# EXERCISE 43

# The Genus *Neisseria*

## Materials and Equipment

- Chocolate agar plates
- Blood agar plates
- Candle jar (optional)
- Sterile cotton swabs and tongue depressors
- Oxidase reagent in dropper bottles
- Gram stain reagents

## Watch This Video

- Oxidase Test

The flora of the upper respiratory tract often includes a number of species of the genus ***Neisseria*** (see **FIGURE 43.1**). These include *N. sicca, N. mucosa*, and *N. subflava*. These organisms are structurally similar to *N. meningitidis*, the agent of meningococcal meningitis, and *N. gonorrhoeae*, the cause of gonorrhea. Another common inhabitant of the upper respiratory tract is *Moraxella (Branhamella) catarrhalis*, which also is a member of the family Neisseriaceae.

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.

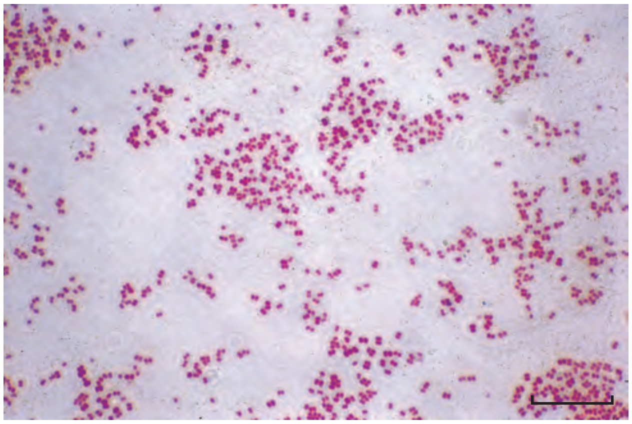


**FIGURE 43.1** Gram-negative diplococci typical of **Neisseria** species. (Bar = 10 μm.).

## THE GENUS *NEISSERIA*

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

| TABLE 43.1 *Neisseria* Species Found in Humans |
|---|
| *N. gonorrhoeae* |
| *N. meningitidis* |
| *N. lactamica* |
| *N. sicca* |
| *N. subflava* |
| *N. mucosa* |
| *N. flavescens* |
| *N. cinerea* |
| *N. polysaccharea* |
| *N. elongata* |
| *N. kochii* |

## PROCEDURE

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

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 lyses 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."
   - Obtain a sterile cotton swab and a sterile tongue depressor.
3. Have a fellow student swab your pharynx (throat) according to the method outlined in Exercise 41.
   - Apply the bacteria on the swab to one small area of the agar plate by rubbing it gently.
   - Perform the **streak plate technique**:
     - Using a sterile loop, streak for isolated colonies as described in Exercises 5 and 41 (**FIGURE 43.2**).
   - 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 $CO_2$ tension and encourage growth of the *Neisseria* species.

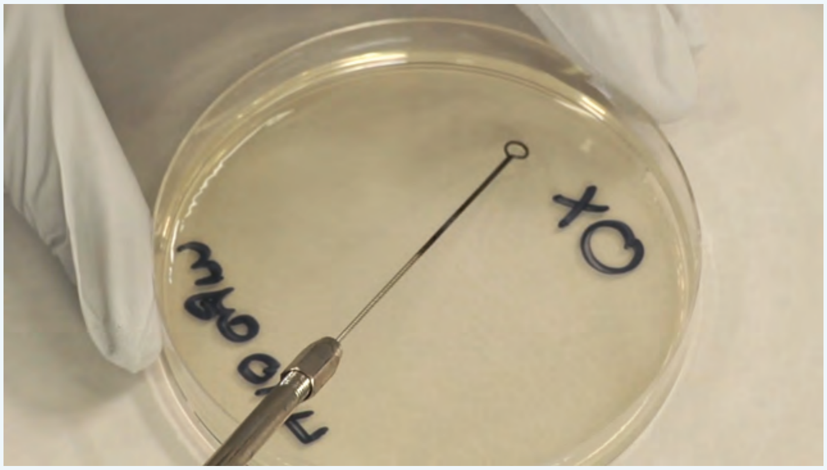


**FIGURE 43.2** Streak the plate for isolated colonies.

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 43.3** 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.

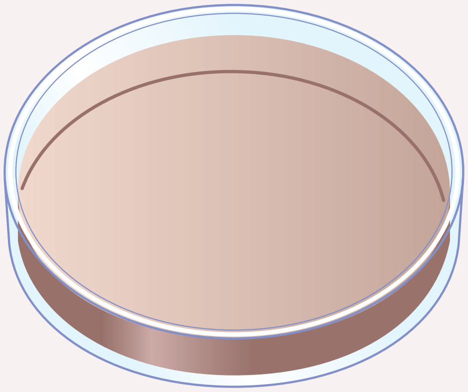
Name: ______________________ Date: __________ Section: __________

# The Genus *Neisseria*

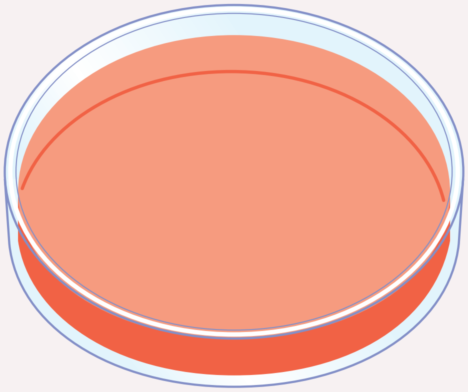
EXERCISE RESULTS 43

## RESULTS

### *NEISSERIA* SPECIES FROM THE UPPER RESPIRATORY TRACT



Chocolate Agar Plate



Blood Agar Plate

Stained Smears of *Neisseria* Species

Source: ______________ ______________ ______________

Magnif.: ______________ ______________ ______________

## 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?