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FREE PHENYTOIN ANALYSIS: EVALUATION OF TWO ULTRAFILTRATION  
SYSTEMS AND ADAPTATION OF THE ASSAY TO THE ROCHE COBAS-BIO®

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Submitted to the faculty of the Graduate School

in partial fulfillment of the requirements

of the Degree

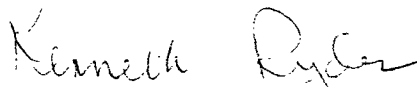
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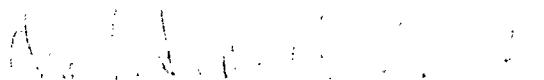
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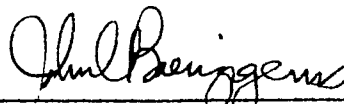
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## ABSTRACT

FREE PHENYTOIN ANALYSIS: EVALUATION OF TWO ULTRAFILTRATION SYSTEMS AND ADAPTATION OF THE ASSAY TO THE ROCHE COBAS-BIO®

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A comparison was made between the Syva Emit® FreeLevel™ System I and the Amicon Centrifree™ Micropartition for the ultrafiltration of free phenytoin. An investigation was performed concerning the effect of various analytical factors on this process. These factors include: temperature, length of centrifugation, centrifugal force, sample volume and storage of the sample prior to ultrafiltration. From this study, optimal conditions for both ultrafiltration techniques were determined to be: centrifugation at 25°C for 10-15 minutes at 1500 xg with a sample volume of 0.2 - 0.5 ml. The specimen can be stored at 24°C, 4°C or -20°C for up to one week without significant loss of concentration. Results of the precision study between the two techniques were comparable and a comparison of patient's samples ultrafiltered by both methods revealed no significant difference between the Syva and Amicon ultrafiltration methods.

An adaptation of the Syva assay protocol to the Roche Cobas-Bio® was also performed. No difference was detected between the two methods in terms of precision, specificity, interferences, or recovery.

A statistical difference was revealed between the assay on the Syva AutoCarousel<sup>TM</sup> system and the assay on the Roche Cobas-Bio<sup>®</sup> in the patient's correlation study. However, this difference was judged to be clinically insignificant.

The recommendation of this study is that the Amicon ultrafiltration procedure in combination with the free phenytoin analysis on the Roche Cobas-Bio<sup>®</sup> using Syva reagents is an acceptable method in terms of accuracy, precision, and cost-effectiveness for free phenytoin determination in the clinical laboratory.

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