retain more liquid in the wells, because a damp environment ensures the most effective plasma treatment. We also tested the effect of a 30 s prewash with MilliQ (MerckMillipore, Darmstadt, Germany) water (after the pulse spin step emptying the assay well contents), prior to rinsing plates with 14% ethanol to test whether this would improve plate cleaning. The test set of 78 active compounds was used to generate a set of used microtiter plates in the biochemical absorbance assay, and these were then cleaned using the modified PlasmaKnife MCS prototype. **Figure 4A** shows that despite these various changes, the cleaning process was still not completely effective, because wells that had contained the highest concentrations of compound X were still inhibiting the assay signal on the cleaned plates when retested in the assay as empty plates. **Figure 4B** shows that these changes to the cleaning protocol still resulted in successful cleaning of compound Y, as no inhibition could be detected after PlasmaKnife MCS cleaning.

Plasma Controller Modifications

During this prototype development, IFS also implemented several hardware enhancements. The original plasma generator produced a single band of plasma under which the microplate moved. As described in the previous section, this was replaced with a full microplate-sized focused plasma generator to increase plasma density and energy and thereby reduce heat. Used assay plates were cleaned as before using the new plasma source. Representative heat maps of assay data from the original used assay plate (**Fig. 2A**) and after plate cleaning (**Fig. 2B**) are shown in **Figure 2**. All cleaned plates had a clear pattern present in the data, with high absorbance raw data values on the left-hand side

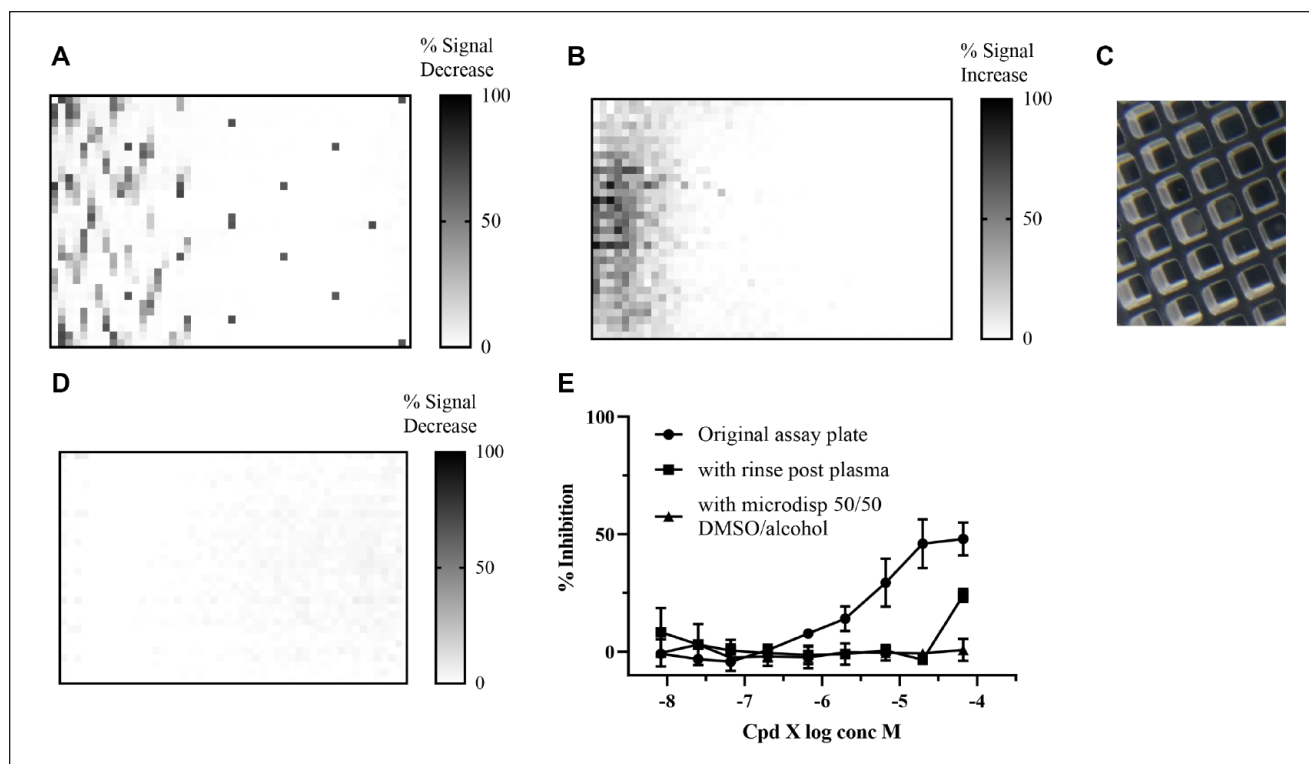


Figure 2. Effect of increasing plasma concentration and addition of DMSO/ethanol microdispense prior to the main wash step to solubilize any precipitated compounds. **(A)** Heat map showing the percentage signal decrease data from the original assay plate used in compound potency testing in concentration response format. **(B)** Heat map showing the percentage signal increase data from the same used assay plate cleaned with the more powerful plasma generator. The increased assay signal was due to interference from contaminating nitric acid generated by the more powerful plasma source. **(C)** White residue found in several wells demonstrating that the standard rinse and plasma treatment process did not effectively remove all material and **(D)** the addition of 50/50 DMSO/alcohol microdispense to solubilize any precipitated compounds prior to the main wash step, combined with a rinse step with 14% ethanol after plasma treatment to restore neutral pH. **(E)** Effect of plate cleaning using compound X. The PlasmaKnife microplate cleaning system cleaned plate was retested in the assay as an empty plate. Using a combination of all of these changes (triangles) showed successful cleaning of compound X, as no inhibition was detected after plate cleaning. Mean and standard deviation of $n = 2$ data are shown.

of each plate (**Fig. 2B**). It is well known that plasma produced from the atmospheric discharge barrier generates nitrogen radicals, which can react with oxygen in its various states within the plasma to generate various nitrogen oxides.¹³ In the presence of water, these nitrogen oxides are present as reactive intermediates and instantly react to become nitric acid. Tests using litmus paper showed that those wells with high absorbance raw data values had pH = 1 to 2, which was attributed to the contaminating nitric acid generated during the plasma treatment. To remove the nitric acid, we added a brief 5 s rinse with 14% ethanol after plasma treatment to ensure wells were a neutral pH. Data are shown in **Figure 2E** for compound X (squares) in plates treated with the more powerful plasma source combined with the post-plasma rinse step; residual compound can still be detected in wells that had contained the highest compound concentration.

We found that the patterning seen on these cleaned plates was due to uneven air flow during plasma treatment with the full microplate-sized plasma generator; there was no air dilution of plasma in the plate center, but this occurred at the plate edges. The production PlasmaKnife MSC now has a single row-type plasma generator, with plasma applied in two passes over the microtiter plate, which is more effective than one pass and eliminates uneven plasma treatment.

Removing Residual Compound Contamination Using Solvent Microdispense

The biochemical absorbance assay used clear microtiter plates, and visual inspection after the wash and plasma treatment process showed white residues in wells 12C and D (**Fig. 2C**), which was present in the same positions on all cleaned plates. The standard wash and plasma treatment

process did not remove this material. Our first step was to identify a solvent that would solubilize the visualized material. We tested alcohol (a 50/50 mix of 100% methanol/100% ethanol), DMSO, and a 50/50 mix of alcohol/DMSO. Using a few microliters of each solvent(s) that covered just the base of the well, all visualized material released from the polymer. A 50/50 mix of MilliQ water and alcohol had no effect. The test microplates were put through the wash process testing one well at a time. It is not known whether the compound was dissolved or simply released due to solvents and the applied centrifugal force. All of the used microplates were then reprocessed using the 50/50 DMSO/alcohol mix between the initial spin and main wash, and there was no visual evidence of residual compound in any of the wells, demonstrating that both compound X and the second compound (shown in **Fig. 3B**) had been completely removed with the additional cleaning procedures (**Fig. 2D**). Data presented in **Figure 2E** showed successful cleaning of compound X (triangles), as no inhibition could be detected in the assay after plate cleaning.

To implement this procedure, an additional microdispenser was added in the rinse station, which could be programmed to optionally dispense DMSO, solvent, or a mix into the wells to help remove any dried or precipitated compounds. It is standard practice in most HTS labs to use acoustic dispensing of compound stocks in DMSO into empty microplates to create assay-ready microtiter plates.¹⁴ From a review of the literature, dried or precipitated compounds are most commonly associated with acoustic dispensing onto dry plates,¹⁵ and the PlasmaKnife MCS needed to address this issue. We routinely use a 2 s microdispense, which adds 1.5 to 2 μ L per well in a 1536-well microtiter plate (hence, four times this volume for 384-well plates), but the dispense time can be adjusted in 1 s increments if another volume is found to work better. The solvent mix is left in the wells for 5 s before it is washed out with the standard 14% ethanol wash. The low volumes of DMSO/alcohol mix will not materially alter the waste stream generated.

Decreasing the Duration of Main Wash Step

After successful complete cleaning of the used microtiter plates, we investigated the effect of decreasing the length of the main wash step with 14% ethanol. This would reduce the length of time for plate processing through the rinse station, speed up the overall process, and positively affect the waste stream by reducing the amount of solvent waste generated. We tested reducing the wash step from 30 s to 20, 10, or 5 s using assay plates generated in the biochemical absorbance assay using the same set of 78 test compounds that had been used in previous experiments. Data in **Figure 5** show that using compound X as an example of a compound that had been difficult to clean, we were able to reduce the

main wash step to 5 s and still maintain the ability to clean plates successfully. The high-pressure wash uses about 60 mL per second, so reducing the wash from 30 to 5 s results in an ~80% waste reduction, which has a positive impact on the waste stream disposal. The system can be set for shorter wash times (minimum 1 s), which could further reduce the waste stream.

Standard Plate-Cleaning Process

All steps in the cleaning process have variables, such as length of dispense time, centrifuge speed, dwell time after all dispense steps, and time of cold plasma treatment, which allow the user to optimize the wash and plasma treatments and create bespoke cleaning protocols for different assay and plate types. We selected a colorimetric detection endpoint as the test assay to develop the PlasmaKnife MCS, as we believe this to be the most sensitive to interference from compounds that have not been successfully cleaned. Typically, compounds contained within pharmaceutical company compound collections interfere more with absorbance assays than fluorescence endpoints.¹⁶ From this work, we have now developed a standard cleaning protocol using the production PlasmaKnife MCS, which has been successfully used to clean a range of standard polystyrene microtiter 1536-well plates (clear, white, and black) used in absorbance, fluorescence, and luminescence biochemical assays within the HTS center, demonstrating that the MCS can be more broadly applied to typical conditions or assays. The standard cleaning process employs a 5 s pulse spin to empty assay contents, a 2 s addition of DMSO/alcohol, followed by 5 s dwell time to aid solubilization of any dried/precipitated compounds. Plates are spun for 5 s and then washed using 14% ethanol for 5 s, before being centrifuged at 1500 rpm (relative centrifugal force = 254g) for 5 s. Plates are then treated with plasma for 60 s, followed by a 5 s rinse with 14% ethanol and left to air dry. It is possible for liquid droplets to be on the vertical edge and/or skirt of the microplate, and so these should be allowed to dry before stacking the cleaned microplates.

Future Considerations

To significantly reduce the amount of microtiter plates going to plastic biohazard waste, there are areas that require further evaluation to widen the application of the PlasmaKnife MCS. During development of the prototype instrument, we investigated only the effect of one cleaning cycle; additional experiments are planned to investigate how many times plates can be cleaned and reused. It would also be interesting to develop and test cleaning protocols using assay plates containing more potent compounds ($pIC_{50} > 7.5$) and to investigate the cleaning of different classes of compounds.

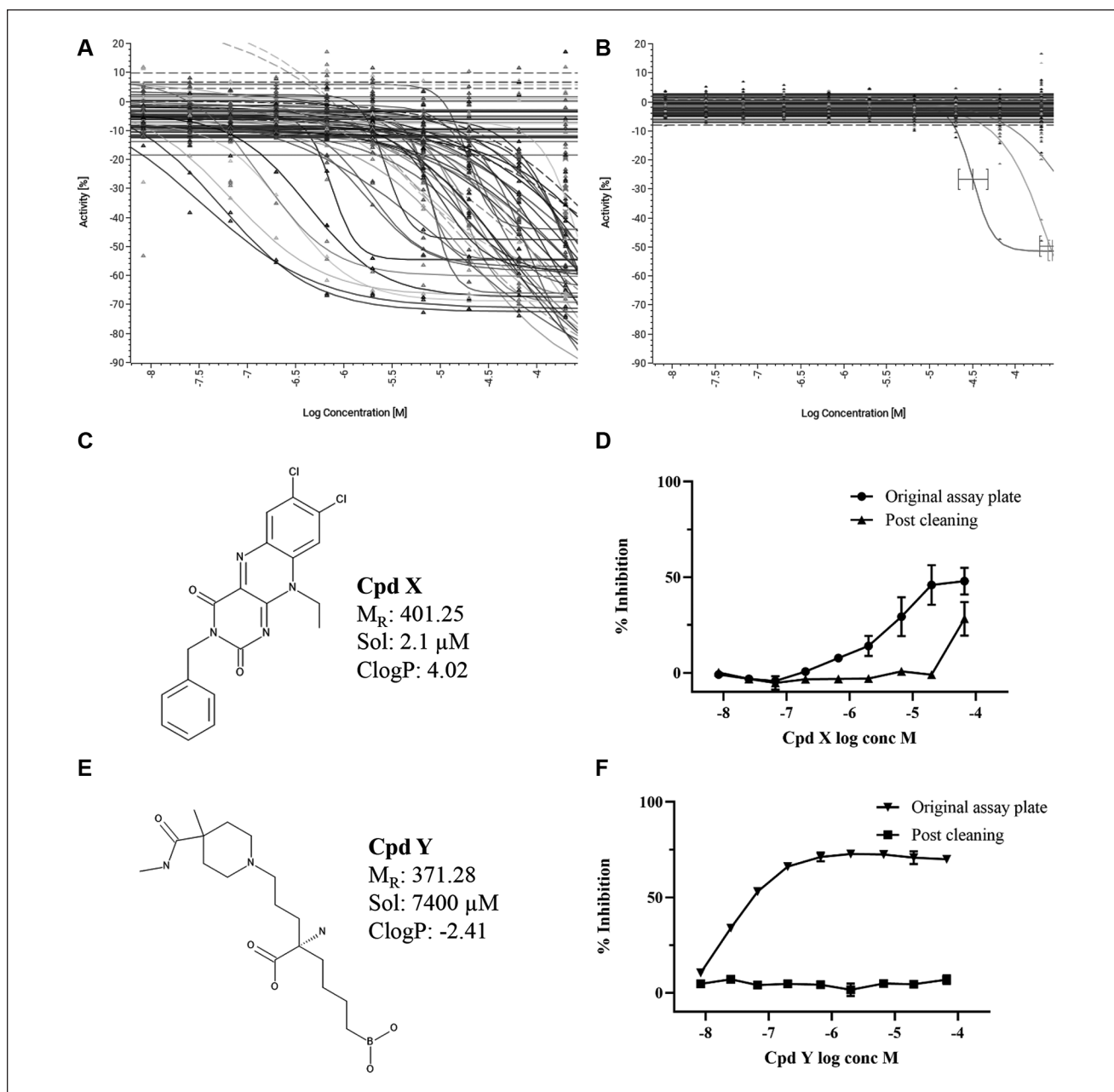


Figure 3. Initial data using the prototype PlasmaKnife microplate cleaning system (MCS) to clean used assay plates. **(A)** IC_{50} data for a set of 78 active known pharmacology compounds tested in the ARG1 biochemical absorbance assay. This microtiter assay plate was then cleaned using the prototype PlasmaKnife MCS and then tested as a cleaned plate (with no further compound additions) in the biochemical absorbance assay. **(B)** Residual activity for two compounds was detected after PlasmaKnife treatment. **(C)** The structure and associated molecular weight, solubility, and cLogP data of compound X, the compound that showed the greater amount of residual activity **(D)** on the cleaned plate. **(E)** The structure and associated molecular weight, solubility, and cLogP data of compound Y, selected as a representative compound that had been successfully cleaned, as no inhibition was detected after PlasmaKnife treatment **(F)**. Mean and standard deviation of $n = 2$ data are shown.

This article describes data from standard polystyrene plates; however, there are many different materials widely used to manufacture microtiter plates, such as cyclic olefin copolymer, and plates used in other assay formats, such as optical

imaging plates or sensor plates with inserts. Cleaning protocols could also be developed for these additional plate types. Such plates tend to be used in lower throughput assays, so there is less impact for reducing plastic pollution; however,

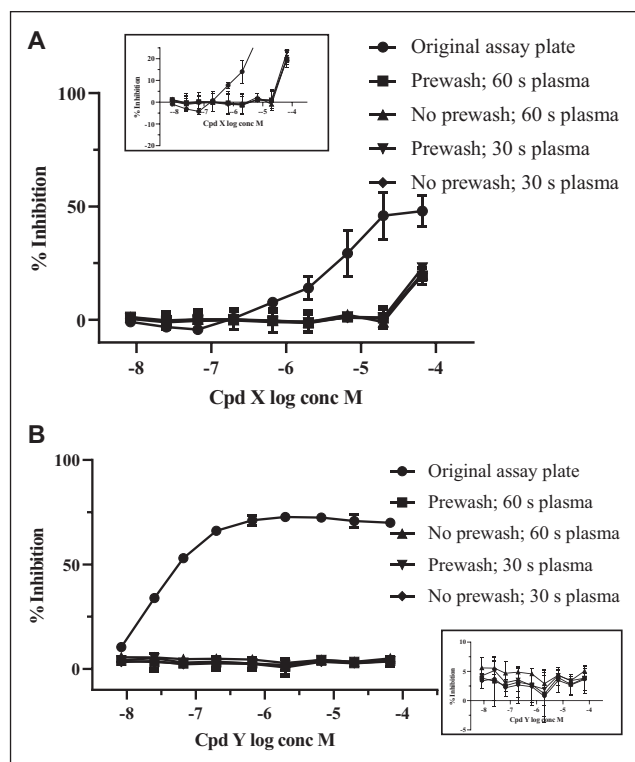


Figure 4. Effect of water prewash and length of plasma treatment on the plate-cleaning process. Optimizing the workflow using the prototype PlasmaKnife microplate cleaning system (MCS) to clean the used assay plate by including a water prewash step and different lengths of cold plasma treatment time. All PlasmaKnife MCS cleaned plates were retested in assay as empty plates. **(A)** Example data showing incomplete cleaning, as compound X is still present in some wells from potency testing and showing inhibition of the assay signal. **(B)** Successful cleaning of compound Y, as no inhibition is detected after PlasmaKnife MCS cleaning. Both insets show an expanded data set at the lower percentage inhibition levels to more easily visualize the overlaid data shown in the main graphs. Mean and standard deviation of $n = 2$ data are shown.

there are potential cost savings if these more expensive plates can be cleaned and reused more than once.

Our evaluation was performed as a manual process, with scientists moving plates between the different devices in the standard workflow. Using the standard plate-cleaning process described earlier, the throughput is ~55 plates per hour. To realize the potential of the PlasmaKnife MCS to clean hundreds of plates, there is a clear requirement to automate the process, and IFS is developing software to facilitate this. Allowing for robotic transit time and data processing of sensor data, we estimated that the overall system could produce a renewed microplate every ~45 s for most assay plates. Handling different plate types is facilitated using custom three-dimensional-printed insets for the plate holders, allowing handling of different plate heights (96-, 384-, or 1536-well plates) as long as they have a standard American National Standards Institute footprint. The PlasmaKnife

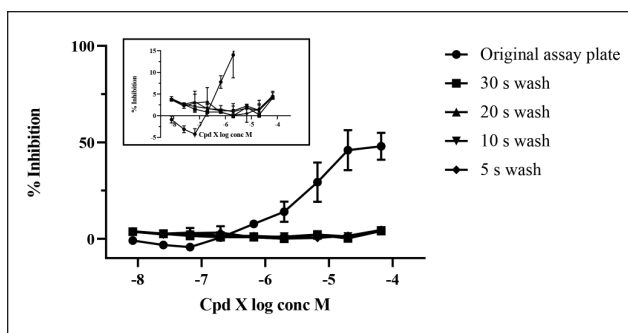


Figure 5. Effect of decreasing the length of wash time with 14% ethanol. Compound X was successfully cleaned by reducing the main wash time to 5 s. The inset shows an expanded data set at the lower percentage inhibition levels to more easily visualize the overlaid data shown in the main graph. Mean and standard deviation of $n = 2$ data are shown.

MCS has been designed so that most standard robot grippers can easily access the plate holder on both the rinse and plasma stations, and most materials used within the rinse station are hydrophobic in nature, although reliability testing would be required for robot handling of potentially wet/damp plates coming out of the rinse station. The automation of plate cleaning could also incorporate technology to monitor the process; for example, microplates could be scanned and recognized by high-resolution video recognition to ensure the correct software and hardware is present on the system to process that particular plate type. Cleaned plates could also be inspected by high-resolution video after plasma treatment for any anomaly and these plates sent to a quarantine location to prevent them from being included for use in future assays.

Cleaning and reusing plates at scale brings other practical considerations into play, such as the length of time between generating assay plates and cleaning them. Our finding generally is that the shorter the interval between use and cleaning, the easier the plates are to clean. Storage duration and conditions for cleaned plates also require consideration before plates are reused in assays. For example, should they be stored vacuum sealed to protect from moisture or dust ingress? Another aspect is the tracking of reused plates. Generally, plate barcoding is used to track information about the identity of compounds in each plate, with a requirement for single-use barcodes. Cleaning plates brings with it a need to rebarcode them, and information technology solutions may be needed to track the number of times plates are cleaned and reused. Another practical aspect to consider when cleaning hundreds of plates is disposal of the solvent waste generated during the process, to reduce the environmental impact. This could potentially be used as a biofuel to retrieve energy and offset the carbon footprint generated by the waste, but this needs to be carefully investigated.

The issue of plastic pollution is of increasing concern within today's society, and sustainability is a clear focus for the pharmaceutical industry. Reducing single-use plastics

within the laboratory is a key aspect for improving sustainability, with particular focus on the large number of plastic microtiter plates generated annually within screening centers such as ours. Initial data indicate that the development of the PlasmaKnife MCS delivers a potential solution to address this issue and highlight some areas for further consideration and development to deliver a solution at scale. It is important to consider all aspects of the plate-cleaning process workflow to ensure that all waste generated (plastic and solvent) is minimized in a manner that does not compromise data quality in future screening assays.

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Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The authors are employed by either AstraZeneca, IonFields Systems, or Talavera Science as appropriate, and their research and authorship of this article was completed within the scope of their employment with either AstraZeneca, IonFields Systems, or Talavera Science as appropriate.

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