Culture System

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- Growth and identification of the infecting agent in vitro is usually the most sensitive and specific means of diagnosis and is thus the method most commonly used.
- Theoretically, the presence of a single live organism in the specimen can yield a positive result.
- Most bacteria and fungi can be grown in a variety of artificial media, but strict intracellular microorganisms (eg, *Chlamydia*, *Rickettsia*, and viruses) can be isolated only in cultures of living eukaryotic cells.

Isolation and Identification of Bacteria and Fungi

- A single bacterium placed in the proper culture conditions multiplies to quantities sufficient to be seen by the naked eye.
- Bacteriologic media are soup-like recipes prepared from digests of animal or vegetable protein supplemented with nutrients such as glucose, yeast extract, serum, or blood to meet the metabolic requirements of the organism.

Broth and Agar Media

- Growth in media prepared in the fluid state (broths) is apparent when bacterial numbers are sufficient to produce turbidity or macroscopic clumps. Turbidity results from reflection of transmitted light by the bacteria; depending on the size of the organism, more than 10^6 bacteria per milliliter of broth are required.
- The addition of a gelling agent to a broth medium allows its preparation in solid form as plates in Petri dishes. The universal gelling agent for diagnostic bacteriology is **agar,** a polysaccharide extracted from seaweed. Agar has the convenient property of becoming liquid at about 95°C but not returning to the solid gel state until cooled to less than 50°C.

Culture Media According to Purpose

Nutrient Media

- The nutrient component of a medium is designed to satisfy the growth requirements of the organism to permit isolation and propagation.
- These media are prepared with enzymatic or acid digests of animal or plant products such as muscle, milk, or beans.
- To this nutrient base, salts, vitamins, or body fluids such as serum may be added to provide pathogens with the conditions needed for optimum growth.

Culture Media According to Purpose

Selective Media

- Selective media are used when specific pathogenic organisms are sought in sites with an extensive normal flora.
- Selective media usually contain dyes, other chemical additives, or antimicrobics at concentrations designed to inhibit contaminating flora but not the suspected pathogen.

Culture Media According to Purpose

Indicator Media

- Indicator media contain substances designed to demonstrate biochemical or other features characteristic of specific pathogens or organism groups.
- The addition to the medium of one or more carbohydrates and a **pH indicator** is frequently used. A color change in a colony indicates the presence of acid products and thus of fermentation or oxidation of the carbohydrate by the organism.
- The addition of red blood cells (RBCs) to plates allows the **hemolysis** produced by some organisms to be used as a differential feature.

Atmospheric Conditions

Aerobic

- Most bacteria that are not obligate anaerobes grow in air; however, CO_2 is required by some and enhances the growth of others. Incubators that maintain a 2% to 5% concentration of CO_2 in air are frequently used for primary isolation, because this level is not harmful to any bacteria and improves isolation of some.
- A simpler method is the candle jar, in which a lighted candle is allowed to burn to extinction in a sealed jar containing plates. This method adds 1% to 2% CO_2 to the atmosphere.

Anaerobic

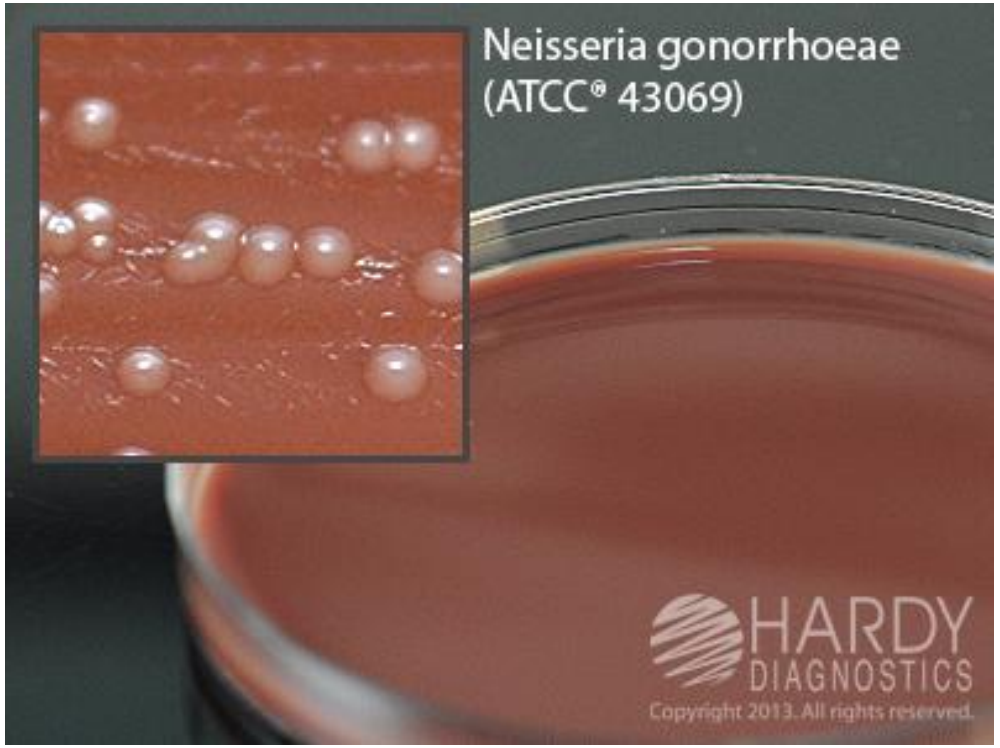
- Strictly anaerobic bacteria do not grow under the conditions just described, and many die when exposed to atmospheric oxygen or high oxidation–reduction potentials.
- Most medically important anaerobes grow in the depths of liquid or semisolid media containing any of a variety of **reducing agents,** such as cysteine, thioglycollate, ascorbic acid, or even iron filings.
- An anaerobic environment for incubation of plates can be achieved by replacing air with a gas mixture containing hydrogen, CO_2, and nitrogen and allowing the hydrogen to react with residual oxygen on a catalyst to form water.

Nutrient broths. Some form of nutrient broth is used for culture of all direct tissue or fluid samples from sites that are normally sterile to obtain the maximum culture sensitivity. Selective or indicator agents are omitted to prevent inhibition of more fastidious organisms.

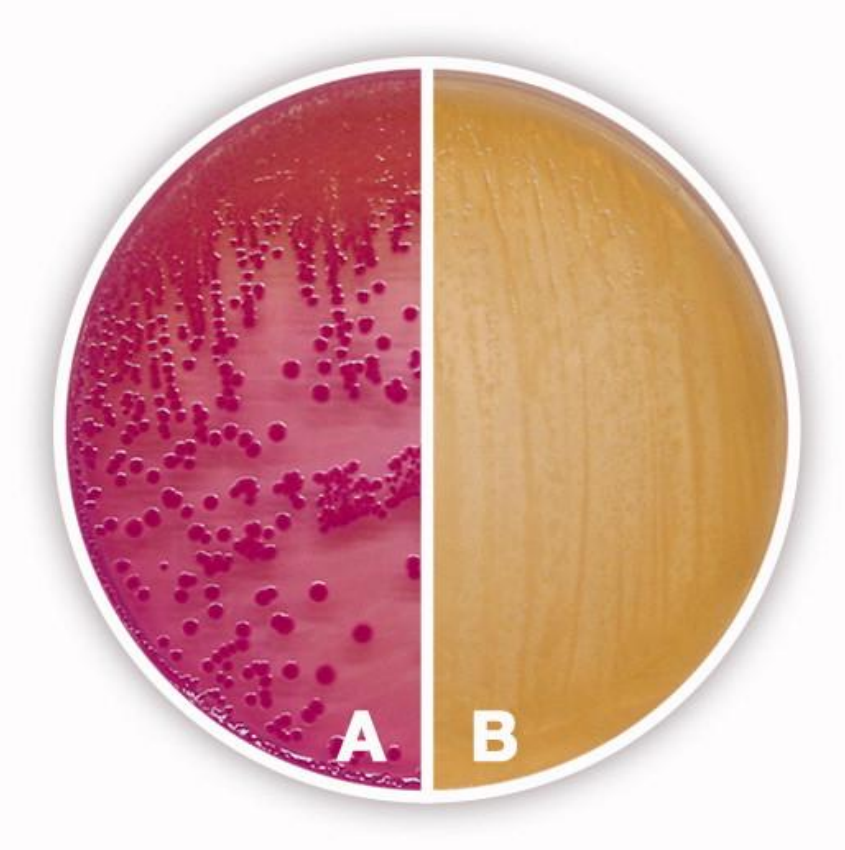
Blood agar. The addition of defibrinated blood to a nutrient agar base enhances the growth of some bacteria, such as streptococci. This often yields distinctive colonies and provides an indicator system for hemolysis. Two major types of hemolysis are seen: β-hemolysis, a complete clearing of red cells from a zone surrounding the colony; and α-hemolysis, which is incomplete (ie, intact red cells are still present in the hemolytic zone), but shows a green color caused by hemoglobin breakdown products. The net effect is a hazy green zone extending 1 to 2 mm beyond the colony. A third type, γ-hemolysis, produces a hazy, incomplete hemolytic zone similar to that caused by α-hemolysis, but without the green coloration.

Chocolate agar. If blood is added to molten nutrient agar at about 80°C and maintained at this temperature, the red cells are gently lysed, hemoglobin products are released, and the medium turns a chocolate brown color. The nutrients released permit the growth of some fastidious organisms such as *Haemophilus influenzae,* which fail to grow on blood or nutrient agars. This quality is particularly pronounced when the medium is further enriched with vitamin supplements. Given the same incubation conditions, any organism that grows on blood agar also grows on chocolate agar.

Martin–Lewis medium. A variant of chocolate agar, Martin–Lewis medium is a solid medium selective for the pathogenic *Neisseria* (*N gonorrhoeae* and *N meningitidis*). Growth of most other bacteria and fungi in the genital or respiratory flora is inhibited by the addition of antimicrobics. One formulation includes vancomycin, colistin, trimethoprim, and anisomycin.



MacConkey agar. This agar is both a selective and an indicator medium for Gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas*. In addition to a peptone base, the medium contains bile salts, crystal violet, lactose, and neutral red as a pH indicator. The bile salts and crystal violet inhibit Gram-positive bacteria and the more fastidious Gram-negative organisms, such as *Neisseria* and *Pasteurella*. Gram-negative rods that grow and ferment lactose produce a red (acid) colony, often with a distinctive colonial morphology.



Hektoen enteric agar. The Hektoen medium is one of many highly selective media developed for the isolation of *Salmonella* and *Shigella* species from stool specimens. It has both selective and indicator properties. The medium contains a mixture of bile, thiosulfate, and citrate salts that inhibits not only Gram-positive bacteria, but members of Enterobacteriaceae other than *Salmonella* and *Shigella* that appear among the normal flora of the colon.

Anaerobic media. In addition to meeting atmospheric requirements, isolation of some strictly anaerobic bacteria on blood agar is enhanced by reducing agents such as L-cysteine and by vitamin enrichment. Sodium thioglycolate, another reducing agent, is often used in broth media.

Anaerobic media

- These media are used to grow anaerobic organisms.

Eg:

- Robertson's cooked meat medium.
- Thioglycolate broth medium.