

# Diagnostic test for bacillus: ascoli's thermo precipitation test essay sample



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Purpose: used to identify anthrax bacilli in animal hides and meat. Principle:

This test was designed to detect B. anthracis antigens in the tissues of animals being utilized in animal by-products and thereby to reveal when these products contained ingredients originating from animals that had died of anthrax. The thermostable antigens involved are common to other Bacillus species so the test depends on the fact that the only Bacillus likely to have proliferated within and throughout an animal depositing extensive precipitating antigens in the tissues is B. anthracis. Procedure:

1. Chop or slice the specimen into fine pieces or strips.
2. Boil approximately 2g of the specimen for 5 minutes in 5mL saline containing 1: 100 (final concentration) acetic acid. Alternately soak in saline containing 0. 5% phenol for 24-48 hours in a refrigerator.
3. After cooling, filter through filter paper until completely clear.
4. Insert a few drops of antiserum in bottom of a small test tube and carefully add some of the filtrate down the side of the tube to form a layer of antigen above antiserum.
5. Include appropriate positive and negative specimen controls.

Reagents used:

\* Acetic acid

\* Saline containing 0. 5% phenol

\* Anthrax antiserum:

Antiserum is prepared in rabbits by the subcutaneous Inoculation of Sterne anthrax vaccine on days 1 and 14. On days 28 and 35, the rabbits receive 0. 5 mL of a mixture of several strains of virulent B. anthracis not exceeding 10<sup>5</sup> colony-forming units (CFU)/ml suspended in saline. Alternatively, the live virulent bacteria can be inactivated by prolonged suspension in 0. 2% formalized saline, but the antigen mass needs to be increased to 10<sup>8</sup> -10<sup>9</sup>

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CFU/ml. The suspension should be checked for inactivation of the B. anthracis before animal inoculation by culture of 0.1 ml into 100 ml of nutrient broth containing 0.1% histidine and, after incubation at 37°C for 7 days, subculture on to blood or nutrient agar. The dose regimen for the formalized suspension after initial vaccination on days 1 and 14 is increasing doses of 0.1, 0.5, 1, and 2 ml given intravenously at intervals of 4–5 days.

Expected results:

\* Positive: Whitish ring appear at the junction of the two fluids \* Negative: No whitish ring appear at the junction of the two fluids Consideration:

The test is not suitable for detection of B. anthracis in environmental specimens; numerous other Bacillus species can be expected to occur in these. GELATIN LIQUEFACTION/GELATIN HYDROLYSIS

Purpose: used to identify Bacillus anthracis and Bacillus cereus which yield positive result in this test. Principle:

This test is used to determine the ability of an organism to produce proteolytic enzymes (gelatinase) that liquefy gelatin. Procedure:

1. Inoculate gelatin deep with 4 to 5 drops of a 24-hour broth culture.
2. Incubate at 35°C in ambient air for up to 14 days. Note: incubate the medium at 25°C if the organism grows better at 25°C than at 35°C.
3. Alternatively, inoculate the gelatin deep from a 24-hour-old colony by stabbing 4 to 5 times ½ inch into the medium.
4. Remove the gelatin tube daily from the incubator and place at 4°C to check for liquefaction. Do not invert or tip the tube, because sometimes the only discernible liquefaction will occur at the top of deep where inoculation occurred.
5. Refrigerate an uninoculated control

along with the inoculated tube. Liquefaction is determined only after the control has hardened (gelled). Expected results:

- \* Positive: partial or total liquefaction of the inoculated tube at 4°C within 14 days.
- \* Negative: complete solidification of tube at 4°C

Quality control:

- \* Positive: *Proteus vulgaris*
- \* Negative: *Enterobacter aerogenes*

CATALASE TEST:

Purpose: used to identify *Bacillus anthracis* which yield positive result in this test. Principle:

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide (30% for the slide test), and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. Method:

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth to the surface of a clean, dry glass slide.
2. Place a drop of 30% hydrogen peroxide onto medium.
3. Observe for the evolution of oxygen bubbles.

Reagents used:

- \* 30% hydrogen peroxide for slide test.

Expected results:

- \* Positive: copious bubbles produced
- \* Negative: no or few bubbles produced

\* Note: some organisms (enterococci) produce a peroxidase that slowly  
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catalyzes the breakdown of hydrogen peroxide and the test may appear weakly positive. This reaction is not a truly positive test. Quality control:

\* Positive: *Staphylococcus aureus*

\* Negative: *Streptococcus pyogenes*

#### VOGES-PROSKAUER TEST

Purpose: used to identify *Bacillus anthracis* which yield positive result in this test. Principle:

This test is used to determine the ability of an organism to produce and maintain stable acid end products from glucose fermentation, to overcome the buffering capacity of the system, and to determine the ability of some organism to produce neutral end products from glucose fermentation.

Method:

1. Inoculate MRVP broth with 1 drop from 24-hour brain-heart infusion broth culture.
2. Incubate at 35° C to 37°C for a minimum of 48 hours in ambient air. Tests should not be made with cultures incubated less than 48 hours, because the end products build up to detectable levels over time. If results are equivocal at 48 hours, repeat the tests with cultures incubated at 35°C to 37°C for 4 to 5 days in ambient air; in such instances, duplicate tests should be incubated at 25°C.
3. Split broth into aliquots for MR test and VP test.

A. VP (Voges-Proskauer) test

1. Add 0.6 mL (6 drops) of solution A (alpha-naphthol) and 0.2 mL (2 drops) of solution B (KOH) to 1 mL of MRVP broth.
2. Shake well after addition of each reagent.

3. Observe for 5 minutes

Reagents used:

\* Potassium Hydroxide

\*  $\alpha$ - naphthol

Expected results:

\* Positive: bright red color indicative of mixed acid fermentation \* Weakly positive: red/orange color

\* Negative: yellow color

Quality control:

\* Positive: *Enterobacter cloacae*

\* Negative: *Escherichia coli*

#### STARCH HYDROLYSIS TEST

Purpose: to identify *Bacillus* species

Principle:

Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including  $\alpha$ -amylase and oligo-1, 6-glucosidase, that hydrolyze starch. Starch molecules are too large to enter the bacterial cell, so some bacteria secrete exoenzymes to degrade starch into subunits that can then be utilized by the organism. Iodine is added to indicate color change occurs in the medium when organisms hydrolyze starch.

*Bacillus* species are known to produce the exoenzyme, amylase. Method:

The action of two bacterial species, *Bacillus subtilis* and *Escherichia coli*, is compared on starch agar. After inoculation in the shape of the corresponding bacterial name initials, EC for *E. coli* and BS for *B. subtilis*, the plates were incubated for 24 hours at 37°C. Iodine, which changes color from a yellow-brown to blue-black in the presence of starch, was applied to the agar

surface and allowed to stand for 10 minutes. If the species produces and releases amylase, starch hydrolysis in the agar should occur. Expected results:

- \* Positive: clear zone around bacterial growth
  - \* Negative: agar plate turned completely from blue to black
- Quality control:
- \* Positive: *Bacillus subtilis*
  - \* Negative: *Escherichia coli*

#### NITRATE REDUCTION TEST

Purpose: to differentiate *Bacillus* species from gram negative rods. Principle: The determination of nitrate reduction on the nitrate medium is used for the determination of the reduction of nitrate to nitrogen.

The basic premise of this test is that the N, N-dimethyl- $\alpha$ -naphthylamine reacts with nitrite imparting a red-violet color. The sulfanilic acid heightens the color response. If there is no color change upon addition of the two reagents, this means that this is a true negative reaction. If one uses a liquid medium with Durham tubes, the production of gas is positive for nitrate reduction to nitrogen. Method:

1. The test organism is inoculated into nitrate reduction broth, an undefined medium that contains large amounts of nitrate ( $\text{KNO}_3$ ).
2. Incubate up to 48 hours.
3. After incubation, alpha-naphthylamine and sulfanilic acid are added.

Reagents used:

- \* Sulfanilic acid reagent
- \* N, N-dimethyl- $\alpha$ -naphthylamine reagent

Expected results:

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\* Positive: red coloration

\* Negative: no color changes

Quality control:

\* Positive: Escherichia coli

\* Negative: Bacillus subtilis

#### REFERENCES:

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