CATACHEM

VetSpec™
BROMIDE (DIRECT METHOD)
MANUAL APPLICATION C424-01, C424-02

Intended Use
For **In Vitro diagnostic** use in the quantitative determination of Bromide in serum or plasma.

Clinical Significance (1)
Bromide and Chloride ions are absorbed from the gastrointestinal tract and distributed throughout the organs. Chloride is displaced by Bromide in intracellular fluids and secretions producing central nervous system depression. Bromide penetrates the erythrocyte cell membrane and it is excreted by the kidney and the intestinal tract.

Bromide will falsely elevate serum chloride and will give rise to a negative anion gap.

Bromide In Canine Samples
Potassium Bromide alone or in combination with Phenobarbital is sometimes used for seizure control in dogs. Sodium bromide is less often used. The half-life of Bromide is 25 days and it can require up to four months to reach steady state. The therapeutic drug level for Bromide is 1-4 mg/mL; ≥ 3 mg/mL usually gives seizure control, but at levels ≥ 4 mg/mL signs of toxicity may be observed. Serum levels of Bromide rise more rapidly at the onset of therapy and steady state is almost reached in 60 days. Serum levels taken at three weeks after the initiation of therapy are likely to be 60-70 % of steady state levels.

Method Principle (1, 2)
Under acid conditions, Chloride ions from gold trichloride displace Bromide ions present in the serum sample. The dissociated Bromide ions then engage in a subsequent reaction with gold to produce a colored complex ($AuBr_3$) with maximum absorption at 380 nm. The intensity of the color thus produced is directly proportional to the Bromide ions in the serum sample. The reaction scheme depicts the steps that occur in this Bromide method.

$$AuCl_3 + 3Br^- \longrightarrow AuBr_3 + 3Cl^-$$

The formation of gold chloride may also be accompanied by the formation of $AuBrCl_2$ or $AuBr_2Cl$.

REAGENTS
Sample Diluent Reagent (R1)
Each liter contains:
Buffer
Stabilizer and nonreactive ingredients

Bromide Color Reagent (R2)
Each liter contains:

Gold Chloride	0.51 mmol
Organic acid	3.8 mol
Stabilizer and nonreactive ingredients	

Precautions
Handle these reagents using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes; if contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage And Stability
Store the Bromide Sample Diluent Reagent and Bromide Color Reagent at 2-8°C. When stored as directed these reagents are stable until the expiration date stated on the label.

Working Reagent Preparation
The Bromide reagents are packaged ready for use. No preparation is required. Once opened the Working Sample Diluent Reagent (R1) is stable until the expiration date indicated on the label. The Bromide Color Reagent (R2) once opened is stable for 60 days at 2-8°C.

Reagent Indications Of Deterioration
- Turbidity
- Quality control values out of assigned ranges

If these reagent characteristics are observed contact your technical representative.

Specimen Collection And Stability
Clear unhemolyzed sera are the specimens of choice. Serum should be separated immediately from the clot and analyzed promptly or stored at 2-8°C. Bromide in serum is stable 7 days at room temperature, 10 days refrigerated at 2-8°C and for many months frozen at -20°C. (2).

Materials Provided
Catachem Bromide Reagents:
Catachem Sample Diluent Reagent
Catachem Bromide Color Reagent

Materials Required But Not Provided
- Manual analyzer
- Catachem Calibrator material with assigned Bromide values
- Catachem Quality Control material with assigned Bromide values

Calibration
Catachem Bromide Calibrator which contains a known Bromide value is recommended.

Calibration Schedule
Calibrate the Catachem Bromide procedure each time the procedure is performed.

Quality Control
To monitor the quality performance of the procedure used, Catachem's Bromide Control Level I and Control Level II with assigned Bromide values should be preferably included in the assay procedure each time the assay is performed.

Interfering Substances
Samples with the following concentration substances have no significant effect on the accuracy of this bromide procedure:

• Bilirubin	≤ 2.5	mg/dL
• Hemoglobin	≤ 100	mg/dL
• Lipemia	≤ 1000	mg/dL

Other substances and certain drugs are also known to influence the Bromide values. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting

D.S. Young et al (4).

Directions For Use
The Catachem Bromide method requires two reagents, R1 and R2. Use as R1 the Bromide Sample Diluent Reagent and as R2 the Bromide Color Reagent. The Working Reagents are ready for use.

Assay Procedure
1. Label cuvettes or appropriate test tubes as: a) Calibrator blank (CAL-BLK), b) Calibrator (CAL), c) Control 1 blank (C-1BLK), d) Control 1 (C1), e) Control 2 blank (C-2BLK), f) Control 2 (C2), g) Sample blank (SAMP BLK), h) Sample (SAMP).
2. Pipette the reagent and sample volumes into the cuvettes or test tubes as shown in table below. Pipette Bromide Sample Diluent (R-1) first, followed by Bromide Color Reagent (R-2) and then the water (for the blank) followed by the calibrator and then samples at 15 second intervals.
3. Use a timer.
4. At the end of the 1.0 minute period read the blank cuvette and then all other cuvettes in sequence at 15 second intervals at 380 nm. Record all absorbencies.

	CAL BLK	CAL	C-1 BLK	C-1	C-2 BLK	C-2	SAMP BLK	SAMP
	ml	ml	ml	ml	ml	ml	ml	ml
RGT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
RGT	0.0	0.25	0.0	0.25	0.0	0.25	0.0	0.25
SAM	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014
H2O	0.25	0.0	0.25	0.0	0.25	0.0	0.25	0.0

Calculations And Results

$$\text{Bromide mg/mL} = \frac{\Delta\text{- Abs. Samp.}}{\Delta\text{- Abs. Cal.}} \times \text{Cal. (mg/mL)}$$

Example:

	Samp. Abs.	Blk. Abs.	Δ-Abs.
Sample	0.400	0.300	0.100
Calibrator	0.150	0.030	0.120

Calibrator assigned value = 1.0 mg/mL

$$\text{Sample Bromide (mg/dL)} = \frac{0.100}{0.120} \times 1.0 \text{ mg/mL}$$

$$= 0.83 \text{ mg/mL}$$

Procedure Limitations
Samples with Bromide values greater than 4 mg/mL should be diluted 1:2 with distilled or deionized water and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

NOTE.
Certain turbid samples may require clearing with a precipitating reagent. Catachem provides this as Catalog number C424-30 (25 ml). (Results require adjustment to account for the dilution effect of the precipitating reagent.)

Canine Target Values:

Minimum:	1.0 mg/mL
Maximum:	3.0 mg/mL

The values given here are only to be used as a guideline. The weight and size of the animal will have a bearing on the correct dosage.

Method Performance Characteristics
Sensitivity: Using a pathlength of 1 cm, a Δ-absorbance of 0.0016-0.0024 per mg/mL should be obtained.
Linearity: In this procedure there is not significant nonlinearity over the range of 0-4 mg/mL.
Precision: Precision data was obtained using three levels of protein based controls and following the NCCLS EP5-T2 procedure (3). The following results were observed.

Precision

Bromide	Within-Run Precision		Total Precision (15 days)	
Mean	SD	CV	SD	CV
mg/mL	mg/mL	%	mg/mL	%
0.50	0.034	6.8	0.039	7.80
1.00	0.034	3.4	0.049	4.90
1.50	0.036	2.4	0.107	7.10

ACCURACY
Correlation studies were carried out between an automated Bromide method (Y) and this manual method (2) as reference (X). Canine serum samples were assayed and the results compared by the least squares regression. The following statistics were observed:

N	=	25
Range	=	56 - 349
Mean Y	=	148.59
Mean X	=	157.77
Y	=	0.985x - 8.8
Sy.x	=	10.25
r	=	0.987

Bibliography

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3. Evaluation of Precision Performance of Clinical Chemistry Devices. Second Edition. NCCLS Document EP5-T2. Vol. 12, No. 4.
4. Young DS, Pestamer LC, Gibberman V. Effects of drugs on clinical laboratory tests. Clin. Chem. 21, No. 5(1975).

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353 Christian Street Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● Fax: (203) 262-9836 ● email: catachem@catacheminc.com