

Abaxis VetScan Phenobarbital Profile: Performance and Reliability

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Introduction

Phenobarbital, a long-acting barbiturate drug, is commonly used as an anticonvulsant in veterinary medicine to prevent seizures in dogs and cats. To be effective, phenobarbital must be present within a therapeutic range in the blood. Monitoring the level of phenobarbital is essential to achieve maximal seizure control, while avoiding harmful side effects. Overdosing and chronic usage can lead to liver damage, so it is crucial to both monitor liver values and to closely monitor phenobarbital levels to ensure a safe and effective treatment.

The VetScan Phenobarbital Profile[®] used with the VetScan VS2[®] Chemistry Analyzer utilizes dry, lyophilized reagents and provides quantitative determination of a full panel of chemistries relevant to phenobarbital monitoring: alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), blood urea nitrogen (BUN), gamma glutamyl transferase (GGT), phenobarbital (PHB), and total bilirubin (TBIL). The profile can be used with heparinized whole blood, heparinized plasma, or serum.

Abaxis has adapted a homogeneous method for phenobarbital (PHB) for use in the VetScan VS2. This assay produces reliable and accurate results for monitoring patients being treated with phenobarbital. Listed below is an evaluation of the:

- Clinical correlation of PHB assay performed on the VetScan VS2 in comparison to the Cobas FARA[™] clinical chemistry analyzer and Antech Diagnostics[®] Reference Laboratories
- Precision of the VetScan Phenobarbital Profile on the VetScan VS2
- Sample type variability
- Exogenous interferences with the PHB assay

Clinical Correlation

Materials & Methods: Serum samples were collected and analyzed for phenobarbital concentrations using the Cobas FARA reference chemistry analyzer and the VetScan VS2 analyzer. All assays were performed according to the manufacturer's specifications. Data was plotted to determine regression statistics.

In addition to these banked samples, field studies were conducted at two companion animal hospitals. Heparinized whole blood (WB), heparinized plasma, and serum samples (canine and feline) were collected from clinic patients and analyzed for phenobarbital concentrations using the Abaxis VetScan VS2. Serum samples were sent-out to Antech Diagnostics reference laboratory for comparison. All methods were compared using linear regression and evaluating clinical concordance utilizing method specific reference intervals.

Results: Results were obtained for 52 dog samples and 3 cat samples for the comparison study on the Cobas FARA. Due to the limited availability in cat samples, the correlation analysis was performed on the dog samples. For the field studies, 8 patient samples were collected at Site 1 and 21 patient samples were

collected at Site 2. A linear regression and calculation of correlation coefficient were used to evaluate the overall agreement of PHB concentrations.

Displayed below are the correlation graphs for PHB results using the VetScan VS2 versus the Cobas FARA (Figure 1), and Antech Diagnostics send-outs (Figures 2&3) listing the slope, y-intercept, correlation coefficient (R) and coefficient of determination (R²).

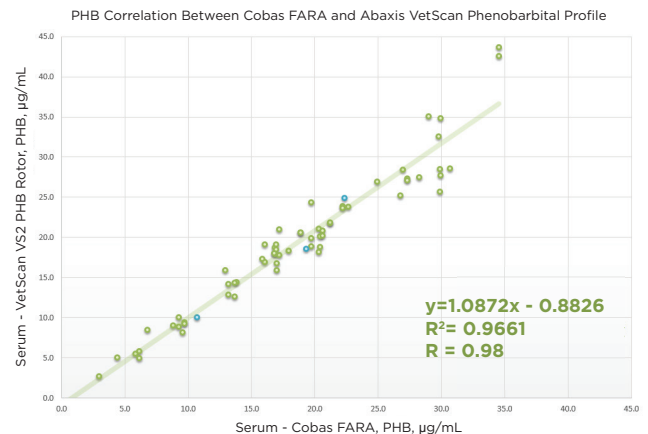


Figure 1. Abaxis VetScan VS2 vs. Cobas FARA
Linear regression plot of phenobarbital concentration on 52 dog samples (green). 3 cat samples (blue) are displayed to show overall agreement.

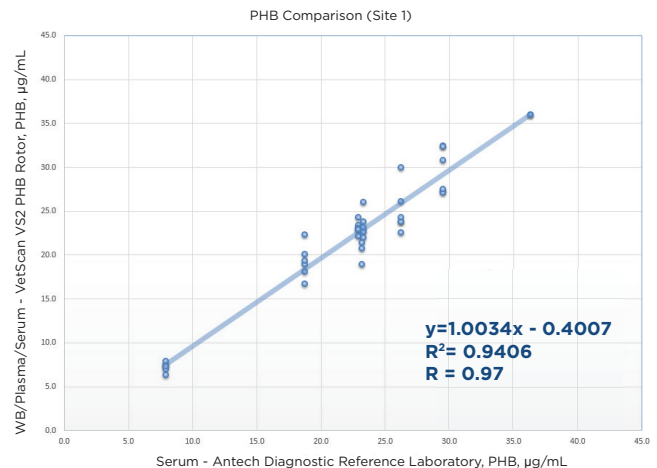


Figure 2. Abaxis VetScan VS2 vs. Antech Diagnostics Site 1
Linear regression plot of phenobarbital concentration at Site 1 with all 3 sample types performed on the VetScan VS2 and serum at Antech.

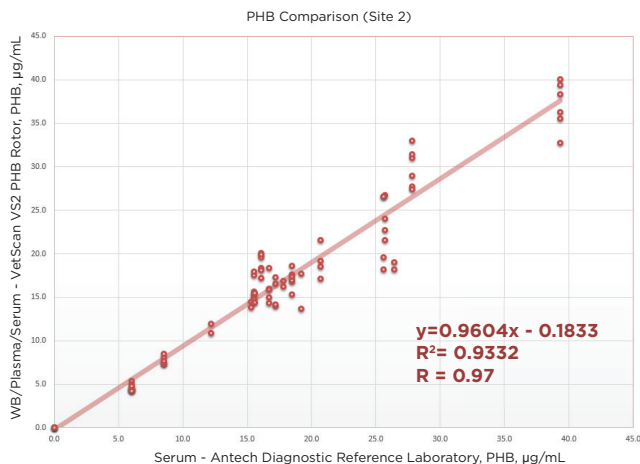


Figure 3. Abaxis VetScan VS2 vs. Antech Diagnostics Site 2

Linear regression plot of phenobarbital concentration at Site 2 with all 3 sample types performed on the VetScan VS2 and serum at Antech.

Precision

Materials and Methods: Precision studies were conducted using the Clinical and Laboratory Standards Institute (CLSI) EP5-A2 method guidelines. Results for within-run and total precision were determined by testing three levels of BioResource Technology™ (BRT) controls (low, normal, high) for PHB. A total of 6 VetScan VS2 analyzers were used, 2 analyzers per control level. There were 2 replicates run both morning and afternoon for 5 days, for a total of 20 replicate runs of each control fluid level per analyzer.

Results: Listed below is a summary of the average results, including mean concentration, standard deviation (SD) and percent coefficient of variation (%CV).

Table 1. Summary of precision results

	Within-Run	Total
Control 1		
Mean	8.3	8.3
SD	0.73	0.92
%CV	8.8	11.1
Control 2		
Mean	22.4	22.4
SD	1.43	1.80
%CV	6.4	8.0
Control 3		
Mean	44.9	44.9
SD	2.99	3.74
%CV	6.7	8.3

Sample Type Analysis

Materials & Methods: Field studies were conducted where 3 sample types: heparinized whole blood, heparinized plasma, and serum samples for dogs and cats. Samples were collected and tested for variability. The overall goal is to evaluate sample type effect on the VetScan Phenobarbital Profile; 2 replicates were run on 2 different VetScan VS2 analyzers for each sample type for a total of 6 replicates per sample type.

Results: Displayed below is a variability chart for each sample type on 7 different patients tested for PHB.

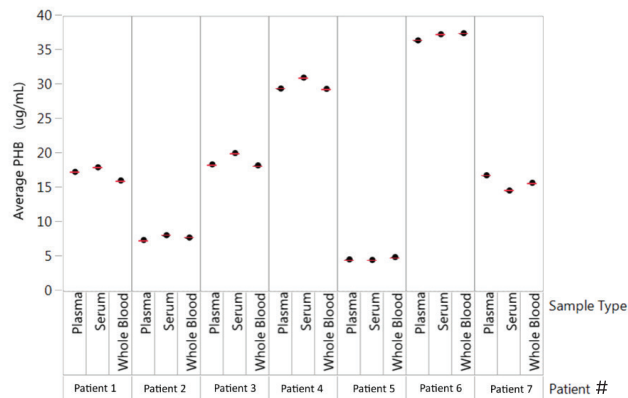


Figure 4. Phenobarbital Sample Type Variability

Sample variability graph for phenobarbital in whole blood, plasma and serum.

Exogenous Interference

Listed below are substances that have been tested for interference in the PHB assay at 2 concentration levels: 28 µg/mL and 55 µg/mL. It was determined that these substances do not interfere with the results of the VetScan Phenobarbital Profile at the tested concentration.

Table 2. Summary of substances at the concentration tested

Substances	Concentration Tested (µg/mL)
Potassium Bromide	3000
Diazepam	0.6
Levetiracetam	45
Zonisamide	40
Valproic Acid	100
Pregabalin	11
Phenytoin Sodium	15
5-(4-Hydroxyphenyl)-5-phenylhydantoin	20
Chlorpromazine hydrochloride	100
Amitriptyline hydrochloride	100

Discussion

Phenobarbital results obtained from running the VetScan Phenobarbital Profile on the VetScan VS2 Chemistry Analyzer are in strong agreement with the Cobas FARA clinical chemistry analyzer and Antech Diagnostics Reference Laboratories. In addition, the VetScan Phenobarbital Profile demonstrates good precision using CLSI EP5-A2 method. Furthermore, sample type analysis between heparinized whole blood, heparinized plasma, and serum indicated low variability among each sample type. Lastly, the VetScan Phenobarbital Profile is not affected by common substances at the stated concentration listed above.

Conclusion

The Abaxis VetScan Phenobarbital Profile shows excellent statistical and clinical agreement with predicate devices and outside reference laboratories. The test produces accurate and precise results for the determination of phenobarbital concentrations in whole blood, plasma, and serum samples from dogs and cats. Also, common substances concurrently used with phenobarbital were tested and showed to have no interference with the PHB assay. Finally, the VetScan VS2 has proven to be an excellent point of care, in-house analyzer that allows for immediate monitoring and titration of phenobarbital for patients affected by seizures.