

REUSE OF FILTER PLATES FOR ELUTION OF DRIED BLOOD SPOT SAMPLES IN NEONATAL ASSAYS

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INTRODUCTION

Millipore MultiScreen™ filter plates are used for elution of dried blood spot samples in neonatal screening assays. A significant concern with the use of these plates is their cost. In this study we have evaluated the practice of reusing the filter plates in the Bio-Rad PKU and total galactose neonatal assays.

MATERIALS AND METHODS

Cleaning procedure

All dried blood spots (DBS) were removed from the wells by shaking or tapping the plate over an appropriate biohazard trash bin. The plates were rinsed under running tap water, ensuring the plates were filled and emptied 3 times. The plates were soaked for four hours by immersing in distilled or deionized water, ensuring that all wells were filled with water. Excess water was shaken out, blotted on paper towel, and dried in a 37 C incubator overnight.

Recovery and carryover

The following recovery and carryover protocols were run on one plate from one lot for 1/8 inch DBS and on one plate from each of three separate lots for 3/16 inch DBS. The protocols were used on both the Bio-Rad PKU and Total Galactose assays.

Testing for recovery between runs

Standard curve DBS were placed into duplicate wells of columns A and B of each filter plate. The rest of the wells of columns B through column G were filled with DBS of standard 2 (middle standard) and standard 4 (high standard) in alternating wells. Column H wells were filled with 2 X DBS of the high Standard. DBS were eluted with the TCA elution buffer and tested according to the assay protocols. After the assay was performed, all wells of the filter plates were washed as described in the cleaning procedure above. Steps 1 through 3 were repeated three more times for each plate.

Testing for carryover between runs

All wells of the test plates used in the recovery study above were washed for a fifth time and then filled with DBS using the zero standard. An unused plate filled with zero standard DBS was used as a control. DBS were eluted and tested according to assay protocols. Results of the fifth run with zero standard on the used plates were compared to zero standard eluted on the unused plate.

RESULTS

Physical damage

There were no plugged wells observed in any of the filter plates at any time. The Eluate flowed through the filters at the same rate for the final use as it did for the first use of the plates. There was no physical damage observed in any of the wells of any of the filter plates.

Recovery between runs

PKU. The Percent OD Recovery from all three lots of filter after four washes, ranged from 90.0% to 102% compared to the initial OD values of the unused plate. CVs on reused plates were comparable to those on the first run. Results were comparable between lots of plates.

Total galactose. The Percent OD Recovery from all three lots of filter plates after four washes, ranged from 91.3% to 105.3% compared to the initial OD values of the unused plate. CVs on reused plates were comparable to those on the first run. Results were comparable between lots of plates.

Carryover between runs

PKU. The mean OD of the zero standard on each plate that had been washed and reused 5 times, ranged from 0.011 to 0.019 OD, compared to the OD range of the unused control plate which was 0.012 to 0.020 OD. Among all lots of plates, the difference between the average OD of the test plate and control plate ranged from -0.001 to 0.000 OD.

Total galactose. The mean OD of the zero standard on each plate that had been washed and reused 5 times, ranged from 0.008 to 0.010 OD, compared to the OD range of the unused control plate which was 0.009 to 0.010 OD. Among all lots of plates, the difference between the average OD of the test plate and control plate ranged from -0.001 to 0.000 OD. Results of both assays showed virtually no carryover between runs, even in wells that had 2X 3/16 inch DBS of the high Standard.

Standard curves

Washing and reusing the filter pates had little or no

effect on the Standard curves for both PKU and total galactose. Results of all lots of plates were similar.

CONCLUSIONS

We have shown that Millipore MultiScreen™ filter plates used in the Bio-Rad PKU and total galactose assays can be washed and reused at least three times before they are discarded, providing a cost savings benefit for neonatal screening labs. Each laboratory should also validate the washing and reusing of these filter plates within their own laboratory environment.