



Caliper Sciclone ALH Carryover Testing Using the Ultrasonic Wash Station and a 384-Cannula Array

Introduction

As an accessory for the Sciclone Advanced Liquid Handler, the Ultrasonic Wash Station is designed to remove contaminating substances from both 96 and 384-cannula arrays. To test this ability, a concentrated fluorescein solution in DMSO was aspirated and dispensed using a 384/1536 cannula array. The array tips were then cleaned using the Ultrasonic Wash Station to determine both external and internal carryover concentrations.

Materials and Methods

Instrumentation

All carryover testing was performed using a Sciclone Advanced Liquid Handler (ALH) with a Low Volume Head, an Ultrasonic Wash Station, and a 384/1536 cannula array. Fluorescence was measured at an excitation of 485nm and an emission of 535nm using a Wallac Victor2V 1420 Multilabel HTS Counter (PerkinElmer-Wellesley, MA).

Consumables

Corning black, untreated, flat bottomed, 384 well plates (Corning # 3710) were used in this study.

Reagents

A 25mM “contaminating” carboxyfluorescein solution was prepared by adding 0.94g of carboxyfluorescein (Molecular Probes) to 100mL of DMSO. A test buffer (2L) was prepared by adding 20mL of DMSO to 1.98L of 10mM sodium phosphate monobasic, monohydrate $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (JT Baker) (pH 7.5) made in deionized water.

Carryover Testing

All testing was performed using a protocol (available upon request) to execute the following three parts: Challenge, Washing, and Carryover Determination.

Part 1: Challenge

- Aspirate 5 μL clean DMSO (DMSO slug), 2 μL air gap, and 20 μL of the 25mM carboxyfluorescein into the 384/1536 array. Next, dispense the 20 μL of carboxyfluorescein into a 384 well plate leaving the DMSO slug and air gap in the array. This step contaminates the internal and external surface of the cannulas.

Part 2: Washing

After contamination, the cannulas were washed to remove the carboxyfluorescein from both the internal and external surfaces of the cannulas.

- 20 μL clean DMSO is aspirated from a reservoir. The entire content of the cannula array is then dispensed into a waste reservoir. This process dispenses the original DMSO slug as well as the 20 μL DMSO aliquot. A total of two cycles (aspirating 20 μL of clean DMSO then dispensing it into the waste reservoir) is then performed. The cannula array is then washed with flowing deionized water in the Ultrasonic Wash Station. A 2 μL air gap is aspirated, and then 27 μL aliquot of fresh deionized water is aspirated from the top of the Ultrasonic Wash Station, close to the inlet source of the flowing deionized water. This aliquot is dispensed at the bottom of the Ultrasonic Wash Reservoir near the drain of the wash station. This process is repeated three times.

Part 3: Internal and External Carryover Determination

- External Carryover is determined by aspirating 20 μ L of buffer from a Buffer Source plate initially containing 40mL of the Test Buffer. Any carboxyfluorescein remaining on the external surface of the cannulas after the wash steps will result in carryover into Buffer Source plate during the aspiration of the 20 μ L. A measurement of the concentration of the carboxyfluorescein present in the Buffer Source plate is used to quantitate the external carry-over for the cannula array.
- Internal Carryover is determined by dispensing the Test Buffer aspirated from the Buffer Source plate into a clean 384 well plate. Any carboxyfluorescein remaining on the interior of the cannula array after the wash steps will be dispensed along with the Test Buffer into the clean plate. A measurement of the concentration of carboxyfluorescein present in the clean plate yields a measurement of the concentration of the carboxyfluorescein and thus a measurement of the internal carry-over for the cannula array.

Results

A calibration curve was generated for the fluorimeter and used to quantitate the carryover in terms of concentration (parts per million). This concentration was then calculated as carryover of the original 25mM contaminating sample. The mean (n=6 plates of 384 wells/plate) external carryover concentration was found to be less than 0.5 ppm while the internal carryover concentration was 10 ppm. The single maximum value for the 2304 wells is also given. Table 1 summarizes the results of the experiment.

	Mean ppm Carryover (n=2304 wells)	Maximum ppm Carryover Observed (n=2304 wells)
External Carryover	0.5 +/- 0.5	3
Internal Carryover	10 +/- 4	100

Table 1. PPM Carryover of 25mM carboxyfluorescein in DMSO. Mean and maxima are for 2304 wells of data

Conclusion

Carryover testing involves exposing the wetted surfaces of the transfer device to a strong analyte. This exposure contaminates the surfaces with an analyte that may be easily measured in subsequent samples. In this study, carboxyfluorescein was used at an excessively high concentration (25mM) at which any residual carboxyfluorescein will be easily detected.

Here we show the average internal carryover to be approximately 10 ppm while the external carryover averages 0.5 ppm. To achieve this level of cleanliness, a slug of clean DMSO was aspirated before the cannulas were challenged with 25mM carboxyfluorescein. This DMSO slug appears to clean the cannulas from the top, thus displacing the contaminating analyte into waste.

The data shown here supports the premise that with proper cannula washing techniques, carryover concentrations approaching 0.5 ppm (external) or 10 ppm (internal) can be readily achieved.



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