

## **Citrate Agar Slant**

### **I. PRINCIPLE**

Some organisms have the ability to utilize citrate, an intermediate metabolite in the Krebs cycle, as the sole external source of carbon. These organisms also utilize inorganic ammonium salts in the medium as the sole source of nitrogen. The resulting production of ammonia creates an alkaline environment that turns the bromthymol blue indicator to an intense blue above pH 7.6.

### **II. REAGENTS AND MATERIALS**

- A. Simmons sodium citrate agar (store at 4°C until expiration date)
- B. Inoculating loop

### **III. QUALITY CONTROL**

- A. Quality control testing is not required. Citrate is listed as an exempt medium in CLSI document M22-A3 (2004).

### **IV. PROCEDURE**

- A. Select a single, well-isolated colony with an inoculating loop.
- B. Streak the surface of the citrate slant (do not stab the agar), and cap loosely.
- C. Incubate at 35°C in non-CO<sub>2</sub>, and examine daily for up to 4 days.

### **V. INTERPRETATION**

- A. Positive: growth with an intense blue color on the agar slant.
- B. Negative: no growth and no color change (green).

### **VI. LIMITATIONS**

- A. Luxuriant growth on the slant without an accompanying color change may indicate a positive test. This should be confirmed by incubating the tube for another 24 h.
- B. The biochemical reaction requires oxygen. Therefore, the medium should not be stabbed, and the cap must be kept loose during incubation.
- C. Carryover of protein and carbohydrate substrates from previous media may provide additional sources of carbon and, therefore, cause false-positive reactions. To avoid this, use only a light inoculum, and inoculate the citrate agar slant first prior to inoculating any other media.

- D. If the inoculum is too heavy, preformed organic compounds within the cell walls of dying bacteria may release sufficient carbon and nitrogen to produce a false-positive result.

## **VII. REFERENCES**

- A. BBL Quality Control and Product Information Manual for Tubed Media: Simmons Citrate Agar. BD Microbiology Systems, Cockeysville, MD. 2001.
- B. Isenberg, H.D. 1992. Clinical Microbiology Procedures Handbook. Vol 1. ASM, Washington, DC, p. 1.19.340-1.19.36.
- B. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn. 1992. Diagnostic Microbiology. 4th ed. J.B. Lippincott Company, Philadelphia, p. 4121-123, 177.

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Updates and Revisions: