

## Abstract

**Background:** Free phenytoin concentration samples require a preanalytic spin through a filtration system to separate free from bound drug. A good filtration system needs to produce an accurate result as well as a high yield.

**Objective:** The objective of this study was to compare the performance of the Vivafree™ 2 centrifugal filters from VivaProducts versus the Centrifree® Ultrafiltration Device from Millipore for free phenytoin testing. Performance was evaluated by comparing the analytical results for the filtered samples and the yield of filtrate.

**Method:** 40 samples were created using patient pools and the Roche COBAS FP Free Phenytoin Calibrators. Mixtures were created to span the AMR of 0.1-4.0 µg/mL. 500 µL of each mixture was then transferred to a filter from each manufacturer and spun for 20 minutes at 1810 g. The samples were transferred to cobas micro sample cups and processed on a Roche cobas Integra 800. Filtration yield was investigated by making a patient pool and spinning 3 of each manufacturers' filters with a range of volumes up to 1 mL. Also, 2 mL of sample in the Vivafree™ 2 filters was tested.

**Results:** Values of <0.1 were interpreted as 0.0 and values >4.0 were interpreted as 4.0. The correlation, slope and intercept are shown in the plot. Comparing the yields, the Centrifree filter produces a higher yield at lower sample volumes, producing 130 µL with 250 µL of sample vs. 50 µL yielded by the Vivafree™. At 1000 µL of sample, the filters yield 180 vs. 187 µL respectively.

**Conclusion:** The Vivafree™ 2 and Centrifree filtration systems produce comparable analytical results. The Centrifree system has a higher yield at lower volumes, but the volumes are comparable at higher sample volumes. If larger volume yields are needed, the Vivafree filters could be utilized.

## Introduction

Phenytoin is a first-generation antiepileptic drug approved by the FDA for the treatment of seizure disorders and a short-term prophylactic agent in brain injury. The unbound (free) fraction is the biologically active portion of the total serum concentration. Protein binding can be reduced due to physiological conditions or coadministration of drugs with similar binding properties, resulting in toxicity even when total serum drug concentration is within the therapeutic range. Seizures in a patient being treated with phenytoin may be a result of subtherapeutic or toxic concentrations. At therapeutic concentrations, the elimination pathway for phenytoin may become saturated (zero-order kinetics), resulting in relatively small changes in dosage or elimination having dramatic effects on plasma concentration. For most patients, therapeutic total serum concentrations range from 10 to 20 g/mL, while the therapeutic range for free serum concentrations range from 1 to 2 µg/mL.

The objective of this project was compare the results and yield produced from the Vivafree™ 2 centrifugal filters from VivaProducts versus the Centrifree® Ultrafiltration Device from Millipore for free phenytoin testing. Results were obtained by running the filtered samples on the Roche Integra 800 using the Free Phenytoin fluorescence polarization assay. Samples were produced to span the analytical measuring range or 0.1 to 4.0 µg/mL.

## Methods

To compare the results produced by each filter, 2 patient plasma pools were created and processed to determine the free phenytoin values. These pools were then mixed with the 6 Roche COBAS FP Free Phenytoin Calibrators to create 40 samples that spanned the AMR. 500 µL of each sample was transferred to each filter type and spun in a Drucker Diagnostics 755VES centrifuge for 20 minutes at 3600 rpm (1810 g). The filtered samples were transferred to cobas micro sample cups and processed on a Roche cobas Integra 800.

To compare the filtered sample yield of each filtration system, a patient plasma pool was created. Sample volumes of 250, 500, 750 and 1000 µL were transferred to 3 of each filtration system. A sample volume of 2000 µL was transferred to the Vivafree™ 2 filters in triplicate. All of the filtration systems were spun for 20 minutes at 3600 rpm. The filtered yield was calculated by pulling out decreasing volumes using MLA pipettes.

Sample preparation was done per laboratory protocol for the Centrifree filters. The methodology was not specifically optimized for the Vivafree™ filters. The manufacturers have differing maximum spin speeds. The Vivafree filters have a maximum spin speed of 5000 g. The Centrifree filters have a maximum spin speed of 2000 g. Both filters have a 30000 molecular weight cutoff.

## Conclusion

The Vivafree™ 2 and Centrifree filtration systems produce comparable analytical results. The Centrifree system has a higher yield at lower volumes, but the volumes are comparable at higher sample volumes. If larger volume yields are needed, the Vivafree™ filters could be utilized.

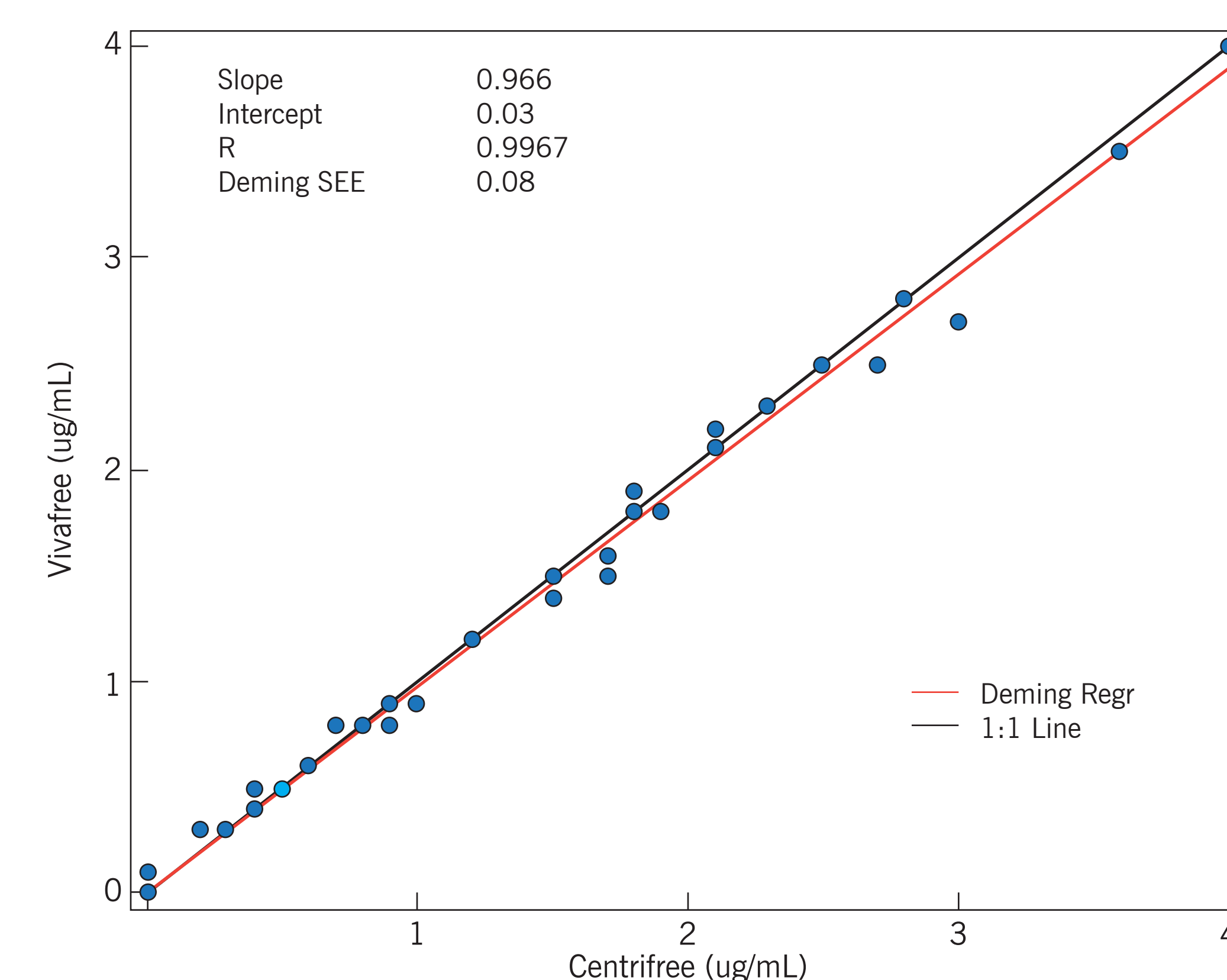
## References

Bishop, M. L., Fody, E. P., & Schoeff, L. E. (2013). Clinical chemistry: principles, techniques, and correlations (7th ed.). Philadelphia: Wolters Kluwer Health/Hippincott Williams & Wilkins.

## Results

Spec ID	X	Y	Bias	Spec ID	X	Y	Bias
S00001	0.5	0.5	0.0	S00021	2.1	2.1	0.0
S00002	0.4	0.4	0.0	S00022	0.4	0.4	0.0
S00003	1.1	1.1	0.0	S00023	1.5	1.5	0.0
S00004	1.8	1.8	0.0	S00024	0.9	0.9	0.0
S00005	0	0	0	S00025	2.7	2.5	-0.2
S00006	0	0.1	0.1	S00026	4.0	4.0	0.0
S00007	1.0	0.9	-0.1	S00027	0.6	0.6	0.0
S00008	1.2	1.2	0.0	S00028	0.4	0.5	0.1
S00009	1.7	1.6	-0.1	S00029	0.6	0.6	0.0
S00010	1.8	1.9	0.1	S00030	0.7	0.8	0.1
S00011	2.8	2.8	0.0	S00031	0.8	0.8	0.0
S00012	2.5	2.5	0.0	S00032	0.9	0.9	0.0
S00013	0.4	0.4	0.0	S00033	0.9	0.9	0.0
S00014	0.3	0.3	0.0	S00034	1.5	1.4	-0.1
S00015	0.6	0.6	0.0	S00035	1.9	1.8	-0.1
S00016	0.8	0.8	0.0	S00036	2.1	2.2	0.1
S00017	0.2	0.3	0.1	S00037	2.3	2.3	0.0
S00018	0.9	0.8	-0.1	S00038	1.7	1.5	-0.2
S00019	0.5	0.5	0.0	S00039	3.0	2.7	-0.3
S00020	1.5	1.4	-0.1	S00040	3.6	3.5	-0.1

**Figure-1: Results (shown in µg/mL) for the 40 mixed samples.** Method X is the result filtered through the Centrifree system. Method Y is the result filtered through the Vivafree™ system. Results less than the AMR were reported at 0. Results greater than the AMR were reported as 4.0.



**Figure-2: Results plotted for the 40 samples.** Method X is the result filtered through the Centrifree system. Method Y is the result filtered through the Vivafree™ system. The resulting slope is 0.966 with an intercept of 0.03 and a R value of 0.9967.

Starting Volume (µL)	Centrifree Yield				Vivafree Yield			
	Filter 1	Filter 2	Filter 3	Average	Filter 1	Filter 2	Filter 3	Average
250	130	130	130	130	50	50	50	50
500	190	130	150	157	100	100	100	100
750	190	200	200	197	170	160	150	160
1000	190	160	190	180	180	190	190	187
2000					210	210	210	210

\*Minimum required volume is 70 µL

**Figure-3: Yields for both filtration systems.** Each system had 3 filters centrifuged with a starting volume of 250, 500, 750 and 1000 µL. The Vivafree™ system has a larger maximum capacity of 2000 µL. The three replicates were averaged for each system.

**Comparing Results** The results from the 2 filtration systems reflect a maximum bias of 0.3. The Deming regression produces a slope of 0.966 with an intercept of 0.03. The allowable variance for free phenytoin according to CAP is 3 SD. This is equivalent to 0.3 µg/mL for a result of 1.8 µg/mL. All of the tested samples across the AMR fall within this variance. Therefore, the filtration systems are found to produce equivalent results.

**Comparing Yields** The yields from the 2 filtration systems are closest at 1000 µL, yielding 180 versus 187 µL from the Centrifree and Vivafree™ systems respectively. Starting with lower volumes, the Centrifree system produces a higher yield than the Vivafree™ system. Starting with 2000 µL, only possible using the larger Vivafree™ system, the highest overall yield is produced of 210 µL.