

### Identification of Bacteria. III. Nitrate Reduction Test.

#### Introduction

The last of the various diagnostic tests one may perform to identify bacteria is the determination of bacterial nitrate ( $\text{NO}_3^-$ ) reduction on nitrate medium. This medium contains a small amount of  $\text{KNO}_3$  (potassium nitrate). Bacteria which reduce nitrate reduce it to either nitrite ( $\text{NO}_2^-$ ) or to gaseous nitrogen ( $\text{N}_2$ ). 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. Some examples of gram negative bacteria and their Nitrate Reduction on nitrate medium reaction are shown in Table 1.

Genus	Tryptic Nitrate Reduction Reaction
<i>Escherichia</i>	+ (red-violet)
<i>Serratia</i>	+
<i>Bacillus</i> *	-

**Table 1.** Tryptic Nitrate Reduction test reactions of several enteric bacteria.

**\* In this table, as well as in the tables in the previous experiments, *Bacillus* (Gram positive rod) is included for variety.**

The purpose of this experiment is to learn how to perform and interpret the Nitrate Reduction reaction for various bacteria on solid media.

## Materials and Methods

### *Materials*

Sulfanilic acid reagent	N,N-dimethyl- $\alpha$ -naphthylamine reagent	Paper towels
Nitrate media	Loop	Bunsen burner
Incubator	Bacteria	Disinfectant

### *Method*

Aseptically streak your Nitrate medium with one of the bacteria. Incubate for 48 hours. Interpret this experiment at your next lab period.

To the colonies, add, directly, one drop of each reagent. A red color is positive; no color change is negative. How do these results compare with the table above?

Draw and label your observations for your bacterium below:

### REFERENCES

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