Plasma-based Cleaning of Pipette Tips Used in Automated CYP450 Assays

Introduction

The assay throughput improvements pioneered by small molecule screening laboratories within the drug discovery environment have made the transition over the past decade into laboratories traditionally focused on the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) aspects of drug development. The Cytochrome P450 (CYP450) assay is often used in these laboratories to evaluate and understand the metabolic fate of drug candidate molecules. CYP enzymes, typically incorporated into the endoplasmic reticulum of hepatocytes or the inner membranes of most mitochondria, exist in several isoform families and are the primary means of modifying drug-like and other molecules for eventual elimination from the host body. The CYP enzymes play important roles in the synthesis and breakdown of hormones, cholesterol and other complex lipids, including those involved in vitamin D metabolism.

CYP450-induced modifications of drug candidate molecules, account for the majority of drug-drug interactions and other deleterious drug side effects. Inhibition of these enzymes often leads to the accumulation of compound within the whole organism, resulting in chronic or acute drug toxicity. Since individual CYP isoforms are derived from different genes, homology alone cannot predict which type will be responsible for the metabolism of a specific compound, consequently, the CYP450 profile for a given candidate molecule must be determined experimentally.

Cytochrome P450 Isoforms

Three CYP subfamily isoforms (CYP3A4, CYP2C9 and CYP2D6) comprise nearly 80 percent of all human isoforms responsible for compound metabolism.The CYP3A subfamily is the most abundant isoform group in humans, representing nearly 30% of the total cytochrome P450 activity in human liver. CYP3A4 has been demonstrated to oxidize the largest range of substrates, and is commonly involved in significant drug-drug interactions.

The CYP2D subfamily, particularly CYP2D6, is most commonly associated with the metabolism of psychotherapeutic compounds. It exhibits significant genetic polymorphism, leading to differential expression within and among distinct populations.

The CYP2C subfamily, represented by CYP2C9, is responsible for the metabolism of a wide variety of compounds, most notably the non-steroidal anti-inflammatory drugs (NSAIDS), and displays polymorphic characteristics similar to those observed with CYP2D6.

Plasma Cleaning of Pipette Tips

The TipCharger™ by IonField Systems™ uses self-contained, low-temperature, atmospheric plasma to clean pipette tips, metal cannula and pin tools associated with automated liquid handlers. Implementation of the TipCharger system provides cleaning equivalent to that of a fresh set of tips, reducing both the direct and indirect costs associated with replacing pipette tips.

Here plasma-based pipette tip cleaning efficacy is demonstrated using three well characterized and fully optimized commercially available Cytochrome P450 kits (Promega Corporation, Madison WI, P450-Glo CYP450 Screening Systems, V9790, V9890 and V9800) (Figure 1).

Atmospheric-Pressure 'Cold' Plasma

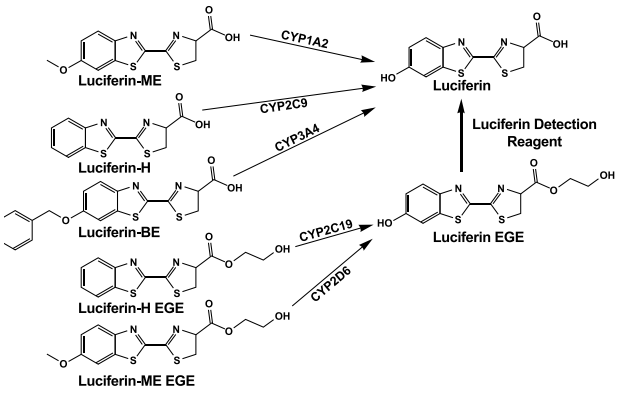


Figure 1. *Cytochrome P450 isoforms and their specific substrates) Promega Corporation, Madison WI).*

TipCharger Integration

The TipCharger system is provided in 8, 96 and 384-well plate densities and is easily integrated into most liquid handling platforms using standard SBS footprints. The TipCharger cleaning station can be taught as either a device or consumable within the liquid handler software. In this study we used a Sciclone ALH 500 (Caliper Life Sciences, Hopkinton MA) and taught the TipCharger TC-96 Cleaning Station as a reagent reservoir.

General Screening Conditions

Luciferin-free water, KPO_4, Luciferin substrate and CYP450 membranes were added sequentially to each well of a 96-well reaction plate (Corning # 3666) with the exception of the negative control wells (A1-H1) which received control rather than CYP450 membranes. The assay plate was incubated for 10 minutes at room temperature (RT). NADPH regeneration system (Luciferin-free water, Solution A and Solution B) was then added to all wells of the reaction plate. The reaction plate was incubated for a total of 30 minutes at 37°C, and the reaction halted by the addition of reconstituted detection reagent to all wells of the reaction plate. Reaction plates were read on a VICTOR Light Luminescence Counter (PerkinElmer, Wellesley MA).

A single box of Caliper 100 µL polypropylene tips (part number #105647) was loaded onto the Sciclone 96-high volume headAssay buffer containing CYP450 was added to each of 4 assay plates; and the tips were inserted into the TipCharger cleaning station for exposure to plasma through 20 mix steps of 100 µL at a rate of 100 µL/second. Using the same tips, Luciferin-free water was added to the remaining plate in place of enzyme .

Similarly, a second plate was prepared within which the substrate was exposed to plasma as above. Luciferin-free water was then added to the test plate (substituting for the substrate). Note: Negative control wells received control membrane with no detectable CYP450. This process (starting with a fresh box of tips) was repeated for each reagent. For each CYP enzyme tested, a total of 5 microplates (Corning #3666) were evaluated with the parameters varying as described in Table 1.

	Control Plate	Plate 2	Plate 3	Plate 4	Plate 5
Luciferin-free water	+	+	+	+	+
KPO_4	+	+	+	+	+
Cytochrome P450	+	-*	+	+	+
Luciferin Substrate	+	+	-*	+	+
NADPH regeneration system	+	+	+	-*	+
Detection system	+	+	+	+	-*

Table 1. *.Matrix approach for validating the TipCharger against Promega Corporation P450-Glo CYP450 screening systems.Plate 2-enzyme/membrane test, Plate 3- substrate test, Plate 4-NADPH regeneration reagents, and Plate 5-detection* reagents.

Results & Conclusion

The TipCharger System effectively and efficiently eliminated each of the potentially cross-contaminating reagents within each CYP450 kit, returning the pipette tips to states indistinguishable from new tips after a 30-second exposure to plasma. The Luciferin-free water used to transport any residual reagent into specific assay plates showed no detectable cross-contamination.

Plasma-based cleaning reduced overall enzyme activity to levels not significantly different from levels attained by the negative controls. Each of the CYP2C9 assay components (0.5 pmol CYP2C9, 100 µM Luciferin-H) in Luciferin-free water (25 mM KPO4), NADPH regeneration system and detection reagents was removed individually after a 30

second plasma exposure. The ~700 CPM observed for the TipCharger treated components represents an 84% reduction with respect to the intact (positive control) reaction. This compares favorably with the negative control, in which an average of ~800 CPM (82% reduction) was observed (Figure 2).

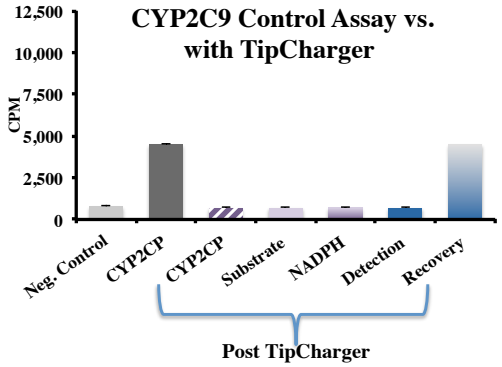


Figure 2. *Pipette tip plasma exposure results for CYP2C9,*

The CYP3A4 assay components (1.0 pmol CYP3A4, 50 µM Luciferin-BE) in Luciferin-free water (200mM KPO4) along with the NADPH regeneration system and detection reagents were removed individually by a 30 second plasma exposure. Here again, the assays that received Luciferin-free water post-TipCharger showed ~11% of the complete reaction, while the negative control plates scored ~1000 CPM (12% of the complete reaction) (Figure 3).

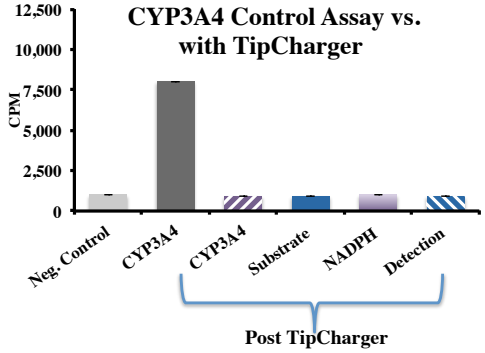


Figure 3. *.Pipet tip plasma exposure results for CYP3A4, substrate, NADPH regeneration system and detection reagent.*

Finally, the CYP2D6 assays that received-Luciferin-free water post-TipCharger showed ~8.6% of the complete intact reaction as compared to the background of ~750 CPM (8.2% of the complete reaction) observed for the control membranes (Figure 4).

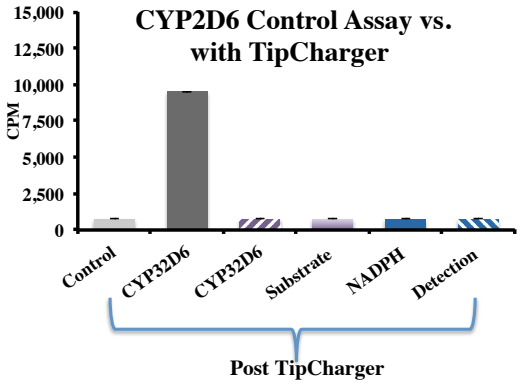


Figure 4. *.Pipette tip plasma exposure results for CYP2D6, substrate, NADPH regeneration system and detection reagent.*

Summary

The cytochrome P450 enzymes play a crucial role in the detoxification and ultimate elimination of natural reactants as well as pharmaceutically active moieties within the body. The biopharmaceutical industry now requires ADMET activities to occur early in the drug discovery process. This investigation strategy results not only in an increasing number of CYP450 assessments, but also in a demand that they be completed more rapidly and with more effective cost control.

The TipCharger by IonField Systems will play an important role in meeting the productivity and cost control demands faced by today's biopharmaceutical industry.

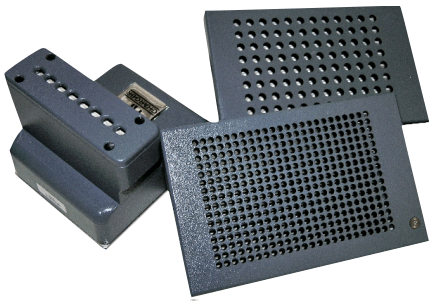
Integrating the TipCharger into Automated Assays Provides the Following Benefits:

Best in Class TipCharger cleans better than any other washing technology - in most applications the TipCharger will clean to background, so there is no difference between plasma cleaning and a new tip.

Cost Benefit TipCharger saves over 90% on the cost of the disposable tips.

Speed Incorporating the TipCharger System can result in a time savings of 10-30 seconds for every microplate processed or rack of tips cleaned.

Convenience Clear away the clutter and save time: Integrating the TipCharger System eliminates the need to store cases of new pipette tips and dispose of racks of hazardous used tips.



TipCharger Plasma Cleaning Stations
Available in 8, 96, 384 and 1536 channel versions

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About the TipCharger™

The TipCharger Cleaning System replaces existing wash stations and easily integrates with most existing and new automation platforms. The system utilizes a low temperature, atmospheric pressure plasma process that cleans metal and plastic pipette tips and pin tools. Treated surfaces are clean, dry and have uniform surface properties.

The TipCharger cleaning process reduces the incidence of micro-bubble formation and other random surface effects that degrade liquid handling precision and accuracy, even with new disposable tips.

IonField Systems' TipCharger improves the reproducibility of process results, shortens automation cycle times, reduces the number of lost runs, and eliminates environmental waste and liquid handling disposables. The overall result is increased confidence in results and a more effective and productive laboratory operation.

About IonField Systems™

IonField Systems is an advanced technology company focused on the development of best-in-class cleaning methods for life science applications.
We provide on-site/phone support and ongoing services to insure a successful installation and exceptional day to day operation of our products.