

**United States Department of Agriculture  
Center for Veterinary Biologics  
Testing Protocol**

**SAM 514**

**Supplemental Assay Method for the Determination of Hydrogen Ion  
Concentration, Total Nitrogen, TCA Nitrogen, Phenol and Clarity in  
Intradermic (Filtrate Produced from Cultures of Pn, C, and Dt Strains of  
*Mycobacterium tuberculosis*) Tuberculin**

Date: **June 17, 2022**

Number: SAM 514.09

Supersedes: SAM 514.08, February 8, 2016

Standard Requirement: 9 CFR 113.406

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## **1. Introduction**

This Supplemental Assay Method (SAM) describes the procedures for determination of hydrogen ion concentration, total nitrogen, trichloroacetic acid precipitable (TCA-ppt) nitrogen, phenol content and clarity in intradermic (filtrate produced from cultures of Pn, C, and Dt strains of *Mycobacterium tuberculosis*) tuberculin; as prescribed in title 9, *Code of Federal Regulations* (9 CFR), part 113.406.

The hydrogen ion concentration shall be determined with a pH meter which has been standardized with a pH 7.0 buffer just prior to use. The total nitrogen content shall be determined by the Kjeldahl method on duplicate 15 mL samples, consisting of 5 mL from each of three vials. The determination of precipitable nitrogen by a final concentration of 4 percent trichloroacetic acid shall be made by the Kjeldahl method on duplicate 15 mL samples, consisting of 5 mL from each of three vials. The phenol content shall be determined by direct titration with a standardized bromide-bromate solution. (A correction factor of 0.04 should be subtracted from the final value in the determination of phenol in tuberculin.) The product shall be optically clear and free from any extraneous particles.

## **2. Materials**

### **2.1 Equipment/instrumentation**

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

**2.1.1** pH meter, with combination pH electrode (i.e., Orion<sup>®</sup> ROSS 8103) capable of measuring from pH 0.0 to 14.0

**2.1.2** Disposable beaker, 5-mL

**2.1.3** Balance, top loading, capable of measuring 0.001 g

**2.1.4** Digestion unit (Büchi)

**2.1.5** Distillation unit (Büchi)

**2.1.6** Volumetric pipettes, Class A, 5-, 10-, and 25-mL

**2.1.7** Volumetric flasks with barrel head glass stopper, Class A, 500-mL and 1-L

**2.1.8** Erlenmeyer flasks, 125-mL

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- 2.1.9 Burets with PTFE stopcocks, precision bore, Class A, 10-, 25-, and 50-mL
- 2.1.10 Graduated cylinders, Class A, 50-, 100-, 250-, 500-mL, and 1 L
- 2.1.11 Glass-stoppered Erlenmeyer flasks, 250-mL
- 2.1.12 Heating/stirring plate with stirring bars
- 2.1.13 Filter paper, 11  $\mu$ m particle retention (Whatman No. 1)
- 2.1.14 Timers, 30 seconds to 1 minute
- 2.1.15 Dropper, i.e., transfer pipette, Pasteur pipette, dropper bottle
- 2.1.16 Pipettor and tips to accurately dispense 100- to 1000- $\mu$ L
- 2.1.17 Small spot light lamp
- 2.1.18 Vortex mixer

**2.2 Reagents/supplies**

Equivalent reagents or supplies may be substituted for any brand name listed below. All chemicals are reagent grade, unless specified.

**2.2.1 Total and TCA-ppt nitrogen**

1. Sulfuric acid ( $H_2SO_4$ ), CAS# 7664-93-9, Purity: Minimum 95.0%, Maximum 98.0%
2. Kjeldahl Catalyst Tablets, 1.5 g  $K_2SO_4$  + 0.075 g HgO
3. Sodium hydroxide (NaOH), CAS# 1310-73-2, Purity: 98.5%
4. Boric acid ( $H_3BO_3$ ), CAS# 10043-35-3, Purity: 99.9%
5. Methyl red, CAS# 493-52-7, Purity: 98.0%
6. Hydrochloric acid (HCl), CAS# 7647-01-0, Assay: 36.5-38.0%
7. Sodium carbonate ( $Na_2CO_3$ ), CAS# 497-19-8, Purity: 99.9%
8. Bromo phenol blue, CAS# 115-39-9, Purity: 98.0%

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9. Trichloroacetic acid (TCA), CAS# 76-03-9, Purity: 98.0%
10. Control Sample – Either a pool of PPD tuberculin products with established protein and phenol values as tested by PL-CAS; or a product produced for use as a control sample and tested by PL-CAS.
11. Protein Standard Reference Material, Bovine Serum Albumin (current lot of SRM 927 from National Institute of Standards and Technology)

**2.2.2 Phenol** (some reagents same as for protein)

1. Hydrochloric acid (HCl), CAS# 7647-01-0, Assay: 36.5-38.0%
2. Water (H<sub>2</sub>O), Purity: distilled, demineralized, reverse osmosis or equivalent.
3. Methyl orange, CAS# 547-58-0, Purity: 98.0%
4. Silicotungstic acid hydrate (H<sub>4</sub>[Si(W<sub>3</sub>O<sub>10</sub>)<sub>4</sub>]\*26H<sub>2</sub>O), CAS# 12027-43-9, Purity: 99.0%. Store at 4°C.
5. Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), CAS# 7664-93-9, Purity: Minimum 95.0%, Maximum 98.0%
6. Arsenic trioxide, anhydrous (As<sub>2</sub>O<sub>3</sub>), CAS# 1327-53-3, Purity: 99.9%
7. Sodium hydroxide (NaOH), CAS# 1310-73-2, Purity: 98.5%
8. Phenol (C<sub>6</sub>H<sub>6</sub>O), CAS# 108-95-2, Purity: ≥ 99.0%  
This can be a purchased NIST standard and diluted, if necessary, to the appropriate level.
9. Sodium bicarbonate (NaHCO<sub>3</sub>), CAS# 144-55-8, Purity: 99.9%
10. Potassium bromate (KBrO<sub>3</sub>), CAS# 7758-01-2, Purity: 98.5%
11. Potassium bromide (KBr), CAS# 7758-02-3, Purity: 99.0%

**2.2.3 Hydrogen ion concentration**

1. Commercial buffers, certified pH 7.00 and pH 4.00

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2. pH electrode storage solution
3. Reference electrode filling solution

**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 and maintained according to manufacturers' recommendations and monitored in compliance with applicable standard operating procedures.

**3.2.1 Hydrogen ion concentration**

Calibrate the pH meter with appropriate electrode according to manufacturer's instructions using either one buffer, pH 7.0 or two buffers, pH 4.0 and pH 7.0.

**3.2.2 Total and TCA-ppt Nitrogen Tests**

1. Prepare digestion and distillation units according to manufacturer's recommendations.
2. Check levels of water and sodium hydroxide tanks on the distillation unit, fill if necessary.
3. Prime the buret by rinsing with standardized HCl.

**3.2.3 Phenol Test**

Prime the buret by rinsing with test fluid.

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### 3.3 Preparation of reagents/control procedures

Reagents are stable for 6 months from date of preparation and stored at room temperature, unless otherwise noted. Prepare reagents in volumes appropriate to demand to minimize waste due to expiration.

Glassware used for preparation of reagents must meet ASTM requirements; measurements are based on the measurements of uncertainty outlined in those requirements.

All references to “water” indicate distilled, demineralized, reduced oxygen or water of equivalent purity (**Section 2.2.2(2)**).

In the following steps the acronym QS is used. It is defined as quantity sufficient; as much as is sufficient.

#### 3.3.1 Total and TCA-ppt Nitrogen Tests

**1. Standard,  $1.0 \pm 0.1$  mg/ml Protein (0.016% nitrogen):** Dilute protein standard reference material (**Section 2.2.1(11)**) to the range of  $1.0 \pm 0.1$  mg/mL protein with water. Prepare sufficient dilution to provide several 10 mL portions. Store at 4°C.

**2. Control Sample:** Either (1) a pool of tuberculin products with established nitrogen and phenol values as tested by PL-CAS; or (2) a product produced for use as a control sample and tested by PL-CAS.  
**(1)** Combine any sample volumes remaining after test completion in a pool. Record all identifying information, CAS protein result and expiration date for each sample. Control sample protein concentration is the mean of CAS results for all samples included in the pool.  
**(2)** Obtain a product produced for use as a control sample. Analyze the product a total of three times, mean of these trials must be  $1.0 \pm 0.1$  mg/mL protein. Expiration date is as indicated on product. Store at  $4 \pm 10^\circ$  C.

**3. 32% Sodium Hydroxide (NaOH): *Caution!! NaOH is caustic-- Avoid contact with skin.*** Dissolve  $640 \text{ g} \pm 1 \text{ g}$  sodium hydroxide in approximately 1.4 L water in a 2-L volumetric flask on a stir plate. *Solution will be HOT!!* Cool to room temperature. QS with water. Store at room temperature.

**4. Saturated Boric Acid ( $\text{H}_3\text{BO}_3$ ):** *Use a container with at least twice as much volumetric capacity as your final volume.* Add  $15.0 \pm 0.1$  g boric

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acid to 100 mL water. Stir, with heat, until all boric acid dissolves. Some boric acid recrystallizes when cool. Store at room temperature.

**5. 0.1% bromo phenol blue:** Dissolve  $0.1 \pm 0.1$  g in 100 mL water. Store at room temperature.

**6. 0.5% methyl red:** Dissolve  $0.5 \pm 0.1$  g in 100 mL ethanol. Store at 4°C.

**7. 4.0% Trichloroacetic acid (TCA):** Dissolve  $4.0 \pm 0.1$  g TCA in 75 mL water in a 100-mL volumetric flask. QS with water. Store at room temperature.

**8. Standardized 0.01 - 0.02N Hydrochloric acid (HCl): *Caution!! Concentrated HCl is corrosive – Handle in fume hood. Avoid contact with skin.***

**Preparation:** Add 1.72 mL hydrochloric acid to approximately 900 mL water in a 1-L volumetric flask. QS with water. Store at room temperature.

**Standardization:** Weigh approximately 0.010 g dried sodium carbonate. Record weight. Dissolve in 25 mL water. Add three drops 0.1% bromo phenol blue (indicator). Titrate with prepared 0.01 – 0.02N hydrochloric acid to an endpoint color of green, not bluish green nor yellowish green. Calculate the normality of the hydrochloric acid solution as below. Perform three trials and use the calculated mean as the normality of the hydrochloric acid solution.

**Calculation:**

$$N_{HCl} = \frac{[(g Na_2CO_3)(1000)]}{[(mL HCl)(52.994)]}$$

### 3.3.2 Phenol test

**1. Standard, 0.50% phenol:** Dissolve  $5.0 \pm 0.01$  g phenol in approximately 500 mL water in a 1-L volumetric flask; QS to 1 L with water.

**2. Control, Pool of Previously Analyzed Samples:**

Either (1) a pool of PPD tuberculin products with established protein and phenol values as tested by PL-CAS; or (2) a product produced for use as a control sample and tested by PL-CAS.

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- (1) Combine any sample volumes remaining after test completion in a pool. Record all identifying information, CAS phenol result, and expiration date for each sample. Control sample phenol concentration is the mean of CAS results for all samples included in the pool.
- (2) Obtain a product produced for use as a control sample. Analyze the product a total of three times, mean of these trials must be  $0.50 \pm 0.04$  % phenol. Expiration date is as indicated on product. Store at  $4^{\circ} \pm 10^{\circ}$  C.
- 3. 20% Hydrochloric Acid (HCl):** In a 1-L volumetric flask, slowly add 200 mL hydrochloric acid to 600 mL water; QS to 1 L with water.
- 4. 0.1% Methyl Orange:** Dissolve  $0.1 \pm 0.01$  g methyl orange in 100 mL water. Filter if necessary.
- 5. Silicotungstic acid solution (SAS):** Dissolve  $60.00 \pm 0.5$  g silicotungstic acid hydrate in 400 mL water in a 500-mL volumetric flask. Add 50 mL sulfuric acid. When cool, QS to 500 mL with water.
- 6. Clarifying solution (CS):** Add 50 mL SAS and 125 mL 20% hydrochloric acid to 325 mL water. Prepare fresh prior to each test.
- 7. "Acid solution" for As<sub>2</sub>O<sub>3</sub> standardization solution:** Add 110 mL hydrochloric acid and 2.5 mL 0.1% methyl orange to 100 mL water.
- 8. 0.050 N Arsenic trioxide (As<sub>2</sub>O<sub>3</sub>):** *CAUTION!! Arsenic trioxide is extremely toxic. Avoid contact; handle in fume hood using gloves, mask, and goggles. Consult the Safety Data Sheet for specific handling instructions before proceeding.* Dissolve  $2.4730 \pm 0.001$  g anhydrous arsenic trioxide in 25 mL hot 1 N sodium hydroxide in a 1-L volumetric flask. Neutralize solution with 25 mL 1 N sulfuric acid. When cool, QS to 1 L with water.
- 9. 1N Sodium hydroxide:** Dissolve  $4.00 \pm 0.01$  g of sodium hydroxide in 60 mL water in a 100-mL volumetric flask; QS to 100 mL with water.
- 10. 1N Sulfuric acid:** In a 100-mL volumetric flask, slowly add 4.904 mL sulfuric acid to 60 mL water; QS to 100 mL with water.
- 11. Test fluid (TF):** Dissolve  $0.30 \pm 0.01$  g sodium bicarbonate,  $1.67 \pm 0.01$  g potassium bromate, and  $15.00 \pm 0.01$  g potassium bromide in water and QS to 1 L with water. **CRITICAL CONTROL POINT: The test fluid must be standardized as described in 3.3.11(1) prior to use.**
1. Standardization

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- a. Prepare standardization solution: Add 25 mL 0.050N arsenic trioxide to 10 mL “Acid Solution.”
- b. Confirm standardization solution by titrating with previous lot of TF. It should take 21.3 mL TF to titrate the standardization solution.
- c. Titrate standardization solution with new lot of TF. The required titration volume is 21.3 mL TF. A first time titration may require less than 21.3 mL TF, in which case, the TF volume must be adjusted by adding the correct volume of water to the TF, continue to **Step 1d** if this is the case. If the first time titration is  $21.3 \pm 0.1$  mL TF, continue to **Step 2**.
- d. Adjust the TF volume. For this step the calculations are shown and an example is used to illustrate.

A = Starting volume of TF (mL)  
B = Titration volume of TF (mL)  
C = Volume of TF left (mL)  
D = Required titration volume (21.3 mL)  
E = Adjusted volume of TF (mL)  
F = Volume of water to be added to volume of TF left to achieve the adjusted volume (mL)

*Example:* Assume the starting volume of TF is 1000 mL and the titration volume is 20.5 mL.

- $A - B = C$

*Example:* (1000 mL) - (20.5 mL) = 979.5 mL

- $\frac{(C)(D)}{(B)} = E$

*Example:*  $\frac{(979.5 \text{ mL})(21.3 \text{ mL})}{(20.5 \text{ mL})} = 1017.7 \text{ mL}$

- $E - C = F$

*Example:* (1017.7 mL) - (979.5 mL) = 38.2 mL

- Add the calculated volume of water (F) to the existing TF and put any TF remaining in the buret back into flask. Continue to **Step 2**.

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2. Repeat **Step 1c** until three consecutive trials produce an average titration volume of 21.3 mL.

### **3.4 Preparation of the sample**

#### **3.4.1 Receipt**

Complete sample receipt as described by standard operating procedures.

#### **3.4.2 Preparation**

Licensed or prelicense biologics products are generally received in sealed serum bottles and stored at  $4^{\circ}\pm 10^{\circ}\text{C}$  prior to testing. Before testing, allow sample vials and reagents to come to room temperature.

#### **3.4.3 Clarity Test**

Remove label from a sealed bottle of tuberculin. When the bottle has warmed to room temperature, make sure the label is dry and peel the label from the bottle carefully. Clean the bottle with alcohol and a lint-free towel.

## **4. Performance of the Test**

### **4.1 Hydrogen ion concentration**

**4.1.1** Place approximately 5.0 mL of tuberculin product in each of two disposable beakers.

**4.1.2** Flush pH electrode in first beaker of tuberculin by means of dipping it several times until the pH readout settles. If using a printer, label this first readout "flush."

**4.1.3** Place electrode in second beaker of tuberculin. Wait until pH readout becomes stable; record pH.

### **4.2 Clarity**

In an area with subdued light, allow your eyes to adjust. Turn on the spotlight lamp, which is positioned upright. Place the unlabeled bottle over the light beam and observe for extraneous particles.

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### **4.3 Total nitrogen**

Analyze duplicate 15 mL samples, consisting of 5 mL from each of three vials. Analyze the control, standard and a blank sample in duplicate each time testing is performed.

**4.3.1** Place one Kjeldahl Catalyst tablet, 1.0 mL sample and 3.0 mL sulfuric acid into a digestion flask. Follow the same procedure for standard and control, use only reagents for the blank.

**Caution: HgO is poisonous – Use gloves, mask, and goggles.**

**Caution: Concentrated H<sub>2</sub>SO<sub>4</sub> is corrosive--Avoid contact with skin.**

**4.3.2** Place the digestion flasks in the digestion unit.

**4.3.3** Digest in a method that results in a fully digested product, i.e., 250°C for 15 minutes, 410°C for 60 minutes, and 500°C for 15 minutes. Final product should be clear to white-cloudy.

**4.3.4** Cool in digestion flasks, add 6 mL water (**Section 2.2.2(2)**), mix (a vortex may be used), and cool again.

**4.3.5** Place digestion flask and a 125-mL Erlenmeyer flask containing 5 mL saturated boric acid solution and 3 drops 0.1% methyl red into the distillation unit. Tilt the flask so the tip of the condenser is immersed in the boric acid.

**4.3.6** Add a sufficient amount of 32% sodium hydroxide to make the solution in the digestion flask alkaline, i.e., 25 mL. A sodium hydroxide pump may be used for this, if the distillation unit is equipped with one.

**4.3.7** Distill for two minutes.

**4.3.8** Titrate collected distillate to endpoint color change of yellow to deep rose (pH 5.0) with standardized hydrochloric acid. Record the volume of hydrochloric acid titrated.

### **4.4 TCA-ppt nitrogen**

Analyze duplicate 15 mL samples, consisting of 5 mL from each of three vials. Analyze the control, standard and a blank sample in duplicate each time testing is performed.

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**4.4.1** Place 5.0 mL of sample and 5.0 mL 4.0% TCA in a 15 mL, screw-capped centrifuge tube. Follow the same procedure for standard and control. Blank sample preparation steps begin with **Section 4.4.7**.

**4.4.2** Vigorously shake centrifuge tube for 1 minute, then let stand for 10 minutes.

**4.4.3** Centrifuge tube under conditions that result in pellet formation, i.e., 2,500 rpm for 10 minutes. Discard supernatant.

**4.4.4** Add 3 mL water (**Section 2.2.2(2)**) and vortex tube until the precipitate at bottom mixes. Transfer mix to digestion flask.

**4.4.5** Repeat **Section 4.4.4** two more times, each time adding to the original digestion flask so the final volume in the digestion flask is approximately 9 mL.

**4.4.6** Add one Kjeldahl Catalyst tablet and 3.0 mL sulfuric acid to the digestion flask.

**4.4.7** Proceed to **Section 4.3.2** and follow the digestion, distillation and titration procedures through **Section 4.3.8**.

#### **4.5 Phenol**

Analyze the control pool and phenol standard in duplicate each time testing is performed. Analyze samples in triplicate.

**4.5.1** Combine 5 mL sample and 100 mL clarifying solution (CS) to a 250-mL glass-stoppered flask. Shake 2 minutes. Filter through filter paper and collect 50 mL of filtrate.

**4.5.2** Transfer 50 mL of filtrate to another 250-mL glass-stoppered flask. Add a stir bar and place flask on stir plate with buret directly above. Add 1 drop 0.1% methyl orange (indicator), stir for a few seconds. Observe the color as pink. *An acceptable alternative to using a stir plate and stir bar would be shaking the flask.*

**4.5.3** Titrate with 2 mL test fluid (TF), stir or shake for a few seconds. Observe the color; if pink, repeat. If colorless, go to **Section 4.5.4**.

**4.5.4** Stir or shake 30 seconds. Add 1 drop indicator, stir for a few seconds. Observe the color. If colorless for  $\leq 10$  seconds or if pink, titrate with 1 mL TF, and repeat. If colorless for  $\geq 10$  seconds, go to **Section 4.5.5**.

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**4.5.5** Stir or shake 1 minute. Add 1 drop indicator, stir for a few seconds. Observe the color. If pink for  $\geq 10$  seconds, titrate with 0.50 mL TF, and repeat. When colorless within 10 seconds, record total volume of TF as the endpoint of titration and use this volume for calculation of percent phenol.

**5. Interpretation of the Test Results**

**5.1 Hydrogen ion concentration**

No calculation is required.

Satisfactory hydrogen ion concentration\*:  $7.0 \pm 0.3$

\*This value is to be used unless otherwise noted in the approved Outline of Production for the product or 9 CFR 113.406.

**5.2 Clarity**

No calculation is required.

Satisfactory clarity: Negative (no insoluble particles observed)

**5.3 Total nitrogen (Report average of duplicates)**

$$\% \text{ total nitrogen} = \frac{(mL_{\text{sample}} - mL_{\text{blank}})(N \text{ HCl})(1.4007)}{1.0 \text{ mL}}$$

$mL_{\text{sample}}$  = Volume of standardized HCl required for sample

$mL_{\text{blank}}$  = Volume of standardized HCl required for blank

N HCl = Normality of HCl

1.4007 = Milliequivalent weight of nitrogen x 100

1.0 mL = Volume of sample

Satisfactory total nitrogen content\*:  $0.18\% \pm 0.06\%$

\*This value is to be used unless otherwise noted in the approved Outline of Production for the product or 9 CFR 113.406.

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**5.4 TCA-ppt nitrogen** (Report average of duplicates)

$$\% \text{ TCA - ppt nitrogen} = \frac{(mL_{\text{sample}} - mL_{\text{blank}})(N \text{ HCL})(1.4007)}{5.0 \text{ mL}}$$

$mL_{\text{sample}}$  = Volume of standardized HCl required for sample

$mL_{\text{blank}}$  = Volume of standardized HCl required for blank

N HCL = Normality of HCl

1.4007 = Milliequivalent weight of nitrogen x 100

5.0 mL = Volume of sample

Satisfactory TCA-ppt nitrogen content\*: 0.047% ± 0.01%

\*This value is to be used unless otherwise noted in the approved Outline of Production for the product or 9 CFR 113.406.

**5.5 Phenol** (Report average of triplicates)

*A correction factor of 0.04 should be subtracted from the final value in the determination of phenol in tuberculin. (9 CFR 113.406, (d), (4))*

Percent phenol = [(vol of test fluid) x (0.04)] – (0.04)

Satisfactory Phenol Content\*: 0.54% ± 0.04%

\*This value is to be used unless otherwise noted in the approved Outline of Production for the product or 9 CFR 113.409.

**5.6 Controls**

Results for controls and standards must be within acceptable limits; otherwise repeat testing.

**6. Report of Test Results**

Report results of the test(s) as described by standard operating procedures.

**7. References**

**7.1** Title 9, *Code of Federal Regulations*, part 113.406, U.S. Government Printing Office, Washington, DC.

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**7.2** AOAC Official Method 960.52, Official Methods of Analysis of AOAC International, Arlington, Virginia, 16th Edition, Pat Cuniff, Editor (1995), Volume I, Chapter 12, page 7.

**7.3** ASTM Standard E969, Standard Specification for Glass Volumetric (Transfer) Pipets.

**7.4** ASTM Standard E288, Standard Specification for Laboratory Glass Volumetric Flasks.

**7.5** ASTM Standard E694, Standard Specification for Laboratory Glass Volumetric Apparatus.

## **8. Summary of Revisions**

### **Version .09**

- The coversheet has been updated

### **Version .08**

- The Contact information was updated.
- The phenol control information was corrected.
- An additional option to purchase phenol standard was added.
- “Reduced oxygen” under Water was changed to “reverse osmosis.”

### **Version .07**

- The document has been revised to reflect changes in instrumentation, personnel, and to provide additional detail.
- **5.3/5.4:** Total and TCA-ppt nitrogen, the calculation was changed to reflect that used in AOAC 960.52 taking into account the blank sample. The satisfactory protein content level did not change.
- A blank sample requirement has been added to the procedure to reflect AOAC Official Method 960.52.

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**Supplemental Assay Method for the Determination of Hydrogen Ion Concentration, Total Nitrogen, TCA Nitrogen, Phenol and Clarity in Intradermic (Filtrate Produced From Cultures of Pn, C, and Dt Strains of *Mycobacterium tuberculosis*) Tuberculin**

- The sampling requirements for total nitrogen and TCA-ppt nitrogen changed from “analyze all samples in triplicate” to “analyze duplicate 15 mL samples, consisting of 5 mL from each of three vials” to reflect the requirements in 9 CFR, Part 113.406, (d), (2), (3).

**Version .06**

- The document number has been changed from TCSAM0514 to SAM 514.

**Version .05**

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.

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