

PW 03: BACTERIAL IDENTIFICATION using VARIOUS MEDIA

1. Introduction

Five commonly used microbiological culture media—**Chapman** medium, **Hektoen** enteric agar, **Simmons citrate** medium, **King A** medium and **King B** medium —are used for different purposes in bacterial identification. Each medium has distinct selectivity, composition, and diagnostic indicators.

2. Chapman medium

Often called *Mannitol Salt Agar*, it is a selective and differential medium primarily used for the isolation and identification of *Staphylococcus* species, especially *Staphylococcus aureus*. Its selectivity comes from the high sodium chloride concentration (about 7.5%), which inhibits most bacteria except salt-tolerant organisms such as staphylococci. The medium is differential because it contains mannitol as a fermentable carbohydrate and phenol red as a pH indicator. Bacteria capable of fermenting mannitol produce acid, which lowers the pH and changes the color of the medium from red to yellow. Colonies of *Staphylococcus aureus* typically appear yellow with a yellow surrounding zone, while non-mannitol-fermenting species such as *Staphylococcus epidermidis* form red or pink colonies with no color change in the medium. The medium therefore distinguishes pathogenic mannitol-fermenting staphylococci from other salt-tolerant bacteria.

3. Hektoen enteric agar

It is a selective and differential medium used for the isolation of enteric pathogens, particularly *Salmonella* and *Shigella* species, from clinical samples such as feces. The medium contains bile salts that inhibit Gram-positive bacteria and reduce the growth of many Gram-negative organisms that are not intestinal pathogens. It also contains carbohydrates (lactose, sucrose, and salicin) and the pH indicator bromothymol blue with acid fuchsin to differentiate fermenters from non-fermenters. Organisms that ferment these carbohydrates produce acid, causing colonies to appear yellow or orange. Non-fermenting bacteria remain blue-green. The medium also includes sodium thiosulfate and ferric ammonium citrate, which allow detection of

hydrogen sulfide (H₂S) production. H₂S-producing bacteria form black precipitates in the colonies. Typical colony appearances are: *Salmonella*—blue-green colonies often with black centers (H₂S positive); *Shigella*—green colonies without black centers; lactose-fermenting enteric bacteria (e.g., *Escherichia coli*)—yellow to orange colonies.

4. Simmons citrate medium

It is a defined differential medium used to determine whether bacteria can utilize citrate as their sole carbon source and ammonium salts as the sole nitrogen source. It is commonly used in the identification of members of the *Enterobacteriaceae* family. The medium contains **sodium citrate** as the only carbon source, **ammonium dihydrogen phosphate** as the nitrogen source, and bromothymol blue as a pH indicator. When bacteria are able to metabolize citrate, they produce alkaline by-products such as sodium carbonate and ammonia, which increase the pH of the medium. As a result, the color changes from green (neutral pH) to blue (alkaline pH), indicating a positive citrate utilization test. If the organism cannot use citrate, no growth occurs and the medium remains green.

5. King A medium and King B medium

Microbiological culture media used mainly for the **isolation and identification of *Pseudomonas* species**, particularly *Pseudomonas aeruginosa*. They are differential media designed to stimulate the production of specific pigments produced by these bacteria.

5.1. King A medium

King A medium is primarily designed to ***promote the production of the pigment pyocyanin***. It's used for the detection and differentiation of *Pseudomonas aeruginosa* by stimulating pyocyanin production (blue-green pigment), it's non-selective medium but favors *Pseudomonas* pigment expression. It enhances the **production of pyocyanin** (blue-green pigment) and sometimes **pyorubin** (reddish pigment). The colonies of *P. aeruginosa* appear blue-green with diffusible pigment in the agar.

5.2. King B Medium

King B medium is designed mainly to ***enhance fluorescent pigment production***, it is used to detect fluorescent *Pseudomonas* species. It stimulates the production of **fluorescein** pigment

(**pyoverdine**), which appears yellow-green with fluorescent under UV light. The colonies may show fluorescent green pigment diffusing into the agar, visible especially under UV light.

5. 3. Key differences between King A and King B

Feature	King A medium	King B medium
Main pigment detected	Pyocyanin	Fluorescein (pyoverdine)
Pigment color	Blue-green	Yellow-green fluorescent
Observation	Visible in normal light	Best seen under UV
Main use	Identification of <i>P. aeruginosa</i>	Detection of fluorescent <i>Pseudomonas</i>
Magnesium salts	Lower	Higher (stimulates fluorescence)