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Testing Protocol

SAM 504

Supplemental Assay Method for the Manual Determination of Protein Content of Veterinary Biologics (Biuret)

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Contact: Brandalyn L. Heathcote, (515) 337-7013
Julia A. Kinker, (515) 337-6226

Approvals: /s/Walter G. Hyde Date: 08Mar16
Walter G. Hyde, Section Head
Chemistry and Analytical Services

/s/Paul J. Hauer Date: 15Mar16
Paul J. Hauer, Director
Policy, Evaluation, and Licensing
Center for Veterinary Biologics

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA 50010

Entered into CVB Quality Management System
by: /s/Linda S. Snavely 16Mar16
Linda S. Snavely Date
Quality Management Program Assistant

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1. Introduction

This Supplemental Assay Method (SAM) describes the classical biuret procedure for the indirect determination of the protein content of various veterinary biologics products (i.e., serum, antiserum and antitoxins). Protein content is often utilized in the evaluation of such products.

2. Materials

2.1 Equipment/instrumentation

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

2.1.1 Spectrophotometer or colorimeter (1-cm or greater path length) with appropriate accessories, i.e., cuvettes

2.1.2 Common laboratory apparatus and glassware – pipettes, pipettors with tips, screw cap tubes, Class A volumetric flasks, linear graph paper

2.1.3 Computer – with linear regression program (optional)

2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

2.2.1 Potassium phosphate, monobasic

2.2.2 Sodium phosphate dibasic, anhydrous

2.2.3 Sodium chloride

2.2.4 Sodium phosphate, dibasic

2.2.5 Copper sulfate, pentahydrate

2.2.6 Potassium sodium tartrate

2.2.7 Sodium hydroxide

2.2.8 Potassium iodide

2.2.9 Water – deionized, demineralized, reverse osmosis or equivalent in purity to water filtered through a Milli-Q purification system

2.2.10 Standard protein solution **–** Crystalline bovine albumin containing a known amount of protein

2.2.11 Bovine serum reference – Normal bovine serum

3. Preparation for the Test

3.1 Personnel qualifications/training

Technical personnel must have experience or training in this protocol. This includes working knowledge of the use of general laboratory equipment, glassware and chemical safety; and specific training in the operation of the laboratory equipment and reagents listed in **Section 2**.

Analysts performing this procedure should first conduct at least 2 trial runs using controls and standards and obtain results within acceptable limits.

3.2 Preparation of equipment/instrumentation

All equipment must be operated according to manufacturers' recommendations and monitored in compliance with applicable standard operating procedures.

Turn on spectrophotometer and allow instrument to "warm up" for at least 30 minutes.

3.3 Preparation of reagents/control procedures

3.3.1 Phosphate buffered saline (PBS), 0.01M, pH 7.2 - 7.4: Dissolve 0.34 g potassium phosphate, monobasic; 1.10 g sodium phosphate dibasic, anhydrous; 8.50 g sodium chloride; and 0.15 g sodium phosphate, dibasic in 400-500 mL water in a 1-L volumetric flask. QS to volume. Transfer to a 1-L glass bottle and sterilize by autoclave. Store at 4°± 5°C, stable for 6 months. (*An acceptable alternative would be to use a prepared product, such as National Centers for Animal Health (NCAH) Media #*30033.)

3.3.2 Biuret Reagent: Dissolve 1.50 ± 0.01 g copper sulfate, pentahydrate in 400 mL water in a 1-L volumetric flask. Add 6.00 ± 0.01 g potassium sodium tartrate and mix until dissolved. In a separate container dissolve 30.0 ± 0.01 g sodium hydroxide in 300 mL water, and then add to previous solution. Add 1.00 ± 0.01 g potassium iodide to and QS to 1 L. Store at room temperature, stable for 6 months. (*An acceptable alternative would be to use a prepared product, such as NCAH Media #10307*.)

Critical control point: Biuret reagent should be replaced when crystals or other precipitates appear in the solution.

3.3.3 Bovine serum – not sterilized: Donor bovine serum, mix thoroughly, dispense 3-mL into 5-mL serum vials. Store at -20°± 10°C, stable for 1 year. (*An acceptable alternative would be to use a prepared product, such as NCAH Media #40032*.)

3.3.4 Standard solutions: Dilute Bovine Albumin with PBS to contain 10 mg/mL protein. Use 10 mg/mL protein solution as stock for curve. Dilute as listed below to establish a working standard curve.

CONC.(mg/mL)	mL STOCK	mL PBS
10	1.0	0
8	0.8	0.2
6	0.6	0.4
4	0.4	0.6
2	0.2	0.8
1	0.1	0.9

3.4 Preparation of the sample

Samples are normally sera, antisera or antitoxins, or serum fractions. Occasionally, biuret tests are run on other solutions, such as antigens. Follow sample receipt procedures as described by standard operating procedures

4. Performance of the Test

4.1 Standard curve

Run duplicate tubes of each standard solution (**Section 3.3.4**) using the test method described in **Section 4.2** to establish a standard curve. Plot average optical density (OD) for each point on graph paper (concentration vs. OD) or enter data into computer program to plot curve and calculate test results. If OD values for any point differ more than 0.05, disregard that point. If more than one point has unacceptable OD variations, rerun standard curve.

Critical control point: A standard curve is accurate for that lot of Biuret reagent. A comparison run or a new curve must be run when a new lot is used.

4.2 Test method

4.2.1 Dilute sample and bovine serum control 1:10 or 1:20 with PBS. Mix gently. (Sample dilution is based on the sample appearance or prior knowledge of sample. Dilute sample so OD falls on standard curve.)

4.2.2 Transfer 1 mL of dilution (**Section 4.2.1**) to tube or cuvette. Run duplicates.

4.2.3 Transfer 1 mL PBS to tube or cuvette for instrument blank.

4.2.4 Add 4 mL biuret reagent to each tube and mix gently. Let stand at room temperature for 30 to 45 minutes to allow for color development.

4.2.5 Read OD at 540 nm, using blank to set zero. Read and record OD.

5. Interpretation of the Test Results

5.1 Calculation

Determine sample value (either read from curve and multiply x dilution or enter data into computer program). Average test results of duplicate tests. Test results are acceptable if the duplicate test results vary no more than 5% from the mean and the protein value for the bovine serum control falls within 5% of the established value.

5.2 Retest

If the OD of the diluted sample reads outside the end points on the standard curve, redilute sample and rerun the test.

6. Report of Test Results

Test results are reported following the current standard operating procedures.

7. References

7.1 Robinson, H. W. and Hogden, C. G. (1940) J. Biol. Chem., vol. 135, pp. 707-725.

7.2 Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) J. Biol. Chem., vol. 177, pp. 751-766.

7.3 Kibrick, A.C. (1958) Clin. Chem., vol. 4, pp. 232-236.

7.4 Kingsley, G. R. (1939) J. Biol. Chem., vol. 131, pp. 197-200.

8. Summary of Revisions

Version .06

- Updated Contact information.
- Replaced "reduced oxygen" method with "reverse osmosis" for Water.

Version .05

- The Contact information has been updated.
- References to specific products, such as media lot numbers, have been changed to optional.
- All instructions for preparation of reagents and standards have been moved from appendices and Section 4.1 to Section 3.3.
- The Quick Reference section has been removed

Version .04

- The document number has been changed from TCSAM0504 to SAM 504.

Version .03

This document was revised to clarify the practices currently in use at the Center for Veterinary Biologics and to provide additional detail. While no significant changes were made that impact the outcome of the test, the name of the contact person has changed.