

EXERCISE 13

Specimen Transport & Ubiquity of Microorganisms

OBJECTIVES

At the conclusion of the exercise, you should...

1. be able to collect a sample with a specimen transport swab.
2. know why specimen transport devices and media are used.
3. understand the purpose of RODAC plates.
4. observe how ubiquitous microbes are.
5. be able to discuss some of the microorganisms found in the environment.
6. perform and discuss the use of Quality control in evaluating Gram stains

INTRODUCTION

Quality Control (QC): As you have learned, one of the most important techniques used in microbiology is the Gram stain. In order to be sure that the Gram stain is performed correctly, you must include quality-control slides each time you stain an organism of unknown identity. Quality control is used for many techniques in the laboratory and in industry to make sure all reagents are working correctly and the techniques are performed accurately. You will prepare your own set of quality-control slides for Gram stains by following the technique described below.

MATERIALS

Cultures:

P. aeruginosa (Gram negative rod)
M. luteus (Gram positive coccus)

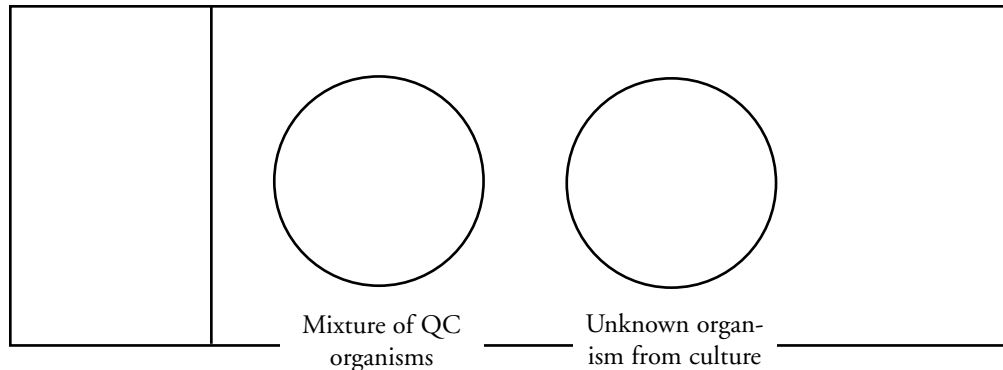
Supplies:

Slides
Gram stain reagents

PROCEDURES:

Day 1

1. Use the known Gram-negative and Gram-positive bacterial cultures provided:
P. aeruginosa and *M. luteus*
2. Label the frosted end of the slide (with the pencil) appropriately (see diagram below).
3. Draw two circles on the non-frosted side of the slide (underneath) with a Gram stain pen. (See diagram below)
4. One area is used for the two quality control organisms mixed together.
5. Follow the proper technique for smear preparation as described in Exercise 7; aseptically add a loopful of each QC organism to this area, mix well and allow to air dry.
6. Aseptically add a loopful of the unknown culture to the second circle (see diagram below).
7. Prepare at least 6 slides (up to 12) with the QC mixture and store for later use.
8. After air drying, heat fix one at a time immediately before staining.
9. Always observe your QC area first to be sure the results are reliable before evaluating the Gram stain of your unknown organism.



INTRODUCTION

Microorganisms are ubiquitous in nearly every area of the environment. Microbiologists have several tools at their disposal to collect and transport samples to the laboratory to quantify and identify them. In this exercise, you will be introduced to two of these tools and practice the proper collection and evaluation of microbiological samples.

Materials

Media:

1 TSA plate

Supplies:

1 Transport swab

1 Rodac plate

A. SWAB

PROCEDURES

Day 1 (collection)

Technical Background

In order to perform laboratory tests on specimens that are collected outside the laboratory, they must be collected and transported properly. It is essential that the container bearing a specimen does not contribute its own **microbial flora**. Also, the original flora should neither multiply nor decrease because of prolonged standing on a hospital ward or in the field.

A variety of containers have been devised for collecting bacteriologic specimens. The most commonly used is a cotton- or Dacron-tipped applicator stick. These must be sterile and remain sterile before specimen collection. One approach uses a sterile disposable culture unit (Culturette, Becton-Dickinson Microbiology Systems), consisting of a plastic tube containing a sterile polyester-tipped **swab** and a small glass ampule of holding medium. This medium maintains a favorable pH and prevents both dehydration of secretions during transport and oxidation and enzymatic self-destruction of any pathogens present. There are other versions of this type of transport media, such as a gel in the bottom of the tube, or a separate test tube into which the specimen swab is inserted.

The unit is removed from its sterile envelope, and the swab is used to collect the specimen. It is then returned to the tube, the ampule is crushed (if there is one), and the swab is forced into the released holding medium. This will provide sufficient moisture for storage up to 72 hours at room temperature. After the specimen arrives in the laboratory, the swab can be removed and used to inoculate the appropriate media.

A variety of transport media is available for prolonging the survival of microorganisms when a significant delay occurs between collection and culturing. Special media is needed for specific types of specimens. One example is for anaerobic specimen transport.

Collecting and Transporting a Specimen

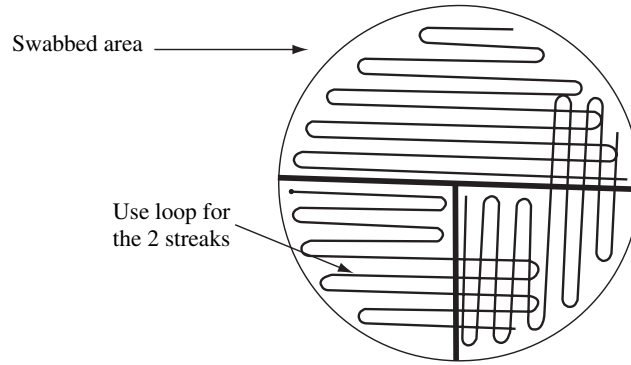
1. Follow the directions on the package to open and use the swab.
2. Use the specimen swab to collect a specimen from somewhere away from the laboratory. Some places to collect samples from home include the following: kitchen, bathroom, bed linens, washer, dryer. Some items that could be sampled include toothbrush bristles, hair brush, cutting board, sink handle, TV remote. Be creative. Because of the danger from highly resistant organisms, if you work or volunteer in a health care setting, please do not collect a sample from this location.
3. Follow directions on the package for transporting the swab back to the laboratory.

PROCEDURES

Day 2 (Inoculation)

Preparing a Streak Plate for Your Specimen

1. Use the specimen swab to streak back and forth onto one third of the TSA plate (see diagram).
2. Flame and cool a loop to streak for isolation from the primary streak area. Streak the Secondary inoculation area by going back into the primary area with your loop only once or twice. Streak this area covering as much agar as possible without overlapping the previous streaks. Flame and cool your loop.
3. Continue streaking the third inoculation by passing into the secondary area once or twice and then streaking out to the end of the third inoculation area. Flame your loop.
4. Incubate the TSA plate upside down at 30°C until the next lab period.



PROCEDURES

Day 3 (evaluation)

Evaluating Your Results

1. If there is a lot of fungus growing on the plate, DO NOT OPEN IT.
2. Use the stereoscope to observe the colonies.
3. Count the total number of colonies obtained and the number of different appearing colonies.
3. Describe the colonies in the table in the evaluation portion of the Data section. Repeat for your lab partner's sample.
4. Using the prepared QC slides from the previous section, Gram stain one or two of the colonies that are not fungus.
5. Record your results in the evaluation portion of the Data section.

B. RODAC PLATES

PROCEDURES

Day 1 (collection and inoculation)

Technical Background

RODAC = Replicate Organism Detection and Counting

RODAC plates are used for the detection and enumeration of microorganisms present on surfaces of sanitary importance. The plates are specially constructed so that the agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial content. After touching the surface to be sampled with the medium, the dish is covered and incubated and kept at room temperature until it is brought to the lab. It then can be incubated at 30°C or 35°C, depending on the organisms that are being looked for. The presence and number of microorganisms is detected by the appearance of colonies on the surface of the agar. Collection of samples before and after cleaning and treatment with a disinfectant permits the evaluation of the efficacy of sanitary procedures. We will use these as another example of transporting specimens to the lab and for demonstrating the ubiquity of microorganisms. Assigned students will take one plate home and use it to touch an area of their choice—either with or without cleaning. Students are encouraged to be creative when they decide to touch the plate. Some examples of places to touch include the refrigerator, kitchen counter, bathroom sink, bowl, etc. Another application is to do a before and after sampling using disinfectant on the lab bench. The media used for these plates consists of a general-purpose medium with some other ingredients to select for certain types of bacteria. The grid on the plate serves as an aid in counting the colonies. Below are the general guidelines for evaluating the number of colonies per plate when testing for sanitary conditions immediately after cleaning:

Colonies per RODAC Plate

GOOD = 0-25

FAIR = 26-50

POOR = 50 and over

Collection and Inoculation of a RODAC Sample

1. Choose an area to which you will touch the RODAC plate. Some examples are a kitchen cutting board or counter, shower, or bathtub floor.
2. Lightly press the plate to the chosen surface.
3. Label the plate.
4. Tape it closed.
5. Incubate the plate at 30°C until the next lab period.

PROCEDURES

Day 2 (evaluation)

Evaluating Your RODAC Plate Culture

1. Examine the RODAC plate for the number of colonies.
2. If there is no fungus growing, open the plate to examine the colony types.
3. (Optional). Using the prepared QC slides from the previous section, Gram stain one or two of the colonies that are not fungus.
4. Record your results as instructed for the transport swab on the table in the evaluation portion of the Data section.

CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. Discuss the results obtained from the different collection sites (how many types of organisms isolated, total quantity, etc).
2. What can be concluded about microbes in the environment?
3. In what areas of a food production plant might the RODAC plate be used?
4. Give some examples of where specimen transport media would be used in a hospital.

