# CYSTINE HEART AGAR w/ RABBIT BLOOD and ANTIBIOTICS

**INTENDED USE**
Remel Cystine Heart Agar w/ Rabbit Blood and Antibiotics is a solid medium recommended for use in qualitative procedures for the cultivation of *Francisella tularensis*.

**SUMMARY AND EXPLANATION**
Tularemia was described in 1911 as the cause of a plaguelike disease in rodents.[1] McCoy and Chapin were the first to grow the bacterium in their laboratory in Tulare County, California and named the organism *Bacterium tularense*. Edward Francis, while studying a plaguelike disease in humans, made the connection between the illness in humans and the disease described by McCoy in rodents.[2] Francis developed blood-dextrose-cystine agar after determining *F. tularensis* would only grow on an artificial medium supplemented with sulfhydryl compounds (i.e., cystine). To recognize the lifetime achievements of Francis in understanding the disease, the name of the organism was changed to *Francisella tularensis*. Shaw modified the method by which blood-dextrose-cystine agar was prepared, and produced a clear, sterile medium with no precipitate.[3] Rhamy further modified the medium to prevent contamination during preparation and reported satisfactory cultivation of *F. tularensis*.[4]

**PRINCIPLE**
Heart Infusion Agar with 1% dextrose and 0.1% cystine supports the growth of gram-negative cocci and other pathogenic microorganisms. When rabbit blood is incorporated in the medium as enrichment, the medium will also support the growth of *F. tularensis* from most clinical specimens. Penicillin inhibits gram-positive organisms and polymyxin B is inhibitory to most of the *Enterobacteriaceae*.

**REAGENTS (CLASSICAL FORMULA)***

| | | | |
|---|---|---|---|
| Casein Peptone | 13.0 g | L-Cystine | 1.0 g |
| Dextrose | 10.0 g | Penicillin | 100,000 U |
| Sodium Chloride | 5.0 g | Polymyxin B | 100,000 U |
| Yeast Extract | 5.0 g | Rabbit Blood | 5 % |
| Beef Heart Infusion | 2.0 g | Agar | 15.0 g |
| | | Demineralized Water | 1000.0 ml |

pH 6.8 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

**PRECAUTIONS**
*F. tularensis* is highly virulent and must be handled with extreme caution. Laboratory infections can be acquired through any manipulation in which aerosols or droplets are produced.[5] Handle clinical specimens suspected of containing *F. tularensis* following Biological Safety Level-2 (BSL-2) procedures. BSL-3 conditions are recommended for all culture manipulations as soon as *F. tularensis* is suspected.[6,7]

**PROCEDURE**
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If material is being cultured directly from a swab, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate the plates aerobically at 33-37°C for 2-4 days for maximum colony formation. Up to 2 weeks incubation may be required for some strains to develop visible colonies.
4. Examine plate for typical colony morphology. At 48 hours, *F. tularensis* colonies are 1-2 mm in diameter, white to grey to bluish-grey, opaque, flat, with an entire edge, smooth, and have a shiny surface. At 24 hours colonies are usually too small to be seen.

**Note:** Isolates suspected of being *F. tularensis* based on Gram stain and colonial morphology, weak-positive or negative catalase, positive β-lactamase test, and negative satellite test, should be sent to the local or state public health laboratory.[5] Do not attempt identification using a commercial identification system because of the potential of generating aerosols and high probability of misidentification.[7]

**QUALITY CONTROL**
All lot numbers of Cystine Heart Agar w/ Rabbit Blood and Antibiotics have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

| CONTROL | INCUBATION | RESULTS |
|---|---|---|
| *Francisella philomiragia* ATCC® 25017 | Ambient, up to 48 h @ 33-37°C | Growth |
| *Escherichia coli* ATCC® 25922 | Ambient, up to 48 h @ 33-37°C | Inhibition (partial to complete) |
| *Staphylococcus aureus* ATCC® 25923 | Ambient, up to 48 h @ 33-37°C | Inhibition (partial to complete) |

**BIBLIOGRAPHY**

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7. Morse, S.A. 2001. Basic Protocols for Level A Laboratories for the Presumptive Identification of *Francisella tularensis*. U.S. Dept. of H.H.S., CDC, Atlanta, GA.


Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.




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