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

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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 Balance, top loading, capable of measuring 0.001 g

2.1.2 Digestion unit (Büchi)

2.1.3 Distillation unit (Büchi)

2.1.4 Volumetric pipettes, Class A, 5, 10, and 25 mL

2.1.5 Volumetric flasks with barrel head glass stopper, Class A, 500 mL and 1 L

2.1.6 Erlenmeyer flasks or collection container, ≥80 mL

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2.1.7 Burets with PTFE stopcocks, precision bore, Class A, 10, 25, and 50 mL

2.1.8 Weigh boats, or equivalent

2.1.9 Graduated cylinders, Class A, 50, 100, 250, 500 mL, and 1 L

2.1.10 Glass-stoppered Erlenmeyer flasks, 250 mL

2.1.11 Heating/stirring plate with stirring bars

2.1.12 Filter paper, 11µm particle retention (Whatman No. 1)

2.1.13 Timers, 30 seconds to 2 minutes

2.1.14 Dropper, for example, transfer pipette, Pasteur pipette, dropper bottle

2.1.15 Pipettor and tips to accurately dispense 100 to 1000 µL

2.1.16 pH meter accurate to at least 0.1 pH
- CVB uses Mettler Toledo automated titrator

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

2.2.1.1. Sulfuric acid (H_2SO_4), CAS# 7664-93-9, Purity: Minimum 95.0%, Maximum 98.0%
Note: EXTREME CAUTION - Sulfuric acid can irritate and burn the skin and eyes, and may lead to blindness. Wear face shields, protective gloves, and chemical-resistant clothing and boots. Must be handled in a chemical fume hood if there is any potential for inhalation exposure.

2.2.1.2. Kjeldahl Catalyst Tablets, 1.5 g K_2SO_4 + 0.075 g HgO

Note: Kjeldahl catalyst tablets may cause damage to organs through prolonged or repeated exposure. Harmful if swallowed or if inhaled. Wear suitable gloves and respiratory protection.

2.2.1.3. Sodium hydroxide (NaOH), CAS# 1310-73-2, Purity: 98.5%
Note: Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs,

2.2.1.4. Boric acid (H_3BO_3), CAS# 10043-35-3, Purity: 99.9%
Note: Boric acid is low in toxicity if eaten or if it contacts skin. People

and diarrhea. Wear face shields, protective gloves, and chemical-resistant clothing and boots. Must be handled in a chemical fume hood if there is any potential for inhalation exposure.

2.2.1.5. Methyl red, CAS# 493-52-7, Purity: 98.0% or Sher indicator (Buchi 3512)
Note: Methyl red Causes eye and skin irritation. Wear protective eyeware and gloves. Use only in well-ventilated areas.

2.2.1.6. Hydrochloric acid (HCl), CAS# 7647-01-0, Assay: 36.5-38.0%
Note: Hydrochloric acid causes acute (short-term) inhalation exposure may cause eye, nose, and respiratory tract irritation and inflammation and pulmonary edema in humans. Wear face shields, protective gloves, and chemical-resistant clothing and boots. Must be handled in a chemical fume hood if there is any potential for inhalation exposure.

2.2.1.7. Sodium carbonate (Na_2CO_3), CAS# 497-19-8, Purity: 99.9%
Note: Inhalation of sodium carbonate can lead to adverse effects such as respiratory tract irritation, coughing, shortness of breath, and pulmonary edema. Use only in well-ventilated areas.

2.2.1.8. Bromo phenol blue, CAS# 115-39-9, Purity: 98.0%
Note: Potential Acute Health Effects - Bromo phenol blue is slightly hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Use only in well-ventilated areas.

2.2.1.9. Trichloroacetic acid (TCA), CAS# 76-03-9, Purity: 98.0%

Note: Trichloroacetic acid (TCA) is a CORROSIVE CHEMICAL and contact can severely irritate and burn the skin and eyes with possible eye damage. Breathing TCA can irritate the nose and throat plus irritate the lungs causing coughing and/or shortness of breath. Wear face shields, protective gloves, and chemical-resistant clothing and boots. Must be handled in a chemical fume hood if there is any potential for inhalation exposure.

2.2.1.10. Control Sample – Either a pool of PPD tuberculin products or a single product with established protein and phenol values defined in **Section 5.**

- The same control may be used for phenol and protein or one control for each. Any controls used must be defined and lot controlled.
- Validation of the control requires at least three replicates with the mean of the replicates falling within the defined range.
- Store at 5° ± 3°C.

2.2.1.11. 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)

2.2.2.1. Hydrochloric acid (HCl), CAS# 7647-01-0, Assay: 36.5-38.0%
Note: Hydrochloric acid causes acute (short-term) inhalation exposure may cause eye, nose, and respiratory tract irritation and inflammation and pulmonary edema in humans. Wear face shields, protective gloves, and chemical-resistant clothing and boots. Must be handled in a chemical fume hood if there is any potential for inhalation exposure.

2.2.2.2. Water (H_2O), Purity: distilled, demineralized, reverse osmosis or equivalent.

2.2.2.3. Methyl orange, CAS# 547-58-0, Purity: 98.0%
Note: Methyl orange is harmful if swallowed. Wear appropriate protective gloves and clothing. Use in well-ventilated areas.

2.2.2.4. Silicotungstic acid hydrate ($H_4[Si(W_3O_{10})_4]*26H_2O$), CAS# 12027-43-9, Purity: 99.0%. Store at 4°C.
Note: Silicotungstic acid hydrate causes skin irritation, serious eye irritation, and may cause respiratory irritation. Precautionary

statement - Avoid breathing dust. Wear face shields, protective gloves, and chemical-resistant clothing and boots. Must be handled in a chemical fume hood if there is any potential for inhalation exposure.

2.2.2.5. Sulfuric acid (H_2SO_4), CAS# 7664-93-9, Purity: Minimum 95.0%, Maximum 98.0%
Note: EXTREME CAUTION - Sulfuric acid can irritate and burn the skin and eyes, and may lead to blindness. Wear face shields, protective gloves, and chemical-resistant clothing and boots. Must be handled in a chemical fume hood if there is any potential for inhalation exposure.

2.2.2.6. Arsenic trioxide, anhydrous (As_2O_3), CAS# 1327-53-3, Purity: 99.9%
Note: Airborne arsenic trioxide may produce a burning sensation to the nose, mouth, and eyes and cause coughing, shortness of breath, headache, sore throat, and dizziness. Wear appropriate protective clothing such as coveralls, gloves, impervious boots, hat, goggles or a face shield. Work is performed in a dedicated and functionally certified fume hood whenever possible.

2.2.2.7. Sodium hydroxide (NaOH), CAS# 1310-73-2, Purity: 98.5%
Note: Contact with very high concentrations of sodium hydroxide can cause severe burns to the eyes, skin, digestive system or lungs, resulting in permanent damage or death. Wear appropriate protective clothing such as gloves, goggles or a face shield. Work in a chemical fume hood with the sash in the down position.

2.2.2.8. Phenol (C_6H_6O), CAS# 108-95-2, Purity: ≥ 99.0%
This can be a purchased NIST standard and diluted, if necessary, to the appropriate level.
Note: Phenol is highly corrosive to the skin and readily absorbed through it, whereupon it can affect the central nervous system and cause damage to the liver and kidneys. When handling, wear a butyl rubber or neoprene apron and the appropriate gloves.

2.2.2.9. Sodium bicarbonate ($NaHCO_3$), CAS# 144-55-8, Purity: 99.9%
Note: Warning Hazard Statements - Sodium bicarbonate may be harmful if swallowed. Causes skin mild irritation. Causes eye irritation. When handling, wear respirators or dust masks, safety goggles and appropriate gloves. Use in well-ventilated areas.

2.2.2.10. Potassium bromate ($KBrO_3$), CAS# 7758-01-2, Purity: 98.5%

Note: Potassium Bromate can irritate the lungs. When handling, wear respirators or dust masks, safety goggles and appropriate gloves. Use in well-ventilated areas.

2.2.2.11. Potassium bromide (KBr), CAS# 7758-02-3, Purity: 99.0%
Note: Potassium bromide exposure may cause coughing, sore throat, shortness of breath and dizziness. When handling, wear respirators, safety goggles and appropriate gloves. Work in a chemical fume hood.

2.2.3 Hydrogen ion concentration

2.2.3.1. Commercial buffers, certified pH 7.00 and pH 4.00

2.2.3.2. pH electrode storage solution

2.2.3.3. 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

3.2.2.1. Prepare digestion and distillation units according to manufacturer's recommendations.

3.2.2.2. Check levels of water and sodium hydroxide tanks on the distillation unit, fill if necessary.

3.2.2.3. Prime the buret by rinsing with standardized HCl.

3.2.3 Phenol Test

Prime the buret by rinsing with test fluid.

3.3 Preparation of reagents/control procedures

Reagents are stable for 6 months from date of preparation and stored at room temperature (22° ± 5°C), 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

3.3.1.1. Standard, 1.0 ± 0.1 mg/ml Protein (0.016% nitrogen): Dilute protein standard reference material (**Section 2.2.1.11**) to the range of 1.0 ± 0.1 mg/mL protein with water. Prepare sufficient dilution to provide several 15 mL portions. Store at 5° ± 3°C.

3.3.1.2. Control Sample: See **Section 2.2**.

3.3.1.3. 32% Sodium Hydroxide (NaOH): ***Caution!! NaOH is caustic--Avoid contact with skin.*** Dissolve 640 g ± 1 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 (22° ± 5°C). QS with water. Store at room temperature (22° ± 5°C).

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3.3.1.4. Saturated Boric Acid (H_3BO_3): *Use a container with at least twice as much volumetric capacity as your final volume.* Add 15.0 ± 0.1 g boric acid to 100 mL water. Heat while stirring to ≥ 50 °C to achieve saturation. Do not boil!!! Some boric acid recrystallizes when cool. Store at room temperature (22° ± 5°C).

3.3.1.5. 0.1% bromo phenol blue: Dissolve 0.1 ± 0.1 g in 100 mL water. Store at room temperature (22° ± 5°C).

3.3.1.6. 0.5% methyl red: Dissolve 0.5 ± 0.1 g in 100 mL ethanol. Store at room temperature (22° ± 5°C).

3.3.1.7. 4.0% Trichloroacetic acid (TCA): Dissolve 4.0 ± 0.1 g TCA in 75 mL water in a 100 mL volumetric flask. QS with water. Store at room temperature (22° ± 5°C).

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

If preparing internally: Add 1.72 mL hydrochloric acid to approximately 900 mL water in a 1 L volumetric flask. QS with water. Store at room temperature (22° ± 5°C).

If standardizing internally: 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

3.3.2.1. Standard, 0.50% phenol: Dissolve 5.0 ± 0.01 g phenol in approximately 500 mL water in a 1 L volumetric flask; QS to 1 L with water.

3.3.2.2. Control Samples: See **Section 2.2**

3.3.2.3. 20% dilution Hydrochloric Acid (HCl): In a 1 L volumetric flask, slowly add 200 mL concentrated hydrochloric acid (36-38%) to 600 mL water; QS to 1 L with water.

3.3.2.4. 0.1% Methyl Orange: Dissolve 0.1 ± 0.01 g methyl orange in 100 mL water. Filter if necessary.

3.3.2.5. Silicotungstic acid solution (SAS): Dissolve 60.00 ± 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.

3.3.2.6. Clarifying solution (CS): Add 50 mL SAS and 125 mL 20% hydrochloric acid to 325 mL water. Prepare fresh prior to each test.

3.3.2.7. "Acid solution" for As_2O_3 standardization solution: Add 110 mL hydrochloric acid and 2.5 mL 0.1% methyl orange to 100 mL water.

3.3.2.8. 0.050 N Arsenic trioxide (As_2O_3): ***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 ± 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.

3.3.2.9. 1N Sodium hydroxide: Dissolve 4.00 ± 0.01 g of sodium hydroxide in 60 mL water in a 100 mL volumetric flask; QS to 100 mL with water.

3.3.2.10. 1N Sulfuric acid: In a 100 mL volumetric flask, slowly add 2.72 mL sulfuric acid to 60 mL water; QS to 100 mL with water.

3.3.2.11. Test fluid (TF): Dissolve 0.30 ± 0.01 g sodium bicarbonate, 1.67 ± 0.01 g potassium bromate, and 15.00 ± 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 3.3.2.11.1 prior to use.***

3.3.2.11.1. Standardization

3.3.2.11.1.a. Prepare standardization solution: Add 25 mL 0.050 N arsenic trioxide to 10 mL “Acid Solution.”

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3.3.2.11.1.b. Confirm standardization solution by titrating with previous lot of TF. It should take 21.3 mL TF to titrate the standardization solution.

3.3.2.11.1.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 3.3.2.11.1.d.** if this is the case. If the first time titration is 21.3 ± 0.1 mL TF, continue to **Step 3.3.2.11.2**.

3.3.2.11.1.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: $\frac{(979.5\ mL)\ (21.3\ mL)}{(20.5\ mL)} = 1017.7\ mL$

- $E - C = F$

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 3.3.2.11.2**.

3.3.2.11.2. Repeat **Step 3.3.2.11.1.c.** until three consecutive trials produce an average titration volume of 21.3 mL.

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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 5° ± 3°C, prior to testing. Before testing, allow sample vials and reagents to come to room temperature (22° ± 5°C).

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.

4.3 Total nitrogen

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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. The digestion method must be validated by controls.

4.3.1 Place one Kjeldahl Catalyst tablet, 1.0 mL sample and 3.0 mL sulfuric acid into a digestion flask.

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

Caution: Concentrated H_2SO_4 is corrosive--Avoid contact with skin.

4.3.2 Place the digestion flasks in the digestion unit.

4.3.3 Digest by a method that results in a fully digested product. For example, a temperature profile of 250°C for 15 minutes, 410°C for 60 minutes, and 500°C for 15 minutes has historically been used at APHIS. Final product should be clear to white-cloudy.

- The controls must be included on every run to assure full digestion (see **Section 5.6**).

4.3.4 Cool in digestion flasks to approximately 60°C, add 6 mL water, mix (a vortex mixer may be used), and allow to cool again on the bench top to ≤ 40°C.

4.3.5 Place digestion flask and a collection container containing 5 mL saturated boric acid solution and 3 drops 0.5% methyl red (or Sher indicator) into the distillation unit. Assure the tip of the condenser tube of the distillation unit is immersed in the boric acid.

4.3.6 Add an appropriate amount of 32% sodium hydroxide to make the solution in the digestion flask sufficiently alkaline to allow distillation of ammonia, for example, ≥ 25 mL. A sodium hydroxide pump may be used, if the distillation unit is equipped with one.

4.3.7 Distill for sufficient time to fully distill product through comparison with known standards, at least two minutes.

4.3.8 Titrate collected distillate with standardized hydrochloric acid to a pH within the range of 4.65 – 5.0 and record volume.

- Option 1: Titrate with a pH meter to a specific pH in the above range. At APHIS, an automated titrator is used to titrate to pH 4.65.
- Option 2: Titrate to endpoint color change of methyl red (yellow to deep rose) or Sher indicator (gray to clear).

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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. The digestion method must be validated by controls.

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.*

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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 pink for at least 10 seconds, titrate with 1 mL TF, and repeat. If colorless within 10 seconds, go to **Section 4.5.5**.

4.5.5 Stir or shake 1 minute. Add 1 drop indicator, stir for a few seconds. Observe the color. If pink for at least 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 ± 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)

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

mL_{sample} = Volume of standardized HCl required for sample
mL_{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% ± 0.06%

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

5.4 TCA-ppt nitrogen (Report average of duplicates)

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

mL_{sample} = Volume of standardized HCl required for sample
mL_{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 113.406.

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

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

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 .10

- Reformatted cover page.
- Updated numbering and formatting throughout document.
- Updated **Sections 2, 3,** and **4**.
- **Sections 2.2.1.5, 4.3.5,** and **4.3.8:** Added optional Sher indicator or an automatic titrator.
- **Section 3.3.2.3:** Noted 20% "dilution" HCl.
- **Section 3.3.2.10:** Corrected volume of concentrated acid to make 1 N Sulfuric Acid.
- **Sections 4.5.4** and **4.5.5:** Phenol titration wording revision "If pink for at least 10 seconds…"
- **Section 5.5:** Corrected typo to reference 9 CFR 113.406.

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.

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- "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.

- 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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