Members of the genus *Neisseria* are small gram-negative diplococci with flattened adjacent sides. They sometimes give the appearance of two tiny beans lying face to face. The organisms grow on enriched media such as *blood agar* and *chocolate agar*, and produce oxidase, an enzyme that changes the color of a special reagent. There are several *Neisseria* species found in the human body. These are listed in **TABLE 43.1**. In this exercise, *Neisseria* species will be isolated from the throat and their properties observed.

**THE GENUS NEISSERIA**

**PURPOSE:** to identify *Neisseria* species isolated from the throat.
PROCEDURE

1. Blood agar plates are prepared according to the method explained in Exercise 41. Chocolate agar is prepared by heating a rich medium such as trypticase soy agar to 80°C for 10 minutes, and then adding defibrinated sheep blood to a 5% concentration. The heat lysed the red blood cells and releases the hemoglobin. The hemoglobin chars and causes the medium to become brown; hence, the name chocolate agar.

2. Select or prepare a plate of blood agar and/or one of chocolate agar. Label the bottom side of each plate with your name, the date, the name of the medium, and the designation “throat swab.”

3. Obtain a sterile cotton swab and a sterile tongue depressor.

   - Have a fellow student swab your pharynx (throat) according to the method outlined in Exercise 41.

   - Using a sterile loop, streak for isolated colonies as described in Exercises 5 and 41 (FIGURE 43.2).

   - Apply the bacteria on the swab to one small area of the agar plate by rubbing it gently.

   - Incubate the plate(s) at 37°C for 24 to 48 hours in the inverted position.

   - If a candle jar is available, it may be used as described in Exercise 41 to increase the CO₂ tension and encourage growth of the Neisseria species.

   - Wear gloves when adding the blood to the agar base to prepare blood or chocolate agar.

<table>
<thead>
<tr>
<th>TABLE 43.1</th>
<th>Neisseria Species Found in Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. gonorrhoeae</td>
<td></td>
</tr>
<tr>
<td>N. meningitidis</td>
<td></td>
</tr>
<tr>
<td>N. lactamica</td>
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<tr>
<td>N. sicca</td>
<td></td>
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<tr>
<td>N. subflava</td>
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<tr>
<td>N. mucosa</td>
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<tr>
<td>N. flavescens</td>
<td></td>
</tr>
<tr>
<td>N. cinerea</td>
<td></td>
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<tr>
<td>N. polysaccharea</td>
<td></td>
</tr>
<tr>
<td>N. elongata</td>
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</tr>
<tr>
<td>N. kochii</td>
<td></td>
</tr>
</tbody>
</table>
4. Observe the plate(s) for grayish white and light yellow colonies, which may be *Neisseria* species. Some colonies may be wrinkled, others mucoid in texture.

- To verify the presence of *Neisseria*, perform the oxidase test as follows (Watch Microbiology Video: Oxidase Test to see this test performed):
  - Place several drops of freshly prepared oxidase reagent (tetramethyl- *p*-phenylenediamine dihydrochloride) onto the colonies (FIGURE 43.3).
  - The oxidase present in *Neisseria* colonies will cause the colonies to become pink, then maroon, and finally blue-black. These changes should occur rapidly, and they will be complete within several minutes.
- Identify colonies of *Neisseria* and prepare representations of the plates in the appropriate space in the Results section.

**Oxidase Test**

**FIGURE 43.2** Streak the plate for isolated colonies.

**FIGURE 43.3** Place several drops of oxidase reagent onto the colonies.
5. Select samples of possible Neisseria species, and prepare air-dried, heat-fixed smears for Gram staining. Small gram-negative diplococci should be observed.

- Draw labeled representations in the Results section.
- If transfers to agar slants are to be made, these should be done immediately after the oxidase reagent has been added, since the reagent will kill the cells in the colonies.
- It should be noted that rod-shaped organisms of the genera Alcaligenes and Pseudomonas will also give a positive oxidase reaction if present on the plates.
- When observed with the light microscope, these bacteria will appear as gram-negative rods.
The Genus *Neisseria*

**EXERCISE RESULTS 43**

**RESULTS**

*NEISSERIA SPECIES FROM THE UPPER RESPIRATORY TRACT*

Stained Smears of *Neisseria* Species

*Chocolate Agar Plate*

*Blood Agar Plate*

Source: [Image of microscope view]

Magnif.: [Image of magnification levels]
Observations and Conclusions

Questions

1. Does the isolation of *Neisseria* species from the pharynx (throat) necessarily mean that a person has gonorrheal pharyngitis or another disease caused by *Neisseria*?

2. What does the word “chocolate” refer to in chocolate agar?

3. Which medium—nutrient agar, blood agar, or chocolate agar—might be expected to yield better growth of *Neisseria* species? Why?
4. Would the observation of oxidase-positive colonies on chocolate agar necessarily represent final proof that *Neisseria* colonies were present?

5. What is the microscopic appearance of Gram-stained cells of *Neisseria* species?