Comparison of PCR-TE (TE-4) Buffer made with hydrochloric acid to PCR-TE (TE-4) Buffer made with glacial acetic acid.

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BACKGROUND

It was discovered that three previous lots of PCR-TE (TE-4) Buffer were made with 1M Tris-CH_3COOH Buffer pH 8 (Tris Acetate) where glacial acetic acid was added to adjust the pH instead of 1M Tris-HCl Buffer pH 8 (Tris HCL) where hydrochloric acid (HCl) is added to bring the solution to a pH of 8. Both Tris HCl and Tris Acetate are commonly used in nucleic acid analysis; however, the ISPFS analytical method only allows for the use of Tris HCl when making PCR-TE (TE-4) buffer. Two of these three lots were used in casework and database processing. Since TE buffer is used mainly in quantitation and the normalization of samples for amplification, it was determined that a test comparing the old lot of TE made with Tris Acetate with a new lot made with Tris HCl would be performed, focusing on these two steps.

OBJECTIVE

Determine if similar results are obtained during quantification and amplification of DNA samples with PCR-TE (TE-4) Buffer made with Tris HCl and PCR-TE (TE-4) Buffer made with Tris Acetate.

METHODS

A dilution series was made with 2800M DNA starting at 5ng/µL and going down to 0.039ng/µL with water as the diluent. The 2800M DNA was not quantitated prior to preparing the dilution series. It was assumed the starting value was 10ng/µL as labeled. This yielded 8 DNA concentrations to be quantitated. Plexor® HY system standards were made in duplicate with one set using the old lot of TE and one set using the new lot of TE. Samples were quantitated using the Plexor® HY system and ABI 7500 Real-Time PCR system. Data was analyzed with Plexor® Analysis Software v1.6.0. The samples were analyzed first with the standards made with the new TE and then with the standards made with the old TE.

Dilutions of the 2800M 5ng/µL, 2.5 ng/µL, 1.25 ng/µL, and 0.625ng/µL concentrations were made in duplicate. The first set was done using the new lot of TE as the diluent and the calculations from using the Plexor® HY system standards made with the new lot of TE. The second set was done using the old lot of TE as the diluent and the calculations from using the Plexor® HY system standards made with the old lot of TE.

The ISPFS analytical method targets a concentration of 0.1 ng/µL when normalizing DNA extract samples. 15µL of the normalized sample is then amplified for an amplification target total of 1.5ng of DNA. To achieve this for samples with a quantitation value >0.1ng/µL, an aliquot of the original DNA extract, typically 5µL, is diluted with TE to bring the concentration of the aliquot to 0.1ng/µL. TE is not added to the entire DNA extract, only to the aliquot for amplification. Samples with a quantitation of <0.1ng/µL do not have TE added as part of the normalization procedure. As such, lower concentrations were not part of this experiment as those samples would have been unaffected by this issue.

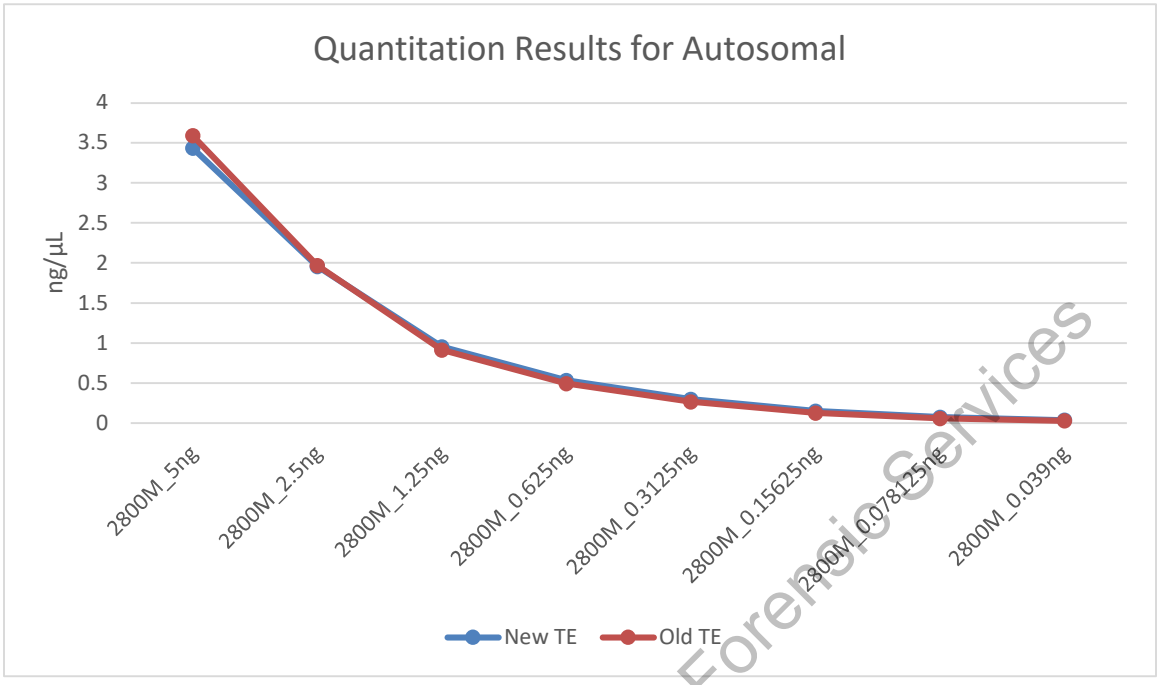
The normalized samples were amplified using the PowerPlex® Fusion 6C kit and were run on an ABI 3500xL Genetic Analyzer. Data were analyzed using GeneMapper™ IDX software version 1.6.

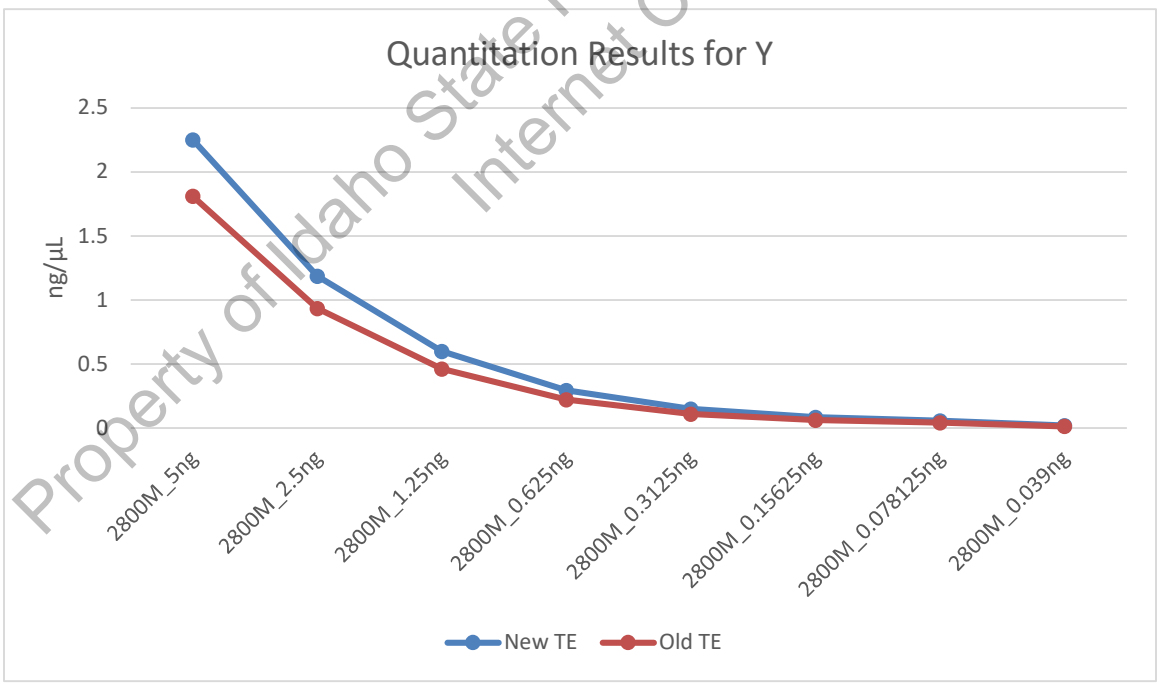
RESULTS

There was a difference between the expected concentrations of the 2800M dilutions, and the results obtained for both the new and old lots of TE. However, the dilutions were made from a tube of 2800M which was labeled as 10ng/µL from the manufacturer, which through previous use is known to not always be accurate. The variations of quantification values obtained between the two TE lots were minimal and are shown in the chart and graphs below:

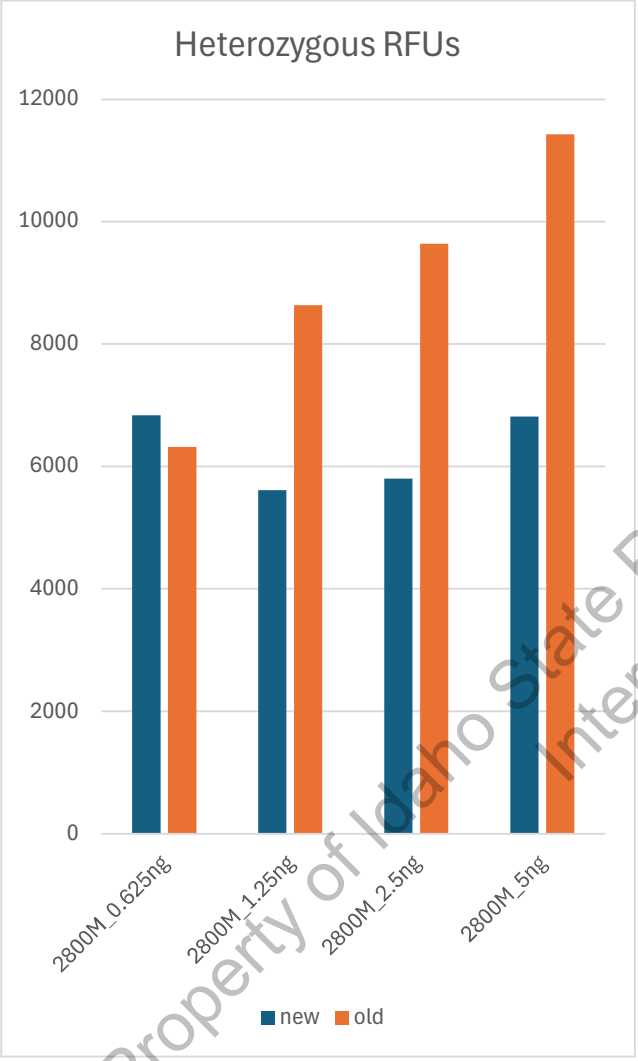
	autosomal DNA			Y DNA		
	New TE	Old TE	Difference (new-old)	New TE	Old TE	Difference (new-old)
2800M_5ng	3.437	3.592	-0.155	2.252	1.811	0.441
2800M_2.5ng	1.956	1.968	-0.012	1.187	0.9355	0.2515
2800M_1.25ng	0.9553	0.9161	0.0392	0.5994	0.4621	0.1373
2800M_0.625ng	0.5343	0.4928	0.0415	0.2936	0.2212	0.0724
2800M_0.3125ng	0.2998	0.266	0.0338	0.1488	0.1097	0.0391
2800M_0.15625ng	0.1509	0.1279	0.023	0.08577	0.06212	0.02365
2800M_0.078125ng	0.07241	0.05839	0.01402	0.05802	0.0415	0.01652
2800M_0.039ng	0.03798	0.02932	0.00866	0.02057	0.01423	0.00634
	Average difference		0.0408975	Average difference		0.12347625

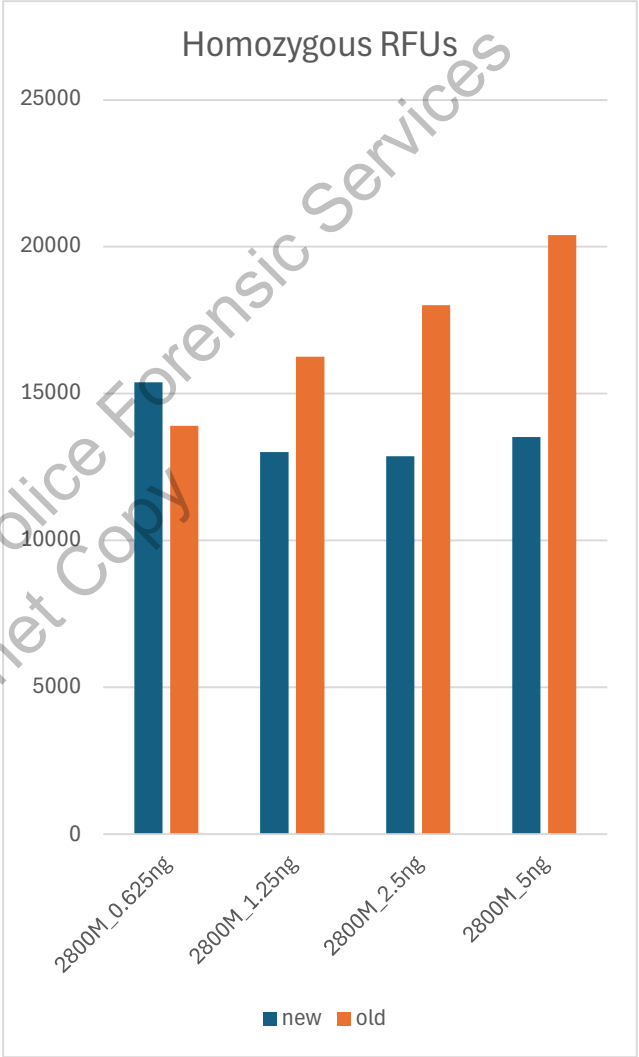
All measurements in ng/µL





Full and correct profiles were developed from all concentrations of the samples amplified in each set. All RFU values were above the lab's analytical threshold of 75 RFU and stochastic threshold of 700 RFU. Some variation in RFU value was observed, which is to be expected due to using different normalization values for each set. Peak height ratios at the heterozygous loci in both sets were >62%. Excessive amplification artifacts that would interfere with data interpretation were not observed in either set of samples.





CONCLUSIONS

The use of glacial acetic acid in making the 1M Tris-HCl Buffer pH 8, creating instead 1M Tris-CH_3COOH Buffer pH 8, appears to have had limited effects on both the quantitation and amplification of DNA samples. Both the quantification as well as the amplification performed as expected with TE buffer made with each acid.