Bacterial Identification

Staining:

How to make smears

Smears should be spread evenly covering an area of about 15–20 mm diameter on a slide.

● *Purulent specimen*: Using a sterile wire loop, make a thin preparation. Do not centrifuge a purulent fluid, e.g. c.s.f. containing pus cells.

● *Non-purulent fluid specimen*: Centrifuge the fluid and make a smear from a drop of the well-mixed sediment.

● *Culture*: Emulsify a colony in sterile distilled water and make a *thin* preparation on a slide. When a broth culture, transfer a loopful to a slide and make a *thin* preparation.

● *Sputum*: Use a piece of clean stick to transfer and spread purulent and caseous material on a slide. Soak the stick in a phenol or hypochlorite disinfectant before discarding it.

● *Swabs*: Roll the swab on a slide. This is particularly important when looking for intracellular bacteria such as *N. gonorrhoeae*(urethral, cervical, or eye swab). Rolling the swab avoids damaging the pus cells.

● *Faeces*: Use a piece of clean stick to transfer pus and mucus to a slide. Decontaminate the stick before discarding it. Spread to make a thin preparation.

Drying and fixing smears

The purpose of fixation is to preserve microorganisms and to prevent smears being washed from slides during staining. Smears are fixed by heat, alcohol, or occasionally by other chemicals.

Heat fixation

This is widely used but can damage organisms and alter their staining reactions especially when excessive heat is used. Heat fixation also damages leucocytes and is therefore unsuitable for fixing smears which may contain intracellular organisms such as *N. gonorrhoeae*and *N. meningitidis*.

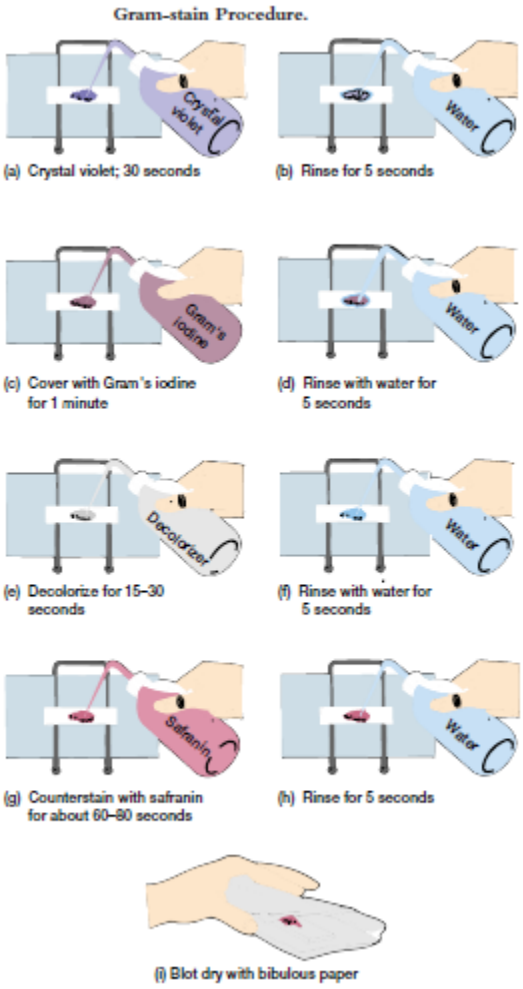
Alcohol fixation

This form of fixation is far less damaging to microorganisms than heat. Cells, especially pus cells, are also well preserved. Alcohol fixation is therefore recommended for fixing smears when looking for Gram negative intracellular diplococci. Alcohol fixation is more bactericidal

than heat (e.g. *M. tuberculosis*is rapidly killed in sputum smears after applying 70% v/v alcohol).

Other chemical fixatives

Other chemicals are sometimes necessary to fix smears which contain particularly dangerous organisms to ensure all the organisms are killed, e.g. 40 g/l potassium permanganate is recommended for fixing smears which may contain anthrax bacilli.



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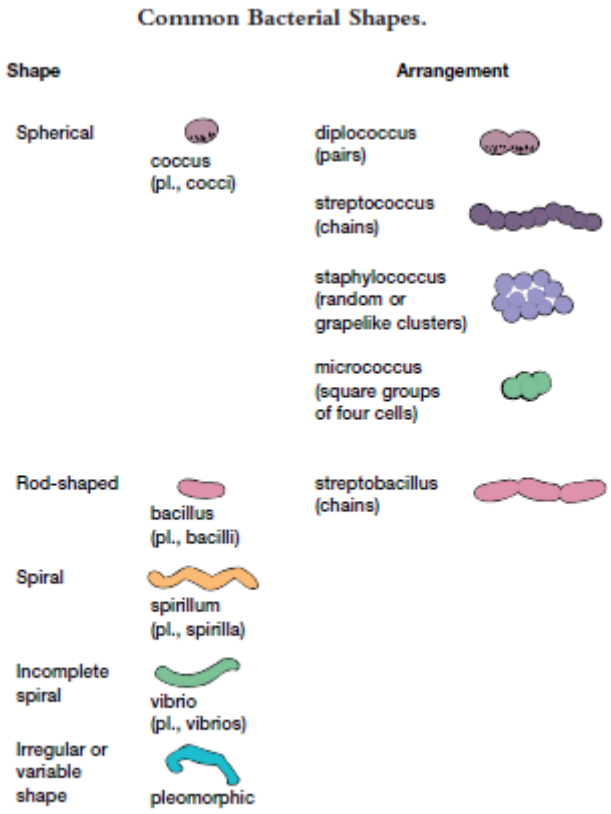
Indistinct Gram-stain results can be confirmed by a simple test using KOH. Place a drop of 10% KOH on a clean glass slide and mix with a loopful of bacterial paste. Wait 30 seconds, then pull the loop slowly through the suspension and up and away from the slide. A gram-negative organism will produce a mucoid string; a gram-positive organism remains fluid.



Q1: Name the reagent used and state the purpose of each of the following in the Gram stain:

a. mordant
b. primary stain
c. decolorizer
d. counterstain

Q2: Which step is the most crucial or most likely to cause poor results in the Gram stain? Why?

Q3: Why must young cultures be used when doing a Gram stain?

Q4: What is meant by gram variable?

Q5: What part of the bacterial cell is most involved with Gram staining, and why?

Culturing:
The purpose of using cultural techniques in microbiology is to demonstrate the presence of organisms which may be causing disease,

and when indicated, to test the susceptibility of pathogens to antimicrobial agents.

Types of Media

For a culture medium to be successful in growing the pathogen sought it must provide all essential nutrients, ions, and moisture, maintain the correct pH and osmotic pressure, and neutralize any toxic materials produced.

It is also essential to incubate the inoculated medium in the correct atmosphere, at the optimum temperature and for an adequate period.

The main types of culture media are:

- Basic
- Enriched
- Selective
- Indicator
- Transport
- Identification

Basic media: These are simple media such as nutrient agar and nutrient broth that will support the growth of microorganisms
that do not have special nutritional requirements.

Enriched media: Enriched media are required for the growth of organisms with exacting growth requirements such as *H. influenzae*, *Neisseria* species, and some *Streptococcus* species . Basic media may be enriched with whole or lyzed blood, serum, peptones, yeast extract, vitamins and other growth factors. An enriched medium increases the numbers of a pathogen by containing all the necessary ingredients to promote its growth. Such a medium is often used for specimens collected from sites which are normally sterile to ensure the rapid multiplication of a pathogen which may be present only in small numbers.

Enrichment media: This term is usually applied to fluid selective media which contain substances that inhibit the growth of unwanted organisms.

Selective media: These are solid media which contain substances (e.g. bile salts or other chemicals, dyes, antibiotics) which inhibit the growth of one organism to allow the growth of another to be more clearly demonstrated. A selective medium is used when culturing a specimen from a site having a normal microbial flora to prevent unwanted contaminants overgrowing a pathogen. Media made selective by incorporating antibiotics are usually expensive.

Other ways to select organisms
Incubation conditions may be used to select organisms, e.g. *P. aeruginosa* is inhibited by anaerobic conditions. Also the pH of a medium may make it selective for a particular organism, e.g. *V. cholera* can be isolated on an alkaline medium such as TCBS agar. Temperature may also help to select an organism e.g. *Listeria monocytogenes* can grow at 4 C whereas other organisms are inhibited. Growth, however, is slow.

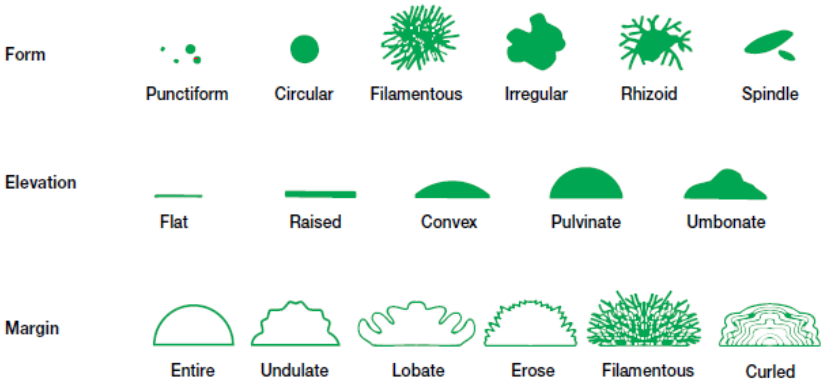
Indicator (differential) media: These are media to which dyes or other substances are added to differentiate microorganisms.
Many differential media distinguish between bacteria by incorporating an indicator which changes colour when acid is produced following fermentation of a specific carbohydrate e.g. MacConkey agar.

Transport media: These are mostly semisolid media that contain ingredients to prevent the overgrowth of commensals and ensure the survival of aerobic and anaerobic pathogens when specimens cannot be cultured immediately after collection. Their use is particularly important when transporting microbiological specimens from health centres to the district microbiology laboratory or specimens to the Regional Public Health Laboratory. Examples of transport media include Cary-Blair medium for preserving enteric pathogens.

Identification media: These include media to which substrates or chemicals are added to help identify bacteria isolated on primary cultures. Examples include peptone water sugars, urea broth, and Kligler iron agar. Organisms are mainly identified by a change in the colour of the medium and or the production of gas. Organisms used to inoculate identification media must be first isolated in pure culture.

Culture media can be classified by consistency as:
- Solid
- Semi-solid
- Fluid

Bacterial Colony Characteristics on Agar Media as Seen with the Naked Eye. The characteristics of bacterial colonies are described using the following terms.



Appearance: Shiny or dull
Optical property: Opaque, translucent, transparent
Pigmentation: Pigmented (purple, red, yellow)
Nonpigmented (cream, tan, white)
Texture: Rough or smooth