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Advanced Diagnostics

Getting reference laboratory results with an in-house blood chemistry analyzer

Alan H. Rebar, DVM, PhD, DACVP, Professor of Veterinary Clinical Pathology, Executive Director of Discovery Park, Senior Associate Vice President for Research, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana

John A. Christian, DVM, PhD, Associate Professor of Veterinary Clinical Pathology; Director, Clinical Pathology Laboratory, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana

Alan H. Rebar
DVM, PhD, DACVP

John A. Christian
DVM, PhD

The nature of veterinary practice makes the likelihood of encountering interferant substances in blood samples very high.[1] Even in human medicine with optimal conditions and trained phlebotomists, as many as 25% to 33% of samples contain lipids, bilirubin, or hemoglobin.[2] The potentially detrimental effects of these and other interfering substances on test results have been well documented[3,4] and reference and academic laboratories go to great lengths to minimize them. The impact of these interfering substances is rarely considered for in-house chemistry analyzers.

It is critical that the impact of interferants on samples be minimized and that in-house blood chemistry analyzers meet basic reference methodology standards for providing consistently accurate results.

ACCURATE AND PRECISE RESULTS

The goal of a chemistry analyzer should be to provide accurate and precise results. Commonly encountered interferants can significantly influence results of routine chemistry tests and subsequent clinical interpretations. Developed to combat interference caused by lipids, hemoglobin, and bilirubin – as well as exogenous substances such as drugs and toxins – dry slide technology minimizes the effects of these substances through a layered design that filters them out before they can impact results. Dry slide technology preserves the underlying accuracy of chemistry analyzer equipment and is available for use in both reference laboratories (Johnson & Johnson® Vitros®) and in-house (IDEXX VetTest Chemistry Analyzer® and, arriving soon, IDEXX Catalyst Dx Chemistry Analyzer™).

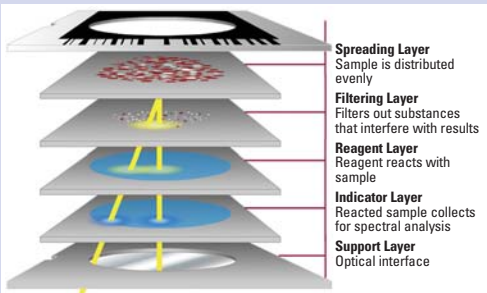
STUDY RESULTS

The impact of lipid, bilirubin, and hemoglobin on dry slide chemical analysis is minimized at the levels tested, preserving accurate results despite the challenge of increasing amounts of interfering substances.

To see the study, please turn the page...

DRY SLIDE TECHNOLOGY

The sample is applied to the top spreading layer to assure even distribution. Before the specimen reaches the reagent layer, a filtering layer removes most interferring substances like lipids, bilirubin, and hemoglobin. Results are optically measured from below after the reaction products reach the indicator layer.



KEY POINTS

- A significant number of samples for chemical analysis is compromised by interfering substances.
- Commonly encountered interferants can significantly influence results of routine chemistry tests and subsequent clinical interpretations.
- The goal of a chemistry analyzer should be to provide accurate and precise results.
- The impact of interfering substances should be considered for all in-house and reference laboratory chemistry analyzers.
- Dry slide technology minimizes the effects of interfering substances through a layered design that filters them out before they can impact results.

STUDY – EFFECTS OF INTERFERANTS ON CHEMISTRY ANALYTES IN ANIMAL SERA*

Objective

To determine the impact of varying amounts of hemoglobin, bilirubin, and lipids on the in-house IDEXX® VetTest® Chemistry Analyzer, 12 clinical chemistry analytes** were tested against increasing interferant levels.

Procedure

- Testing conducted at Purdue University.
- A series of test samples varying only in the concentration of the interferant was prepared by standard methods and run on the IDEXX VetTest® Chemistry Analyzer.[5]
- Pooled canine, feline, and equine samples were collected and stored frozen.
- Analyzer and all test materials were maintained according to manufacturer's specifications, including sample handling and quality control.
- Pooled sera were analyzed for interfering substances and basal values noted.
- 5 aliquots of serum containing increasing levels of the interferant were run in triplicate for each analyte.†
- Lipid and bilirubin were added to pooled sera; hemoglobin was prepared from lysed bovine red blood cells and added to pooled sera.
- All samples were analyzed in random order.

Results and Discussion

- The impact of various interferant concentrations on the IDEXX VetTest® Chemical Analyzer is relatively insignificant.
- Although increasing or decreasing trends appear for many of the analytes, no biological significance is associated with them and many also lack statistical significance. The relatively dramatic interference from hemolysis on bilirubin is well known with most methods of measurement.[6]

CLINICAL IMPLICATIONS

- Interferant impact may have significant clinical implications for both presentation and follow-up treatment of disease.
- Reference laboratories manage interferants and their impact on sample quality with ultracentrifugation, chemical modification, and use of sample and reagent blanks to minimize hemolytic, lipemic, and icteric interferences. Many of these methods are impractical in the in-house setting.
- It is critical that interferant impact is minimized for in-house chemistry analyzers. This is especially important when whole blood is used because plasma physical features are not visualized, and the operator may have a false sense of assurance of quality results if the interference is not observed.

LIPEMIA

Lipid	OD	B/L††	1.3	1.9	4.0	5.9
ALB	g/dL	2.5	2.6	2.6	2.6	2.5
ALKP	U/L	182	184	182	173	173
ALT	U/L	26	25	24	23	23
AMYL	U/L	8	8	8	7	7
BUN	mg/dL	21	21	21	21	21
CA	mg/dL	12.4	12.9	12.9	12.5	12.6
CHOL	mg/dL	96	97	97	98	99
CREA	mg/dL	1.3	1.3	1.3	1.3	1.3
GLU	mg/dL	107	107	106	106	105
PHOS	mg/dL	3.3	3.3	3.3	3.3	3.3
TBIL	mg/dL	1.2	1.4	1.4	1.5	1.4
TP	g/dL	6.2	6.6	6.6	6.5	6.4

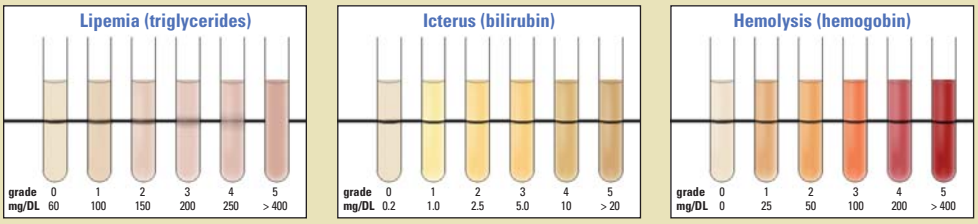
ICTERUS

Bilirubin	mg/dL	B/L††	1.2	2.5	7.7	10.3
ALB	g/dL	2.5	2.5	2.5	2.5	2.5
ALKP	U/L	66	64	62	57	58
ALT	U/L	41	40	39	36	39
AMYL	U/L	950	953	962	978	978
BUN	mg/dL	19	19	19	19	19
CA	mg/dL	10.7	10.7	10.6	10.5	10.4
CHOL	mg/dL	147	146	144	142	143
CREA	mg/dL	1.1	1.0	1.0	1.0	1.0
GLU	mg/dL	71	71	72	72	73
PHOS	mg/dL	5.0	5.1	5.1	5.1	5.0
TP	g/dL	7.1	7.2	7.1	7.2	7.2

HEMOLYSIS

Hemoglobin	mg/dL	B/L††	62.5	125	250	500
ALB	g/dL	2.7	2.7	2.7	2.9	3.0
ALKP	U/L	75	66	56	48	47
ALT	U/L	34	33	32	30	28
AMYL	U/L	739	749	770	805	831
BUN	mg/dL	18	18	19	20	20
CA	mg/dL	10.6	10.6	10.5	10.4	10.3
CHOL	mg/dL	145	145	145	145	145
CREA	mg/dL	1.0	1.0	0.9	0.9	0.8
GLU	mg/dL	69	69	69	70	71
PHOS	mg/dL	5.2	5.2	5.2	5.3	5.3
TBIL	mg/dL	0.1	0.1	0.4	1.0	1.4
TP	g/dL	7.1	7.2	7.4	7.8	8.1

†† B/L = Baseline



When a blood sample is centrifuged prior to analysis, lipemia, icterus, and hemolysis are visually apparent. The grading of samples is subjective and often noted on a 0 to 5+ scale (0 = no interfering substance; 5+ = extremely contaminated). Values noted are respresentative.

* Complete materials and methods can be found at ***www.idexx.com/dryslide***.

** Albumin (ALB), Alkaline Phosphatase (ALKP), Alanine Aminotransferase (ALT), Amylase (AMYL), Blood Urea Nitrogen (BUN), Calcium (CA), Cholesterol (CHOL), Creatinine (CREA), Glucose (GLU), Phosphorus (PHOS), Total Bilirubin (TBIL) and Total Protein (TP)

† TBIL was not run with the bilirubin interferant.

The IDEXX® VetTest® Chemistry Analyzer provides point of care testing in a timely manner.

REFERENCES

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METHODS & MATERIALS

- The study followed CLSI protocol EP7-A2.[1]
- Pooled samples from canine, feline, and equine patients were collected and stored frozen. The samples were visibly clear of interferants. A unique pool was used for each interferant panel: lipemia (equine), icterus (feline), and hemolysis (canine).
- All testing was run on the IDEXX VetTest® Chemistry Analyzer.
- Analyzer and all test materials were maintained according to manufacturer's specifications, including sample handling and quality control.
- Testing was conducted to determine the effects of interference from lipemia, hemoglobin, and bilirubin.
- A series of test samples, systematically varying only in the concentration of the interferant, was prepared by standard methods and run on the IDEXX VetTest® Chemistry Analyzer.[1]
- Basal values for the pooled sera were analyzed for interfering substances and 0.1 OD lipid, 0.1 mg/dL bilirubin, and 0.1 mg/dL hemoglobin were noted.
- For each of the three interfering substances, 5 levels of serum containing increasing levels of the interferant were run in triplicate for each analyte.
- Lipid (Intralipid 30%® IV Solution, Baxter IV Solutions) was added to pooled sera (0 to 5 OD at 660 nm).
- Bilirubin (dissolved in ditaurobilirubin disodium salt, JBL Scientific) was added to pooled sera (0 to 10 mg/dL).
- Hemoglobin was prepared from lysed bovine red blood cells (Sigma) and added to pooled sera (0 to 500 mg/dL).
- All samples were analyzed in random order.
- Results were tabulated.
- 12 analytes were evaluated: Albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), amylase (AMYL), blood urea nitrogen (BUN), calcium (CA), cholesterol (CHOL), creatinine (CREA), glucose (GLU), phosphorus (PHOS), total bilirubin (TBIL), and total protein (TP).
- Results for the 12 analytes are given at each of the corresponding levels of interferant (lipid, bilirubin, and hemoglobin) as the concentration of the interferant increases. TBIL was not run with the bilirubin interferant.

[1] Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition*. CLSI document EP7-A2 (ISBN 1-56238-584-4). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.