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Reducing Disposable Plastic Waste from Protein Quantitative Assays

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Reducing Disposable Plastic Waste from Protein Quantitative Assays

A Capstone Project

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Abstract

Protein concentration quantification is important for many areas of biology and biochemistry. This quantification happens in large quantities in laboratories and needs to be done accurately and quickly. The Bradford assay is one of the most common ways of accomplishing this task (1). This assay relies on the binding of the protein and the Coomassie Blue G250 dye. The anionic blue form binds to the protein and has a maximum at 595 nm. This allows for accurate quantification by reading how much of the sample absorbs at 595 nm (2).

In 2014, it was estimated that 5.5 million tons of plastic waste was generated in all research laboratories around the world, this is equivalent to 83% of all the plastic recycled in 2012 (3). The purpose of this project is to determine laboratory techniques that can reduce the plastic waste of 96-well plates and 100 µL pipette tips by washing and reusing these plates and tips while still maintaining satisfactory results. One of the problems with the Bradford assay is that the dye used stains the 96-well plates making reuse difficult. Using a simple washing method, however, both the 96-well plates and the 100 µL pipette tips showed no statistical variation from a given standard even after significant reuse.

Introduction

Plastic waste is one of the most important issues facing the world today. Plastic waste, compared to other types of waste, poses a significant environmental issue as it does not necessarily decompose in the environments it will contaminate. This poses a serious problem as these plastics will simply accumulate choking out the natural resources the ecosystems of the world need. Accumulating plastics in the environment also presents sever human health risks. These plastics, when ingested or inhaled, expose humans to physical, chemical, and pathogenic risks. Plastics

themselves can disrupt systems within the human body, but also leach toxic chemicals used in plastic production as well as acting as hydrophobic substance for other harmful chemicals to sorb to and bacterial, viral, and parasitic pathogens to collect on. By having an ever-increasing number of plastics in water and air ways, the risk of harmful exposure only increases(4). Reducing plastic waste is then paramount.

In an effort to hasten this reduction, countries like England are implementing bans for common single-use plastic items such as plates, bowls, cups, and cutlery (5). These bans can go a long way towards shifting the culture away from using these types of single-use items and more towards long-lasting or easily recyclable ones.

Laboratories, however, are often full of single-use plastics like pipette tips, gloves, tubes, and plates, despite the importance of reducing plastic waste. These single use items quickly add up and through just one day of regular use a laboratory scientist could go through hundreds of grams of plastic (6). Some laboratories have shifted to more environmentally friendly policies, changing the materials and methods they use to reduce as much single-use and non-recyclable plastics and materials as they can (7).

The easiest thing to do is use less plastic by switching to glass or other long-lasting or recyclable materials, but this is not always cost effective or even possible depending on the procedure. The next best thing is to find ways to reuse the plastics that do get used and then to recycle what plastics to get used that cannot be reused. Another angle to reduction is to be more efficient with the plastics used. As an example, when using 96-well plates, a laboratory might not use the entire plate before throwing it away. The reasons for not using all 96 wells could be related to the specific method being used or a worry about contamination. Regardless of the reasons, the low percentage of the plate being used means that the plastic waste being generated is not only

high but somewhat unnecessary. A great way to change this is to shift away from the traditional 96-well plate structure and move towards a modular frame structure. A traditional 96-well plate is made of one solid piece of plastic, but the modular frame design only has a solid frame with 12 strips of eight wells each that slot into the frame. This design works the same for analysis but can fix the issue of having to throw away the entire plate. The frame will almost never be thrown away and with the layout of the wells and slight method adjustment laboratories could approach using the entire plate all the time, reducing plastic waste significantly for this one item.

This waste reduction can be expanded both for plates and tips if a simple washing process is adopted for reuse of these plastics. Pipette tips are used once and immediately thrown away to avoid contamination. This, however, is unnecessary depending on the solution being pipetted. The same thing is true for many applications of a 96-well plate. A simple washing process can be employed to allow for these items to be reused while maintaining both high accuracy of results and low contamination. A more rigorous cleaning process might be employed to allow for even further long-term use of these typically single-use items, but that will often be outside of the ability of most laboratories. Finding a way to reuse both types of items is important as they are used in everyday functions of laboratories everywhere, and if they could be reused just once that would be a 50% reduction in plastic waste resulting from pipette tips and 96-well plates. Ideally, the plastics would be able to be used indefinitely, but even only being able to be used 5 times is an 80% reduction and there are steep diminishing returns on plastic waste reduction for reuse. The focus then should be on simple reuse practices and reduction of plastic use by increasing efficiency and overall shift away from plastics.

A common analytical method used in many laboratories is the Bradford assay. The Bradford assay works well to study the reuse of common plastics due to the staining properties of

the chemicals involved. The Bradford assay uses a Coomassie Blue G250 dye. This dye will bind to proteins found in a sample. This causes a color change in the dye from blue to purple. This color change can be used to determine the concentration of a protein in a sample by using UV-Vis analysis. When the dye binds its absorption maximum increases from 465 nm to 595 nm, using a calibration curve, a sample can be tested to determine how much of the dye is bound to a protein by reading at 595 nm (2).

Traditionally this would be done using quartz cuvettes reading each one at a time. Due to the quantity of samples analytical labs go through this process was adapted to 96-well plates where a UV-Vis plate reader can be employed to read a whole plate very quickly. This also allows for easily setting up duplicates for the calibration curve and samples all on a single plate that can be read in one operation. The downside for 96-well plates is that they are plastics and often single-use due to the nature of chemical contamination. Additionally, the dye used in the Bradford assay does stain the wells it is used in. If a simple washing method is able make these 96-well plates usable again further Bradford assays, that could cut down on plastic waste substantially without having to change the method drastically.

Materials

The materials used for the project consisted of 100 µL pipette tips bought from Fischer Scientific used to investigate the efficacy of washing tips for reuse. Modular 95-well plates bought from Greiner (Item No.: 762070) used to determine if a 96-well plate could be reused after a being used for a Bradford assay. Bovine serum albumin (BSA) bought from Sigma Aldritch used primarily at a concentration of 2.0 mg/mL. Phosphate buffered saline (PBS) bought from Boston BioProducts. Coomassie Plus™ Protein Assay bought from Thermo Scientific used as the primary staining reagent in the Bradford assay. Statistical analysis was performed using GraphPad's Prism

(version 9.5.1) to perform one-way ANOVA tests with a Dunnett's multiple comparison test with a sample size of 8 and a level of significance of 0.05.

Methods

The BSA solution was made up at 2.0 mg/mL in PBS using 0.0798 mg of BSA which was dissolved in 40 mL of PBS.

When investigating the efficacy of reusing tips, two methods were used. The first was to determine if the tips would deform after many uses. To test this, tips were used at a BSA concentration of 2.0 mg/mL, over a range from 0-25 uses. Each level of use had 8 tips used at that level. To test the deformation each tip was used to bring up 100 µL of the BSA and then dispense back a number of times according to the level of the designated usage.

The second method was to determine if a simple washing method would eliminate contamination. A set of 8 tips were used to bring up and dispense back 10 µL of 2.0 mg/mL BSA. These tips were then used for a row of a Bradford assay using deionized water instead of a protein solution. This process was repeated but before the next set of tips was used, each was washed once by bring up and dispensing 10 µL of deionized water. The next set did this washing process twice and the final set did this washing process three times. All of these sets were compared to a control group of fresh tips that were also used for the assay.

To test 96-well plate reuse, four strips of a modular 96-well plate were designated. The first was a control group. The second was to be reused once. The third was to be reused 3 times. The fourth was to be reused four times. The first strip was set aside and the second, third, and fourth strips all had Bradford assays performed in each of the eight wells at 2.0 mg/mL. Afterwards, each well was washed with 100 µL of deionized water three times. The second strip was now set aside,

and this process was repeated for the third and fourth strip two more times. Then the third strip was set aside, and the process was repeated for the fourth strip two more times. Finally, all 4 strips had a final Bradford assay performed using each of their eight wells and the results of the second, third, and fourth strips were compared to the control group in the first strip. Data was only acquired and analyzed from the final Bradford assay.

The Bradford assays were analyzed using a UV-Vis plate reader and reading each well's absorbance at 595 nm. This data was statistically analyzed using GraphPad's Prism software. Each data set had a one-way ANOVA test done to determine if there were any statistically significantly different data sets. Then, a Dunnett's multiple comparison test was done as a post-hoc test for further analysis to compare each data set to a given control to determine which data sets, if any, were significantly different.

Results and Discussion

To analyze tip deformation, the tips were used to perform a Bradford assay at 2.0 mg/mL using one well per tip. The data obtained from the UV-Vis analysis was statistically analyzed by one-way ANOVA with a Dunnett's multiple comparison test. The 0 use tips were used as the control for this analysis. Reusing tips 5, 10, and 25 times all showed no statistically significant difference from the control group, as shown in Figure 1. This shows tip deformation does not occur even at high levels of reuse (e.g., 25 uses or a 25 fold reduction in plastic tip waste). Figure 2 and the other tip analysis shows that contamination can be minimized or eliminated with a simple washing method. When compared to the control group the only group that showed statistically significantly different results than the control group were the tips that did not use the washing method. The tips that were only washed once still fall within error but do vary enough to warrant

using the two or three wash method, both of which are well within error. Thus, the 3 separate wash method would be considered appropriate for most lab environments.

The data acquired from the final Bradford assay of the 96-well reuse study was statistically analyzed by one-way ANOVA with a Dunnett's multiple comparison test and is shown in Figure 3. There is no statistical variation between any of the three levels of reuse and the control group, indicating that a 96-well plate that has been washed can be reused up to at least 5 times without losing any accuracy. This results in an 80% reduction of plastic plate waste that is generated from 96-well plates.

Conclusion

Reducing plastic waste in laboratories is an incredibly important goal. The results of this project show that there are simple and effective ways to do so using tools and materials that laboratories already have at their disposal. These methods most likely will have the greatest impact in university teaching and research laboratories, where having to spend a significant amount of time or money on a system to wash or recycle the plastic waste in a lab would be cost prohibitive. In industry, moreover, such methods could play a role in non-critical testing or in developing countries where resources are also limited. Switching to modular 96-well plates and using a simple wash method only requires changing a product order and using deionized water, items laboratories can easily handle. In certain scenarios, switching to modular plates over the traditional design would not only save plastic but be more efficient as rather than giving each student or researcher a plate to perform a single example assay on, they can now use a few rows. This would save both time and material while still allowing the user to have hands-on experience performing an analytical process.

Although the methods discussed here do have incredible results for plastic waste reduction, some of that reduction is limited by the addition of chemical waste. The chemical waste produced, however, is very limited. The Bradford assay only requires 10 µL of protein per well and only around 100 µL of deionized water per wash. This results in very limited volumes of chemical waste being produced especially compared to the amount of plastic waste reduced when using this method. Additionally, the chemical waste produced is very low level and can be handled through waste management systems better than plastic waste.

The scope of this project was limited. Yet, the results demonstrated that significant plastic waste can be reduced in typical settings using very straightforward techniques accessible to nearly every laboratory. There are multiple aspects of these methods and materials that this project did not address, such as the longevity of these materials as they are reused. The assumption was nothing discussed in this report would be reused outside of the day it was initially used (in our experience, this is the likely scenario for most laboratories), but it is possible that the plastics do not degrade over appreciable time for both the tips and the 96-well plates. It is feasible that longer time horizons could be achieved with our methodologies. These aspects will be addressed in future research projects.

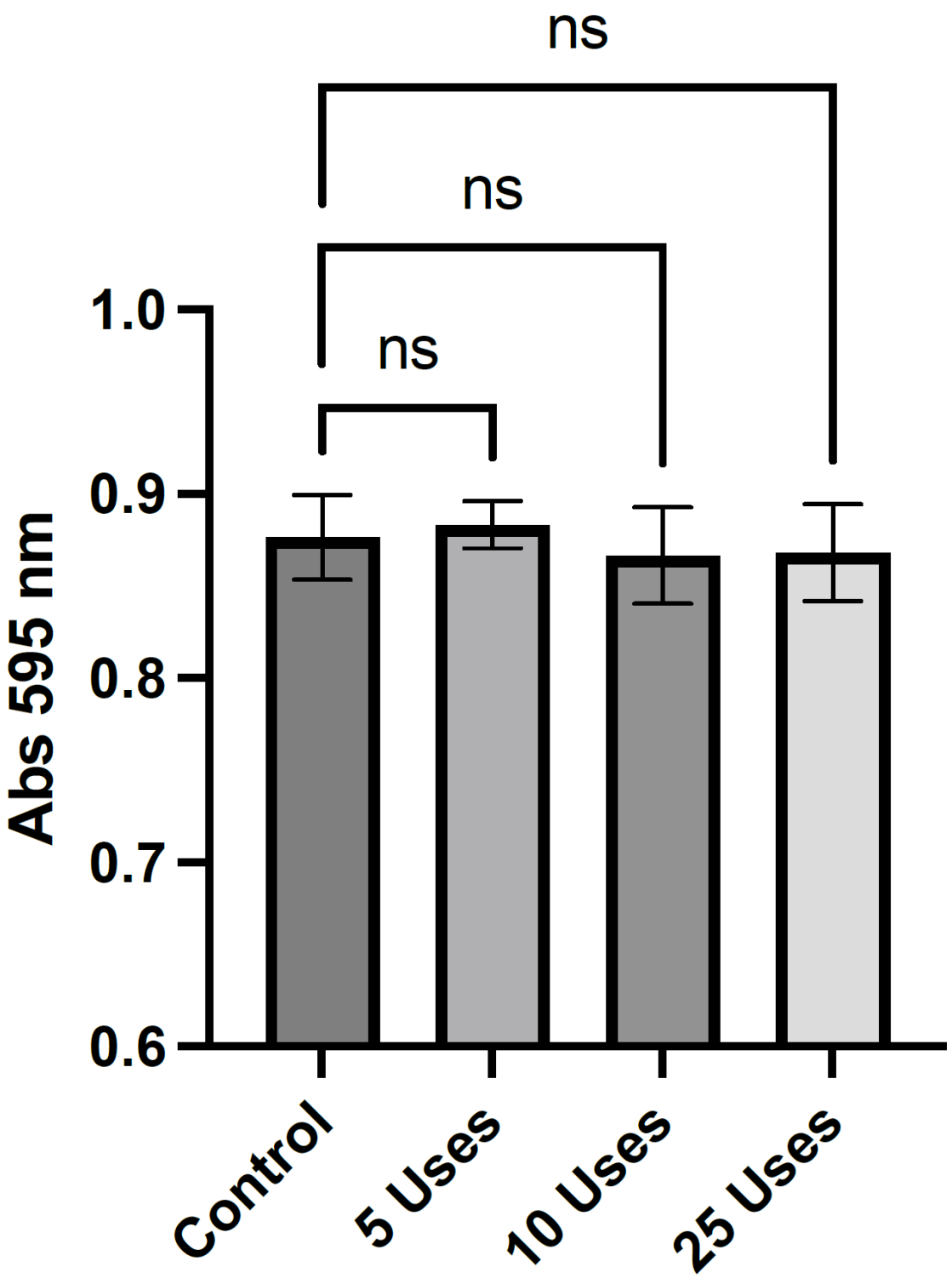

Figure 1. UV-Vis data obtained from Bradford assay analysis of tip reuse study. “ns” standing for “no significance.”

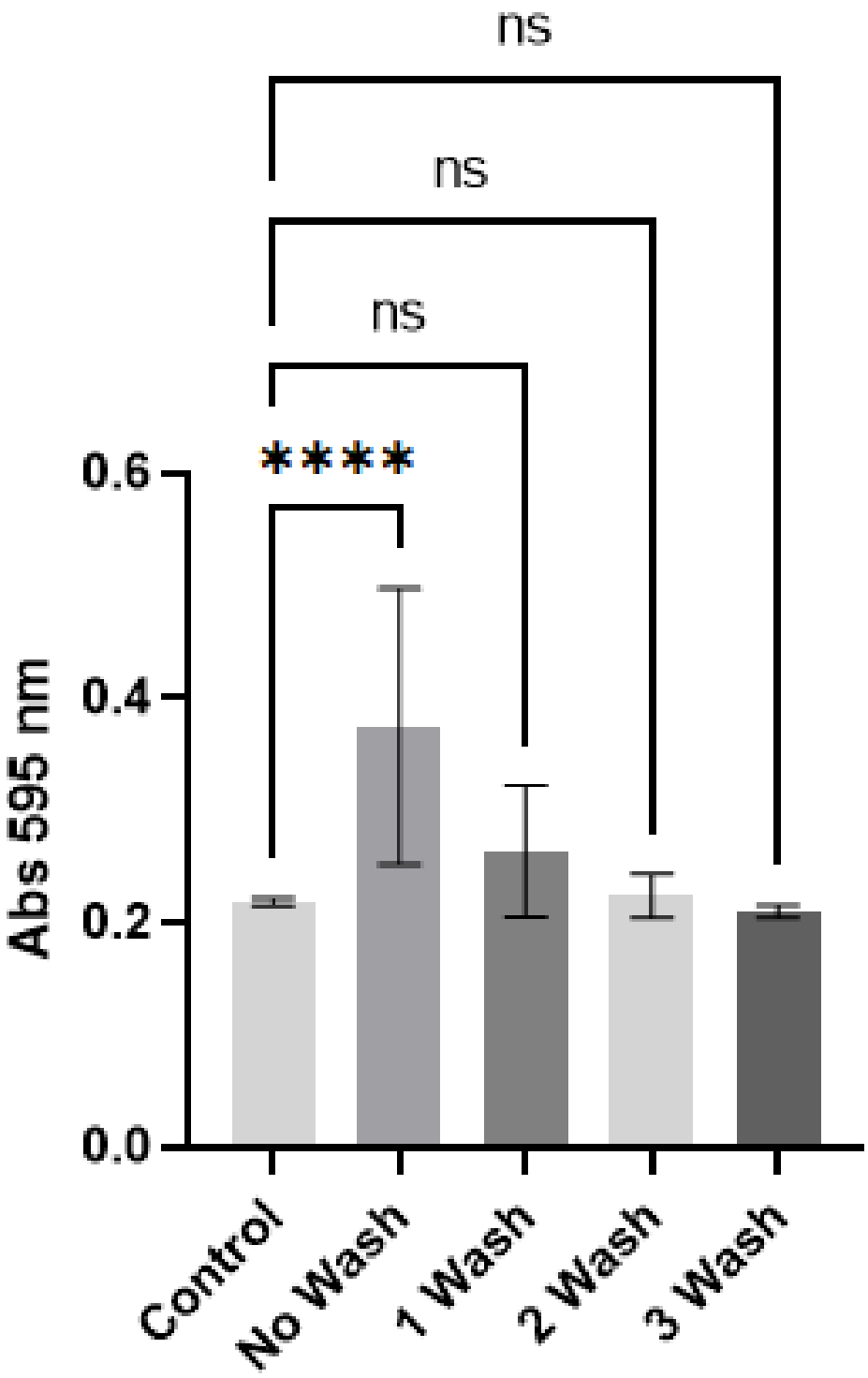

Figure 2. UV-Vis data obtained from Bradford assay analysis of tip wash study. “ns” standing for “no significance” and “****” showing a statistically significant difference when compared to the control.

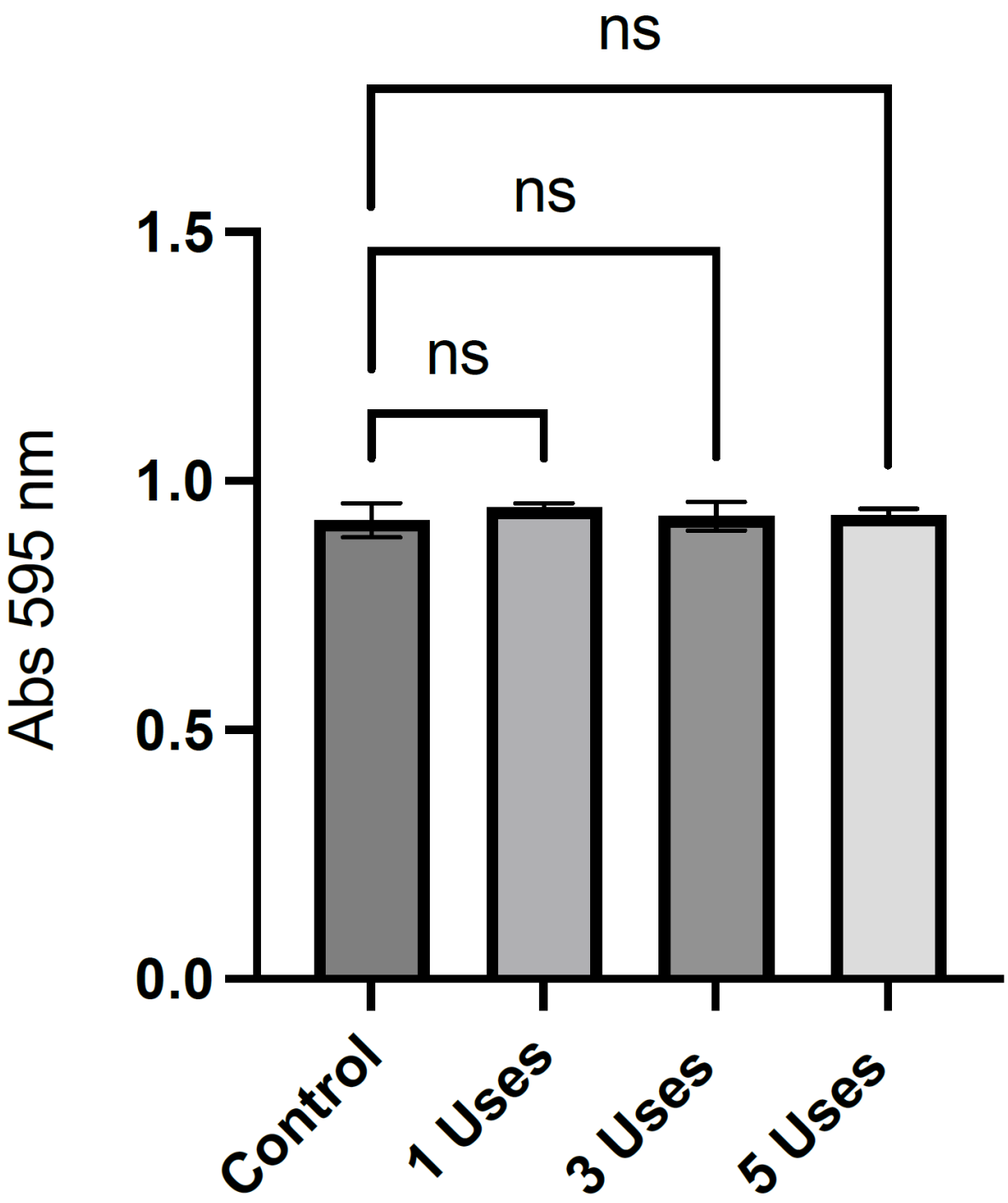

Figure 3. UV-Vis data obtained from the final Bradford assay analysis of 96-well plate reuse study. “ns” standing for “no significance.”

Table 1. UV-Vis absorbances obtained from Bradford assay analysis of tip reuse study.

	Group A	Group B	Group C	Group D
	Control	5 Uses	10 Uses	25 Uses
2	0.875	0.885	0.819	0.851
3	0.884	0.887	0.873	0.874
4	0.914	0.884	0.872	0.831
5	0.869	0.883	0.866	0.891
6	0.890	0.889	0.851	0.882
7	0.878	0.880	0.890	0.897
8	0.832	0.902	0.906	0.885

Table 2. One-way ANOVA analysis results for the UV-Vis absorbance data obtained from the tip reuse study.

	Ordinary one-way ANOVA ANOVA results					
4	**ANOVA summary**					
5	F	0.9454				
6	P value	0.4320				
7	P value summary	ns				
8	Significant diff. among means (P < 0.05)?	No				
9	R squared	0.09198				
10						
11	**Brown-Forsythe test**					
12	F (DFn, DFd)	1.114 (3, 28)				
13	P value	0.3599				
14	P value summary	ns				
15	Are SDs significantly different (P < 0.05)?	No				
16						
17	**Bartlett's test**					
18	Bartlett's statistic (corrected)	3.645				
19	P value	0.3025				
20	P value summary	ns				
21	Are SDs significantly different (P < 0.05)?	No				
22						
23	**ANOVA table**	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
24	Treatment (between columns)	0.001470	3	0.0004899	F (3, 28) = 0.9454	P=0.4320
25	Residual (within columns)	0.01451	28	0.0005181		
26	Total	0.01598	31			
27						
28	**Data summary**					
29	Number of treatments (columns)	4				
30	Number of values (total)	32				

Table 3. Dunnett's multiple comparison test for the UV-Vis absorbance data obtained from the tip reuse study.

	Ordinary one-way ANOVA Multiple comparisons								
1	Number of families	1							
2	Number of comparisons per family	3							
3	Alpha	0.05							
4									
5	**Dunnett's multiple comparisons test**	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**	**A-?**		
6	Control vs. 5 Uses	-0.006625	-0.03489 to 0.02164	No	ns	0.8882	B	5 Uses	
7	Control vs. 10 Uses	0.01000	-0.01826 to 0.03826	No	ns	0.7125	C	10 Uses	
8	Control vs. 25 Uses	0.008750	-0.01951 to 0.03701	No	ns	0.7837	D	25 Uses	
9									
10	**Test details**	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
11	Control vs. 5 Uses	0.8766	0.8833	-0.006625	0.01138	8	8	0.5821	28
12	Control vs. 10 Uses	0.8766	0.8666	0.01000	0.01138	8	8	0.8786	28
13	Control vs. 25 Uses	0.8766	0.8679	0.008750	0.01138	8	8	0.7688	28

Table 4. UV-Vis absorbances obtained from Bradford assay analysis of tip wash study.

	Group A	Group B	Group C	Group D	Group E
	Control	No Wash	1 Wash	2 Wash	3 Wash
1	0.217	0.427	0.218	0.219	0.204
2	0.215	0.252	0.318	0.207	0.206
3	0.216	0.341	0.274	0.212	0.207
4	0.217	0.350	0.225	0.225	0.204
5	0.215	0.232	0.226	0.210	0.209
6	0.219	0.630	0.214	0.222	0.222
7	0.219	0.363	0.379	0.222	0.213
8	0.225	0.398	0.253	0.269	0.211

Table 5. One-way ANOVA analysis results for the UV-Vis absorbance data obtained from the tip wash study.

Ordinary one-way ANOVA ANOVA results					
ANOVA summary					
F	9.855				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.5297				
Brown-Forsythe test					
F (DFn, DFd)	4.321 (4, 35)				
P value	0.0060				
P value summary	**				
Are SDs significantly different (P < 0.05)?	Yes				
Bartlett's test					
Bartlett's statistic (corrected)	76.47				
P value	<0.0001				
P value summary	****				
Are SDs significantly different (P < 0.05)?	Yes				
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Treatment (between columns)	0.1495	4	0.03737	F (4, 35) = 9.855	P<0.0001
Residual (within columns)	0.1327	35	0.003792		
Total	0.2822	39			
Data summary					
Number of treatments (columns)	5				
Number of values (total)	40				

Table 6. Dunnett's multiple comparison test for the UV-Vis absorbance data obtained from the tip wash study.

Ordinary one-way ANOVA Multiple comparisons								
Number of families	1							
Number of comparisons per family	4							
Alpha	0.05							
Dunnett's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**	**A-?**		
Control vs. No Wash	-0.1563	-0.2350 to -0.07749	Yes	****	<0.0001	B	No Wash	
Control vs. 1 Wash	-0.04550	-0.1243 to 0.03326	No	ns	0.3954	C	1 Wash	
Control vs. 2 Wash	-0.005375	-0.08413 to 0.07338	No	ns	0.9991	D	2 Wash	
Control vs. 3 Wash	0.008375	-0.07038 to 0.08713	No	ns	0.9961	E	3 Wash	
Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
Control vs. No Wash	0.2179	0.3741	-0.1563	0.03079	8	8	5.075	35
Control vs. 1 Wash	0.2179	0.2634	-0.04550	0.03079	8	8	1.478	35
Control vs. 2 Wash	0.2179	0.2233	-0.005375	0.03079	8	8	0.1746	35
Control vs. 3 Wash	0.2179	0.2095	0.008375	0.03079	8	8	0.2720	35

Table 7. UV-Vis absorbances obtained from the final Bradford assay analysis of 96-well plate reuse study.

	Group A	Group B	Group C	Group D
	Control	1 Uses	3 Uses	5 Uses
1	0.941	0.955	0.927	0.922
2	0.932	0.963	0.946	0.951
3	0.942	0.942	0.943	0.927
4	0.941	0.939	0.860	0.946
5	0.943	0.946	0.939	0.931
6	0.860	0.941	0.944	0.930
7	0.939	0.944	0.929	0.927
8	0.873	0.947	0.946	0.915

Table 8. One-way ANOVA analysis results for the UV-Vis absorbance data obtained from the final Bradford assay analysis of 96-well plate reuse study.

Ordinary one-way ANOVA ANOVA results					
ANOVA summary					
F	1.68				
P value	0.1930				
P value summary	ns				
Significant diff. among means (P < 0.05)?	No				
R squared	0.153				
Brown-Forsythe test					
F (DFn, DFd)	0.753 (3, 28)				
P value	0.5300				
P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
Bartlett's test					
Bartlett's statistic (corrected)	15.5				
P value	0.0015				
P value summary	**				
Are SDs significantly different (P < 0.05)?	Yes				
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Treatment (between columns)	0.00280	3	0.000933	F (3, 28) = 1.68	P=0.1930
Residual (within columns)	0.0155	28	0.000554		
Total	0.0183	31			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	32				

Table 9. Dunnett's multiple comparison test for the UV-Vis absorbance data obtained from the final Bradford assay analysis of 96-well plate reuse study.

Ordinary one-way ANOVA Multiple comparisons								
Number of families	1							
Number of comparisons per family	3							
Alpha	0.05							
Dunnett's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Below threshold?**	**Summary**	**Adjusted P Value**	**A-?**		
Control vs. 1 Uses	-0.0258	-0.0550 to 0.00347	No	ns	0.0932	B	1 Uses	
Control vs. 3 Uses	-0.00788	-0.0371 to 0.0213	No	ns	0.8427	C	3 Uses	
Control vs. 5 Uses	-0.00975	-0.0390 to 0.0195	No	ns	0.7456	D	5 Uses	
Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
Control vs. 1 Uses	0.921	0.947	-0.0258	0.0118	8	8	2.19	28
Control vs. 3 Uses	0.921	0.929	-0.00788	0.0118	8	8	0.669	28
Control vs. 5 Uses	0.921	0.931	-0.00975	0.0118	8	8	0.829	28

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