United States Department of Agriculture
Center for Veterinary Biologics
Testing Protocol

SAM 512

Supplemental Assay Method for the Determination of Formaldehyde in Veterinary Biologics (Ferric Chloride Test)

Date:	April 5, 2022
Number:	SAM 512
Supersedes:	SAM 512.05, February 8, 2016
Standard Requirement:	9 CFR 113.100 (f), 9 CFR 113.200 (f)
Contact:	Email: Methodsrequest.notification@usda.gov Phone: Center for Veterinary Biologics, 515-337-6100

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

Supplemental Assay Method for the Determination of Formaldehyde in Veterinary Biologics (Ferric Chloride Test)

Table of Contents

Supplemental Assay Method for the Determination of Formaldehyde in Veterinary Biologics (Ferric Chloride Test)

1. Introduction

This Supplemental Assay Method (SAM) describes how total formaldehyde is determined based on the reaction of formaldehyde with Methylbenzothiazolone hydrazone hydrochloride (MBTH). The method involves: a) the combination of MBTH and formaldehyde to give one product; b) the oxidation of excess MBTH to give another product; and c) the combination of these two to give a blue chromophore which is measured at 628 nm.

2. Materials

2.1 Equipment/instrumentation

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

2.1.1 Spectrophotometer 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

2.2 Reagents

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

2.2.1 Ferric chloride-sulphamic acid reagent. A solution containing 10 g/L of ferric chloride and 16 g/L of sulphamic acid.

2.2.2 Methylbenzothiazolone hydrazone hydrochloride reagent. (MW 233.7). (CAS 149022-15-1). 3-Methylbenzothiazol-2(3H) one hydrazone hydrochloride monohydrate. An almost white or yellowish crystalline powder. mp: about 270°C. A solution containing 0.5 g/L.

WARNING: This solution is not stable and should be prepared fresh prior to testing.

2.2.3 Suitability for determination of aldehydes. To 2 mL of aldehyde-free methanol, add 60 µL of a 1 g/L solution of propionaldehyde in aldehyde-free methanol and 5 mL of a 4 g/L solution of Methylbenzothiazolone hydrazone hydrochloride. Mix; allow to stand for 30 minutes. Prepare a blank omitting the propionaldehyde solution. Add 25.0 mL of a 2 g/L solution of ferric chloride to the test solution and to the blank, dilute to 100.0 mL with acetone R and mix. Measure absorbance of the test solution on a spectrophotometer at 660 nm in a

1-cm cell using the blank as compensation liquid. The absorbance of the test solution must be greater than or equal to 0.62 absorbance units.

2.2.4 Formaldehyde solution, containing not less than 34.5 percent w/v and not more than 38.0 percent w/v of formaldehyde (CH_2O)

2.2.5 Isopropyl myristate, analytical grade

2.2.6 Hydrochloric acid (1 M), analytical grade

2.2.7 Chloroform, analytical grade

2.2.8 Sodium chloride (9 g/L and 100 g/L aqueous solutions), analytical grade

2.2.9 Polysorbate 20, analytical grade

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 and glassware; and specific training in the operation of the laboratory equipment listed in **Section 2**.

3.2 Preparation of Standards

Prepare formaldehyde standards of 0.25, 0.50, 1.00 and 2.00 g/L by diluting formaldehyde solution (**Section 2.2.4**) with water in suitable volumetric flasks.

3.3 Preparation of vaccines containing oil emulsion

If vaccine to be examined is an oil emulsion, the emulsion should be broken by a suitable method. The formaldehyde concentration in the aqueous phase should be measured. The following separation techniques have been shown to be appropriate.

3.3.1 Add 1.00 mL of vaccine to 1.0 mL of isopropyl myristate and mix. To the mixture, add 1.3 mL of 1 M hydrochloric acid, 2.0 mL of chloroform, and 2.7 mL of 9 g/L sodium chloride. Mix thoroughly. Centrifuge at 15,000 g for 60 minutes. Transfer the aqueous phase to a 10-mL volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde. If this procedure described fails to separate the aqueous phase, add 100 g/L of polysorbate 20 to the sodium chloride solution and repeat the procedure, but centrifuge at 22,500 g.

3.3.2 Add 1.00 mL of vaccine to 1.0 mL of a 100 g/L solution of sodium chloride and mix. Centrifuge at 1000 g for 15 minutes. Transfer the aqueous phase to a 10-mL volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde.

3.3.3 Add 1.00 mL of vaccine to 2.0 mL of a 100 g/L solution of sodium chloride and 3.0 mL of chloroform and mix. Centrifuge at 1000 g for 5 minutes. Transfer the aqueous phase to a 10-mL volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde.

Note: Volumes used for breaking emulsions are for the purpose of illustration. Volumes may differ subject to proportional adjustment of the volumes of other reagents used in the extraction process.

4. Performance of the Test

4.1 To 0.50 mL of a 1 in 200 dilution of the vaccine to be examined (if emulsion, use 0.50 mL of a 1 in 20 dilution of the diluted aqueous phase), and to 0.50 mL of 1 in 200 dilution of each of the formaldehyde standards, add 5.0 mL of the Methylbenzothiazolone hydrazone hydrochloride reagent. Close the tubes, shake, and allow to stand for 60 minutes.

4.2 Add 1 mL of ferric chloride-sulphamic acid reagent and allow to stand for 15 minutes.

4.3 Measure absorbance of vaccines and standards on a spectrophotometer at the maximum at 628 nm in a 1-cm cell, using the reagent blank as compensation liquid.

5. Calculations and Interpretation

Use linear regression to fit the standard curve; a valid standard curve has a coefficient of determination (R^2) equal to or greater than 0.97. If the standard curve is valid, calculate total formaldehyde concentration (g/L) by interpolation from the standard curve.

6. Report of Test Results

Test results are reported following the current standard operating procedures.

Supplemental Assay Method for the Determination of Formaldehyde in Veterinary Biologics (Ferric Chloride Test)

7. References

7.1 Title 9, *Code of Federal Regulations*, parts 113.100(f) and 113.200(f), U.S. Government Printing Office, Washington, DC.

7.2 Testing of Residual Formaldehyde, VICH GL25 (Biologicals: Formaldehyde), Final, April 2002.

8. Summary of Revisions

Version .06

- Coversheet updated.
- **5.** Revised to correct terminology.

Version .05

- Updated Contact information.

Version .04

- The Contact information has been updated.
- **3.1:** Personnel qualifications/training has been updated.
- **7:** The Reference of "Testing of Residual Formaldehyde" has been added.
- The entire document has been revised to reflect current practices.

Version .03

- The document number has been changed from TCSAM0512 to SAM 512.

Version .02

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.