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**SDA (Schwarz Differential Agar) Instructions**

**Derived from Siebel Institute of Technology**

Propose: Used for detection and enumeration of Bacteria and Yeast

1. Dilute the sample, if necessary, to obtain dilutions containing between 100 and 900 bacterial cells per ml.

**Example:** If pitching yeast (about 15% solids) contains about 0.1 % bacteria, perhaps 10% of the bacteria are viable. Hence the yeast will contain about  $3 \times 10^5$  live bacteria per ml. If diluted  $10^{-3}$ , a plate inoculated as directed below should show about 30 bacterial colonies. On the other hand, a pure or mixed bacterial culture, after full growth in a broth, should be diluted  $10^{-6}$  and or  $10^{-7}$  before testing. The aim should be to have about 25-50 colonies per spread plate.

2. Pipette 0.1 to 0.2 ml aliquots of the sample and/or its dilutions onto SDA/LMDA.
3. Rotate the petri dish and disperse the sample on the surface of the medium evenly with a disposable sterile cell spreader. Spread the sample near, but not onto the edges, of the plate.
4. Cover and invert the plates and incubate at 86 °F in an anaerobic environment to detect beer-spoiling bacteria. To detect yeast and wort spoiling bacteria, incubate aerobically.
5. Examine the plates after a 4 to 7 day incubation period. It may be possible to observe the growth of some bacterial colonies on SDA plates earlier, but the morphological characteristics of some bacterial colonies may not be completely developed. The differentiation may not be as easy or reliable as it would be after a 4 to 7 day incubation period.

If in doubt about identity, use the Gram stain, together with the test for catalase and/or oxidase to confirm identity of brewing bacteria.