# Rapid Diagnostic Methods in Medical Microbiology

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with 31 Contributors



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# Foreword

Nosocomial infection, of prime importance to surgeons in the sixties, looms ever more formidable for the ensuing decade because of the increased use of immunosuppressive agents to assist in allogeneic transplantation. Thus it becomes more important than ever to diagnose rapidly such antibiotic refractory microorganisms as "white" Serratia, the pseudomonads, *Pneumocystics carinii* and the opportunistic fungi, organisms bred and fostered by antimicrobials and all destined seemingly to undo surgical and medical heroics in behalf of the patient.

This volume is timely. Rapid methodology has always been the demand of the clinician. The contributors are all "hard-core" bench microbiologists, some of whom have worked very productively with me in the field of burn research. They represent the best of our time. Their methods are dependable and reproducible. The book should bring to the physician and his supporting scientists new and proven techniques for easier and quicker diagnoses.

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# Preface

The last chapter of this book reports current attempts by microbiologists to institute rapid diagnostic methods by using instrumentation in medical microbiology. It seems likely that quantitation of microorganisms, if not already achieved, will soon be successful, but species identification remains a difficult, unyielding Gordian knot. Some of the methods for genera delimitation (gas chromatography, infrared, etc.) are available today, but time presently consumed in their use hardly warrants calling such procedures rapid.

For years, most of the contributors to this book have known of methods, having little to do with instruments, which permitted faster reporting of results to the clinician. This book represents a distillate of such methods compiled by individuals who continue to spend much time at the laboratory bench.

No claim is made that for every microorganism discussed here that a rapid method is recorded. In some cases, examination of the "state of the art" for genera and species identification is the sole contribution. Admittedly, none of us knows how to grow *Treponema pallidum* rapidly, or at all, or to speed up *Mycobacterium tuberculosis* cultivation or to shorten lead time on viral reports.

This book is written, however, to advise the physician that he need not and should not wait for a week to be told a patient has bacteriologic evidence of a *Salmonella typhi* sepsis, and to spur the technician into trying some of these methods. The caveat that rapid methodology is synonymous with inaccuracy simply is not true. Most statements to this effect emanate from microbiologists who have never worked in a hospital laboratory.

Today it is also becoming apparent that the young physician of the 1970's will know less and less about microbiologic diagnostic testing. New core curricula used in most medical schools have fostered this trend by virtually eliminating laboratory exercises in microbiology, thereby assuring a lack of understanding of conventional testing and creating impatience on the part of the physician.

Perhaps an interim solution to speed up things microbiologic while we await the sophistication of instrumentation to solve our problem is to do what the English have been doing.

In England in recent years, the Society for Applied Bacteriology has scheduled each fall at their meetings a demonstration of newer methods and techniques. These methods are published as the *Proceedings of the Society of General Microbiology*. Publication of such methods, not always concerned with rapid methodology, has led to updating of techniques.

It is suggested that the American Society for Microbiology, through its subcommittee on diagnostic microbiology, undertake a similar forum at the annual ASM meeting and publish the proceedings. This would simplify the compilation attempted by this book.

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# Contents

# Section One

# BACTERIOLOGY

# 1

# The Staphylococci and the Streptococci

JAY O. COHEN*

## LABORATORY IDENTIFICATION OF STAPHYLOCOCCI AND STREPTOCOCCI

The staphylococci and streptococci are among the most frequently encountered microorganisms in the clinical bacteriology laboratory. These two groups include important pathogens and microorganisms commonly found on man and in his environment. Although these groups have some superficial resemblances, the two genera can be distinguished readily. The most important pathogens are *Staphylococcus aureus* and *Streptococcus pyogenes*. *S. aureus* is the causative agent of many skin infections, such as boils and furuncles, and is responsible for such serious infections as osteomyelitis, a form of pneumonia and septicemia. The Group A streptococci (*S. pyogenes*) cause streptococcal throat, impetigo and other infections. Both rheumatic fever and acute glomerulonephritis have been shown to be sequelae of streptococcal infection.

Aside from the acute diseases caused by *S. aureus* and *S pyogenes*, chronic infections resulting from other streptococci, such as the viridans and enterococcal groups, are common. *Staphylococcus epidermidis*, usually thought of as nonpathogenic, has been shown to cause some cases of subacute endocarditis. When the laboratory reports its results, the source of the microorganism is important in judging its significance. In the past,

* This chapter was written by Jay O. Cohen in his private capacity. No official support or endorsement by the Public Health Service or Department of Health, Education, and Welfare is intended or should be inferred.

errors have been made when the laboratory personnel assumed certain microorganisms to be chance contaminants when in reality they were from infectious processes.

Gram positive cocci belonging to both streptococcal and staphylococcal groups can be readily isolated from blood agar plates and cultured in broth such as thioglycolate broth. Selective media for isolating staphylococci from highly contaminated specimens such as food or feces have been developed which contain as much as 7.5% NaCl. The *Staphylococcus* is salt tolerant. Two such media are mannitol salt agar and staphylococcus 110 agar. Even when a selective medium is used for the isolation of staphylococci, some streptococci, notably the Group D enterococci, may grow out and be temporarily confused with staphylococci. The first task then is to distinguish these two genera.

### Colonial Appearance on Blood Agar Surfaces

The general appearance of the colonies provides a frequent clue to their identification as staphylococci or streptococci. This is not reliable in itself and should be followed by other tests and procedures. Both staphylococci and streptococci may be hemolytic or nonhemolytic. Staphylococcal colonies are usually larger and more raised than are streptococcal colonies. Pigmentation is not readily apparent on blood agar, and superficially both may appear to be white or gray. The fact that the colony contains Gram positive cocci should be determined by the Gram stain. Whether the microorganism grows in chains or clumps cannot be ascertained by staining material from colonies on agar.

## THE GRAM STAIN (HUCKER MODIFICATION)[4]

Ammonium Oxalate Crystal Violet

| *Solution A* | | *Solution B* | |
|---|---|---|---|
| Crystal violet (90% dye content), 2 g | | Ammonium oxalate | 0.8 g |
| Ethyl alcohol, 20 ml | | Distilled water | 80 ml |

Mix Solutions A and B.

Gram's Modification of Lugol's Solution

| | |
|---|---|
| Iodine | 1 g |
| Potassium iodide | 2 g |
| Distilled water | 300 ml |

Counterstain

| | |
|---|---|
| Safranin O (2.5% solution in 95% ethyl alcohol) | 10 ml |
| Distilled water | 100 ml |

*Staining Schedule*

1. Stain smears 1 minute with ammonium oxalate crystal violet.
2. Wash with tap water, gently so as not to wash off the smear.

3. Immerse in or flood with iodine solution.
4. Wash off carefully and gently blot away excess water.
5. Decolorize with gentle agitation in 95% ethyl alcohol, about 15 seconds, but stop decolorizing when no further leaching of violet color by the alcohol appears to be taking place.
6. Counterstain for about 20 seconds with safranin solution.
7. Wash gently in tap water, then dry and examine. Do not blot roughly.

When the slide is examined under the oil immersion lens of the microscope, Gram positive organisms will appear violet, and Gram negative ones will appear red. Occasionally, because of possible mistakes in the procedure or because of the age or metabolic condition of the culture, a Gram negative result will be obtained for strains that are actually Gram positive. If there is reason to doubt the result, the stain should be repeated with a new smear from a fresh culture. From the above test, one can determine whether Gram positive cocci are present. If the test is made from a broth culture, some streptococci will appear in chains, whereas staphylococci will appear as pairs and clumps. This gives only a presumptive differentiation, because some strains of streptococci do not form well defined chains. There is a simple cultural test that will differentiate the two groups. This is the catalase test.

### Catalase Test

Add several drops of fresh 3% $H_2O_2$ to a plain agar slant or plain broth culture of the organism to be tested. Observe for the liberation of bubbles or foaming. If bubbles are formed, the microorganism is catalase positive. Staphylococci and micrococci are catalase positive; streptococci are catalase negative. If there is an initial question as to whether a microorganism is a staphylococcus or streptococcus, this test should resolve the question.

Warning: one should not use 30% $H_2O_2$ as it may foam out of the tube and create a contamination hazard.

The catalase test should always be performed on an extra slant or broth of the culture, since the hydrogen peroxide will render the culture unsuitable for further work and probably will eventually kill the microorganisms in the culture. Media containing any body fluids will produce false results.

## THE STAPHYLOCOCCI

Once it is determined whether the microorganism is a *Staphylococcus* or *Micrococcus,* the next step is to find out if it is coagulase positive or negative. The more pathogenic *S. aureus* is coagulase positive. There are two tests currently being used: first, the clumping factor test which is presumptive for coagulase positivity, and second, the tube coagulase test.

*Slide Test for Clumping Factor*[3]

1. On a slide, emulsify the microorganisms from a single *Staphylococcus*-like colony or from a loopful of growth from a slant in a small drop of 0.85% saline. The suspension should be quite heavy.

2. With an inoculating loop, mix into the emulsion a small drop of coagulase plasma.

The slide should be read within 5 seconds. A distinctly cheese-like clump is positive.

Most coagulase positive staphylococci are clumping factor positive. If the test is negative, the tube coagulase test should be run to determine whether the strain may be coagulase positive but clumping factor negative. The clumping factor test is useful for screening large numbers of cultures. If a coccus is positive for clumping factor, one may assume that it will be coagulase positive.

*Coagulase Test*

Rabbit plasma should be used. Coagulase plasma which has been standardized is commercially available.[1] The coagulase plasma is dispensed in 0.5 ml amounts to sterile 12 × 75 mm tubes. Using a capillary pipette, add 2 drops of a broth culture to 0.5 ml of coagulase plasma. Incubate at 37 C for 3 hours. Examine for clotting. Often the entire coagulase plasma tube will be clotted with the consistency of hardened gelatin. However, any degree of clotting is considered positive.

Usually, suspected staphylococci are reported as coagulase positive or negative. The coagulase negative, catalase positive cultures may be either *S. epidermidis* or *Micrococcus* species. These may be distinguished by testing for the fermentation of glucose anaerobically.

*Anaerobic Fermentation of Glucose*[6]

A tube of glucose containing nutrient broth with phenol red or bromcresol purple indicator is inoculated. The broth is overlaid with 2 or 3 ml of sterile mineral oil or sterile liquid petrolatum to set up anaerobic conditions. Fermentation of glucose is a property of staphylococci. Negative cultures are probably micrococci. Coagulase negative staphylococci have been isolated from blood samples, and they occasionally cause subacute endocarditis. Micrococci are likely to be chance contaminants, but *S. epidermidis* is an occasional pathogen.

Other special media have been devised for isolating or identifying certain staphylococci. A medium containing sodium tellurite has been used, but this is not more selective than simpler media such as trypticase soy agar and blood agar. Some staphylococci are lipolytic. These have been identified by special media such as egg yolk agar. Such differentiation is not well coordinated with pathogenicity. Not all coagulase positive

staphylococci clear egg yolk agar, and this medium is useful for special studies only.

Staphylococcal cultures on agar plates and slants will often remain viable for several months at room temperature. When such cultures are isolated from infections and personnel of a hospital, it is advantageous to hold them in the laboratory for several months. If evidence of an epidemic should become apparent, the cultures should be submitted to the state health department or to another available laboratory for phage typing. The typing results might be invaluable to the hospital authorities for understanding and combating the epidemic.

## THE STREPTOCOCCI

The catalase negative, Gram positive cocci are streptococci. They may be isolated from throat cultures, blood specimens, and various exudates of humans. Streptococci recovered from the blood are important regardless of which group or which type of hemolysis is seen. From throat cultures, positive cultures should be beta hemolytic Group A.

The streptococci are identified first according to the type of hemolysis seen on sheep blood agar, then according to the Lancefield group to which they belong.[7] Streptococci belonging to Group A can be further identified according to type either by the Lancefield system of M protein precipitin reactions[10] or by the Griffith system of T agglutination.[5] Usually typing is done at a central public health laboratory. Cultures are forwarded to such laboratories when this information is needed. The clinical laboratory should be prepared to identify the type of hemolysis, whether it is probably Group A, and if possible to which of the Lancefield groups the culture belongs.

Group A streptococci are responsible for pyogenic infections in man, such as streptococcal throat, impetigo and boils. Rheumatic fever and glomerulonephritis are both sequelae to Group A infection. Group B streptococci cause mastitis in cattle, and certain types are responsible for puerperal fever in man and infections of the newborn. Group D streptococci and other alpha hemolytic streptococci and nonhemolytic streptococci have been isolated from the blood and shown to cause septicemia and subacute endocarditis. All streptococci, whether Group A or not, or whether beta hemolytic or not, should be reported, unless they are isolated from throat swabs. From throat swabs, only Group A beta hemolytic streptococci are reported because many of the other species of streptococci are found often in normal throats.

### Determination of Hemolysis

The streptococci are subdivided according to their reaction on sheep blood cells. This is best seen in deep colonies in blood agar pour plates.