Bactria Classification

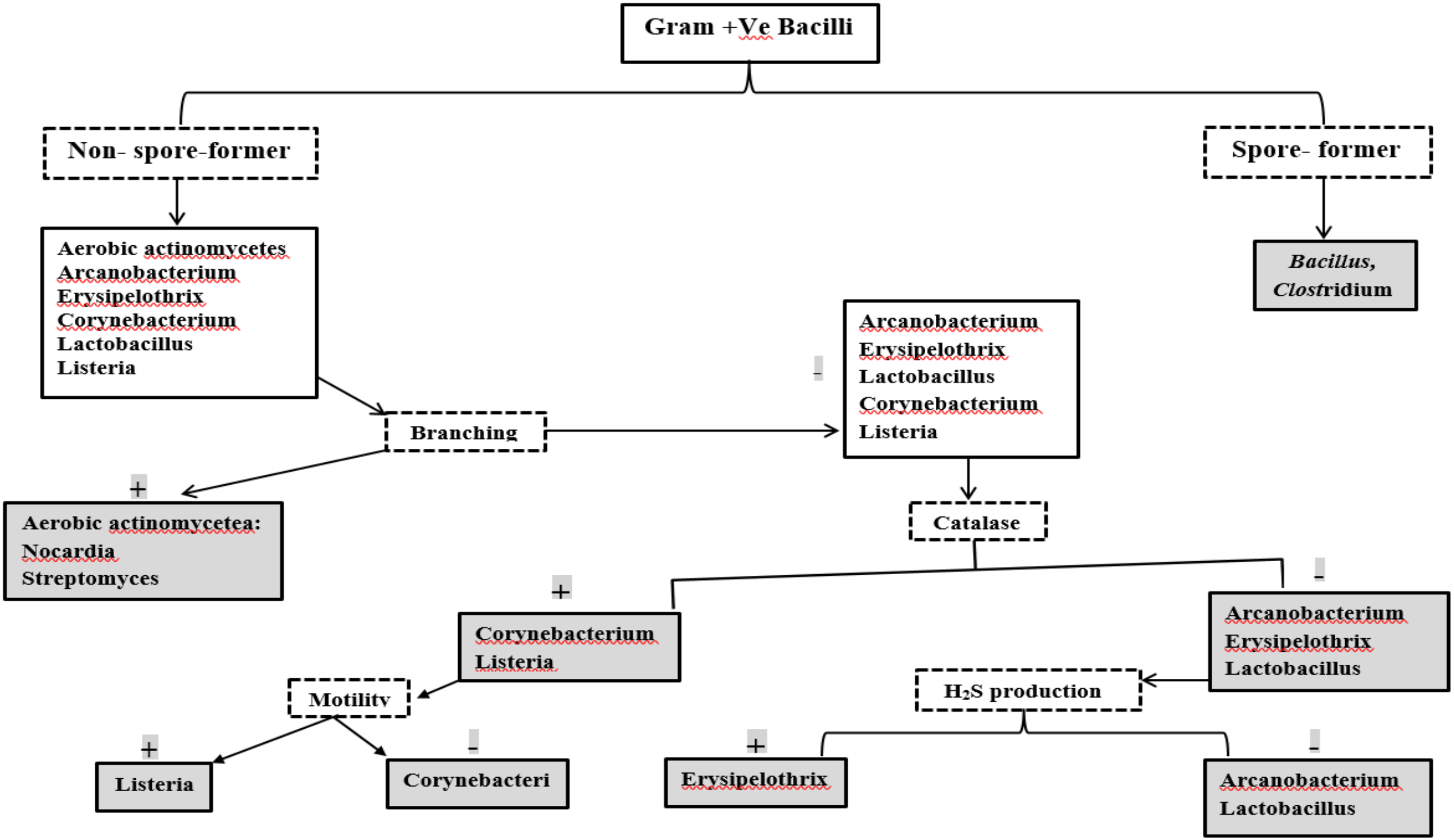
Corynebacterium diphtheriae

Lab 5

Aerobic Non–Spore-Forming Gram-Positive Bacilli

- ***Corynebacterium*,**
- ***Listeria***
- ***Erysipelothrix***
- ***Actinomycetes* ,and Related Pathogens.**

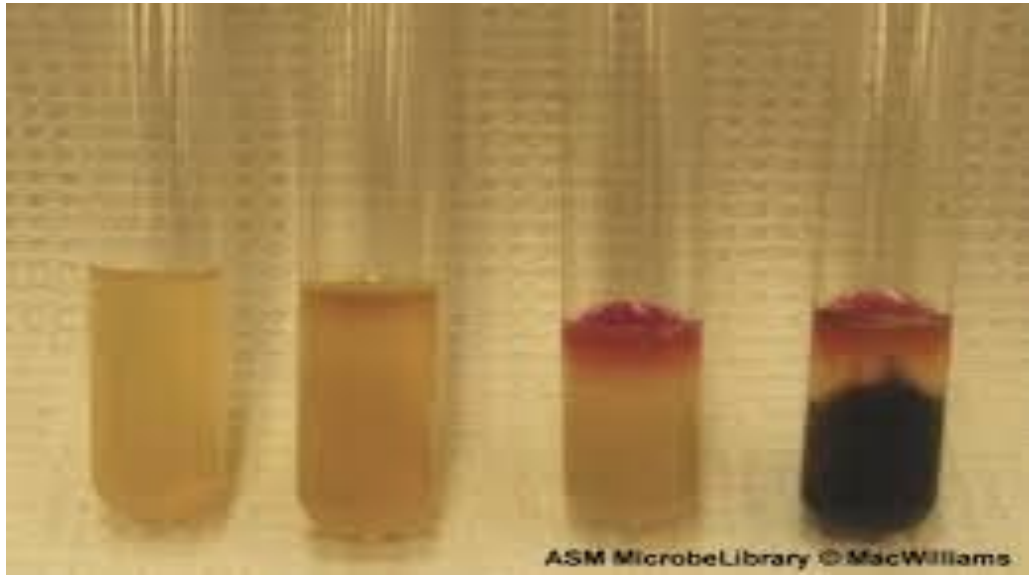
Domain: Bacteria
Phylum: Actinobacteria
Class: Actinobacteria
Order: Actinomycetales
Family: Corynebactericeae
Genus: Corynebacterium
Species: *Corynebacterium diphtheriae* …….. Causes *diphtheria.*


Gram +Ve Bacilli
Non- spore-former
Spore- former
Aerobic actinomycetes
Arcanobacterium
Erysipelothrix
Corynebacterium
Lactobacillus
Listeria
Bacillus,
Clostridium
Branching
-
Arcanobacterium
Erysipelothrix
Lactobacillus
Corynebacterium
Listeria
+
Aerobic actinomycetea:
Nocardia
Streptomyces
Catalase
+
-
Corynebacterium
Listeria
Arcanobacterium
Erysipelothrix
Lactobacillus
Motility
H2S production
+
-
+
-
Listeria
Corynebacteri
Erysipelothrix
Arcanobacterium
Lactobacillus

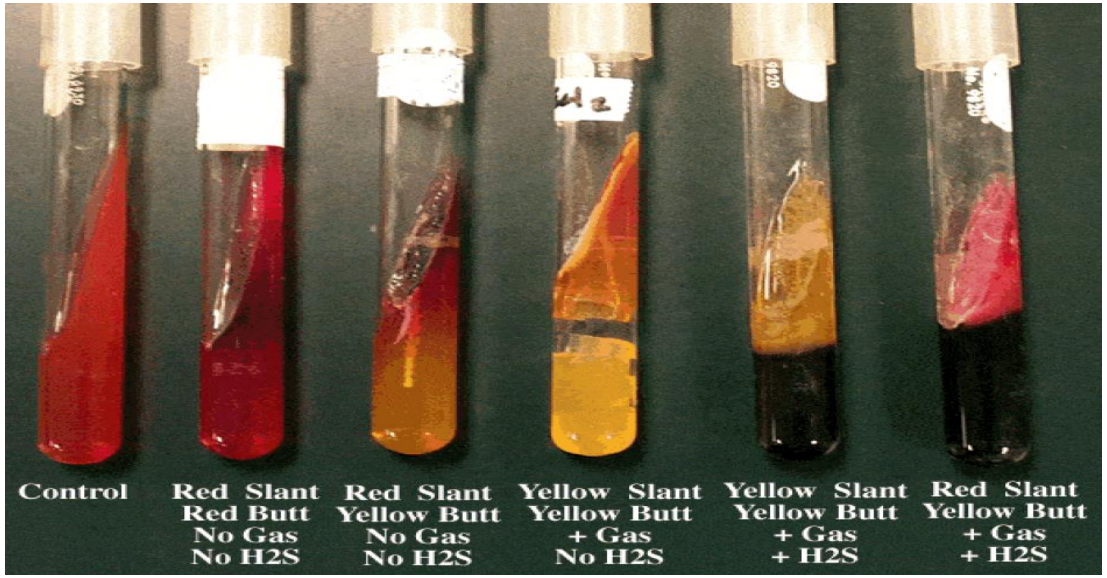
Hydrogen Sulfide (H_2S) Test

Several media containing ***iron compounds*** allow detection of **hydrogen sulfide** production:

1. Kligler iron agar (**KIA**).
2. Triple sugar iron agar (**TSI**).
3. Lysin iron agar (**LIA**).
4. Sulfide-Indole-Motility (**SIM**) medium.

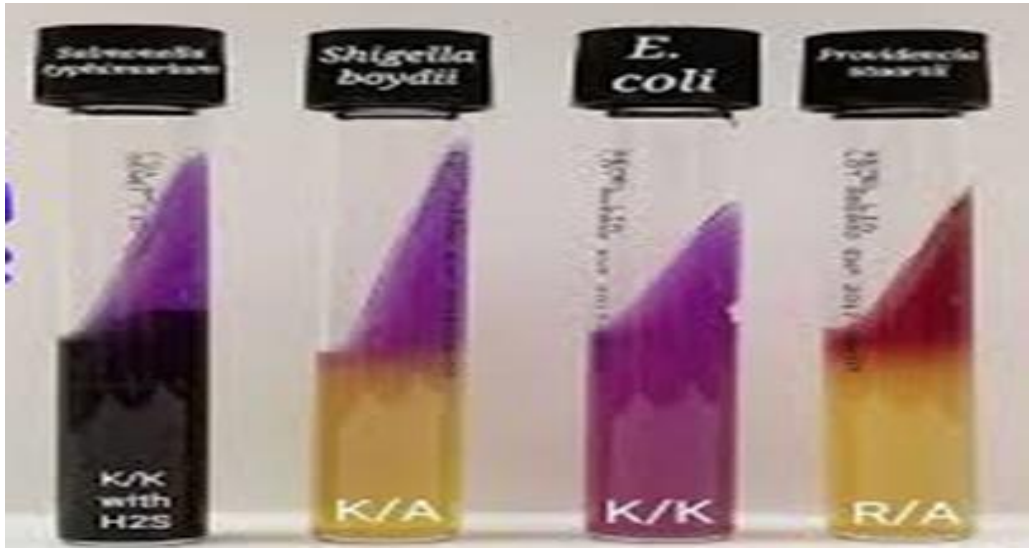


Sulfide-Indole-Motility (**SIM**) medium



Triple sugar iron agar (**TSI**).

Kligler



Lysin iron agar (**LIA**).

Kligler's Iron Agar (KIA):

- **KIA** is primarily used to differentiate members of *Enterobacteriaceae* and to distinguish them from other Gram- negative bacilli such as *Pseudomonas* or *Alcaligenes*

- **Principle**

KIA is a rich medium designed to differentiate bacteria on the basis of **glucose** fermentation, **lactose** fermentation, and **sulfur** reduction. In addition to the two carbohydrates, it includes beef extract, yeast extract, and peptone as carbon and nitrogen sources, and sodium thiosulfate as an electron acceptor. **Phenol red** is the pH indicator and **ferrous sulfate** is the hydrogen sulfide indicator.

- **The medium is prepared as a shallow agar slant with a deep butt, thereby providing both aerobic and anaerobic growth environments. It is inoculated by a stab in the agar butt followed by a fishtail streak of the slant.**

- **The incubation period is 18 to 24 hours for carbohydrate fermentation and up to 48 hours for hydrogen sulfide reactions. Many reactions in various combinations are possible.**

https://www.youtube.com/watch?v=JbJecCTwSUY

- **When KIA is inoculated with a glucose-only fermenter, acid products lower the pH and turn the entire medium yellow within a few hours. Because glucose is in short supply (0.1%), it will be exhausted within about 12 hours.**

- **As the glucose is used up, the organisms located in the aerobic region (slant) will begin to break down available amino acids, producing NH_3 and raising the pH (alkaline). This process, which takes 18 to 24 hours to complete, is called a reversion and only occurs in the slant because of the anaerobic conditions in the butt.**

- **Thus, a KIA with a red slant and yellow butt after a 24-hour incubation period indicates that the organism ferments glucose but not lactose.**

- **Organisms that are able to ferment glucose and lactose also turn the medium yellow throughout.**

- **However, because the lactose concentration is ten times higher than that of glucose, greater acid production results and both slant and butt will remain yellow after 24 hours.**

- ***Therefore, a KIA with a yellow slant and butt at 24 hours indicates that the organism ferments glucose and lactose*. Gas produced by carbohydrate fermentation will appear as fissures in the medium or will lift the agar off the bottom of the tube.**

- **Hydrogen sulfide (H_2S) may be produced by the reduction of thiosulfate in the medium or by the breakdown of cysteine in the peptone. Ferrous sulfate in the medium reacts with the H2S to form a black precipitate, usually seen in the butt.**

- **Acid conditions must exist for thiosulfate reduction; therefore, black precipitate in the medium is an indication of sulfur reduction *and* fermentation. If the black precipitate obscures the color of the butt, the color of the slant determines which carbohydrates have been fermented (*i.e.,* red slant _ glucose fermentation, yellow slant _glucose and lactose fermentation).**

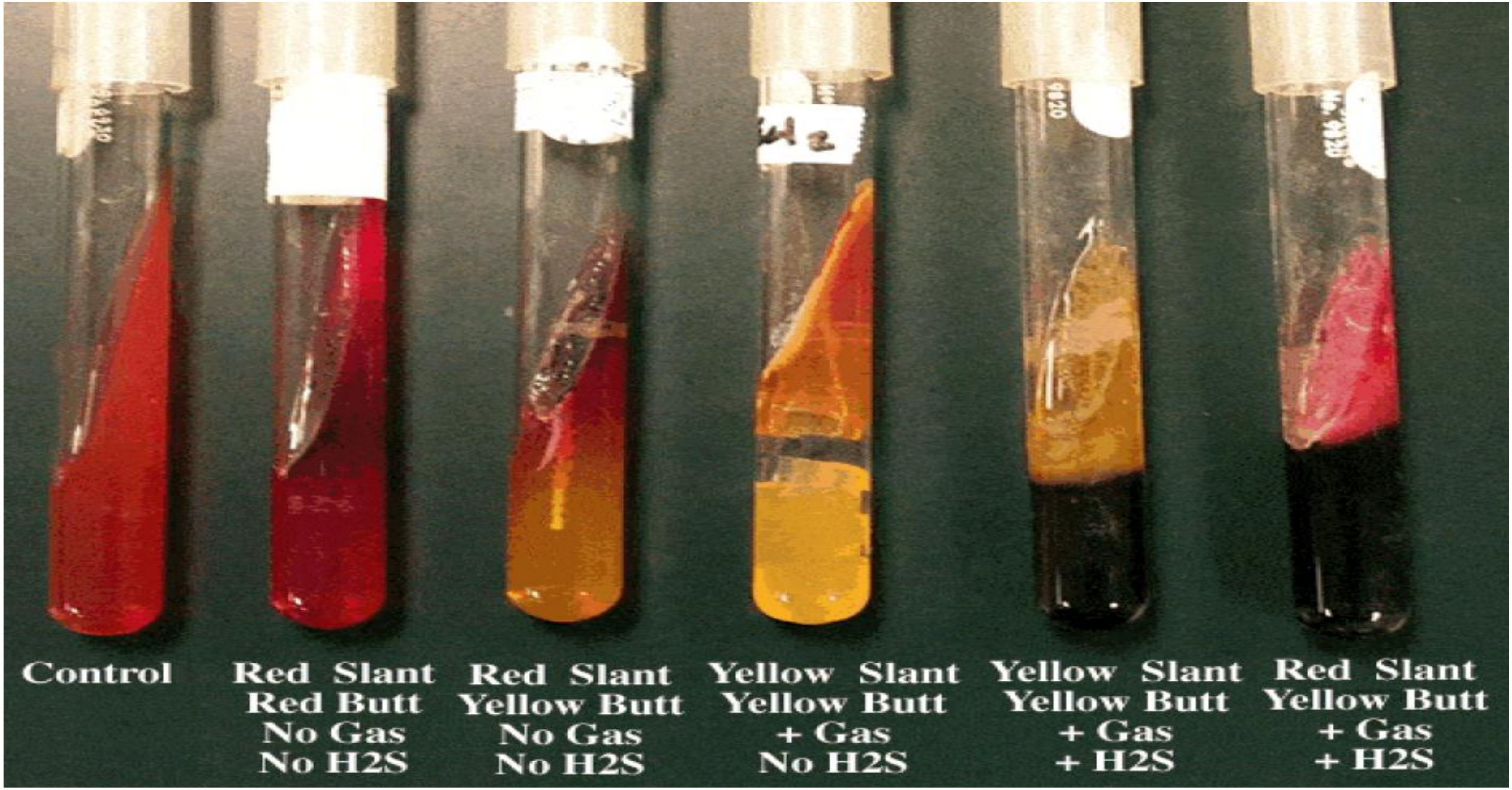
- **An organism that does not ferment either carbohydrate but utilizes peptone and amino acids will alkalinize the medium and turn it red. If the organism can use the peptone aerobically and anaerobically, both the slant and butt will appear red. An obligate aerobe will turn only the slant red.**

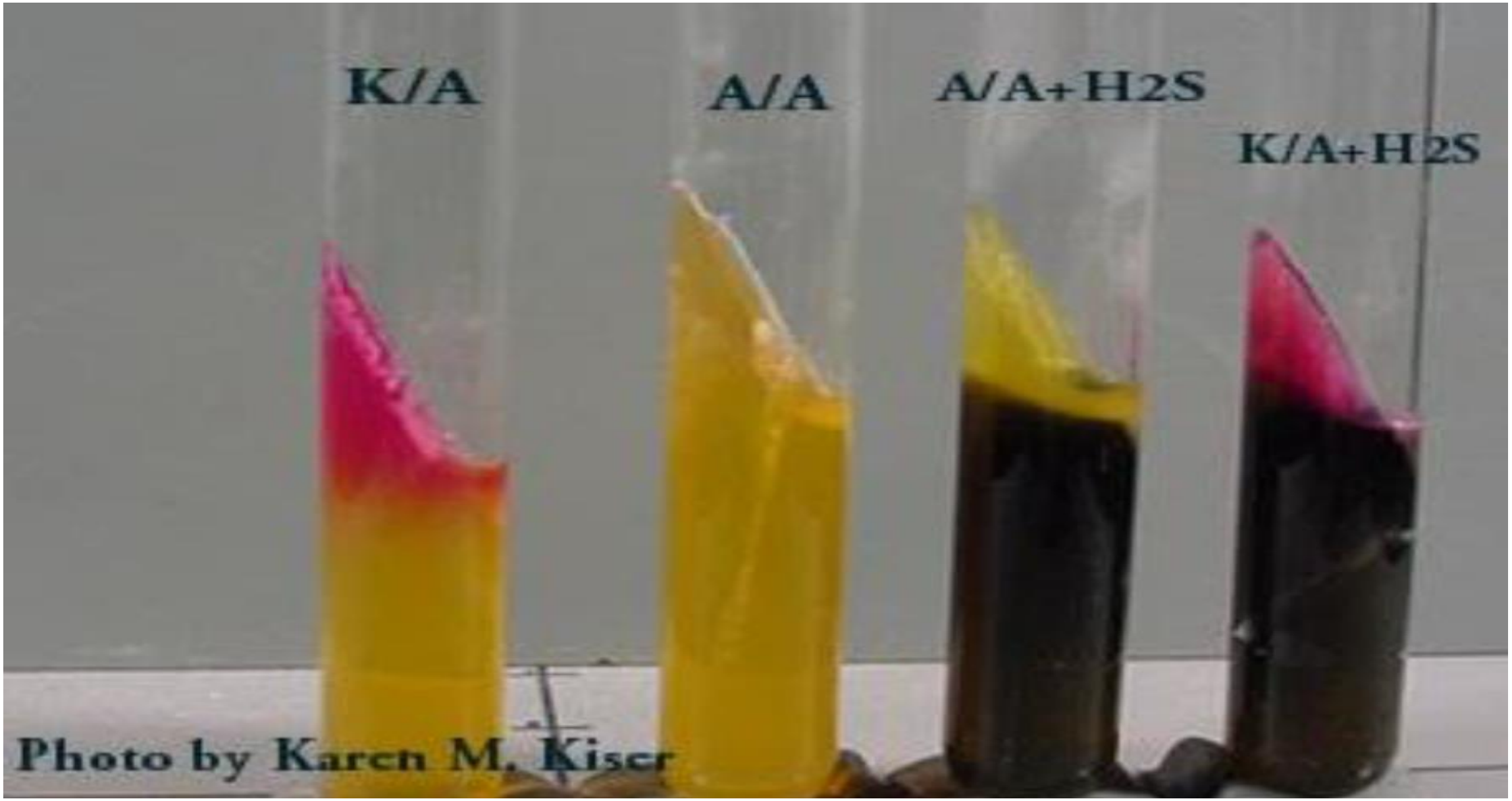
- **Timing is critical in reading KIA results:**

An early reading could reveal yellow throughout the medium, leading one to conclude that the organism is a lactose fermenter when it simply may not yet have exhausted the glucose.

- **A reading after the lactose has been depleted could reveal a yellow butt and red slant leading one to falsely conclude that the organism is a glucose-only fermenter.**
- **Tubes that have been interpreted for carbohydrate fermentation can be reincubated for 24 hours before H_2S determination.**

one to falsely conclude that the organism is a glucose-only fermenter. Tubes that have been interpreted for carbohydrate fermentation can be reincubated for 24 hours before H_2S determination.


Control
Red Slant
Red Butt
No Gas
No H2S
Red Slant
Yellow Butt
No Gas
No H2S
Yellow Slant
Yellow Butt
+ Gas
No H2S
Yellow Slant
Yellow Butt
+ Gas
+ H2S
Red Slant
Yellow Butt
+ Gas
+ H2S


K/A
A/A
A/A+H2S
K/A+H2S
Photo by Karen M. Kiser

Refer to Table 1. for information on the correct symbols and method of reporting the various reactions.

TABLE OF RESULTS

Result	Interpretation	Symbol
Yellow slant/yellow butt	Glucose and lactose fermentation with acid accumulation in slant and butt.	A/A
Red slant/yellow butt	Glucose fermentation with acid production. Proteins catabolized aerobically (in the slant) with alkaline products (reversion).	K/A
Red slant/red butt	No fermentation. Peptone catabolized aerobically and anaerobically with alkaline products. Not from Enterobacteriaceae.	K/K
Red slant/no change in butt	No fermentation. Peptone catabolized aerobically with alkaline products. Not from Enterobacteriaceae.	K/NC
No change in slant/ no change in butt	Organism is growing slowly or not at all. Not from Enterobacteriaceae.	NC/NC
	Sulfur reduction. (An acid condition, from fermentation of glucose or lactose,	