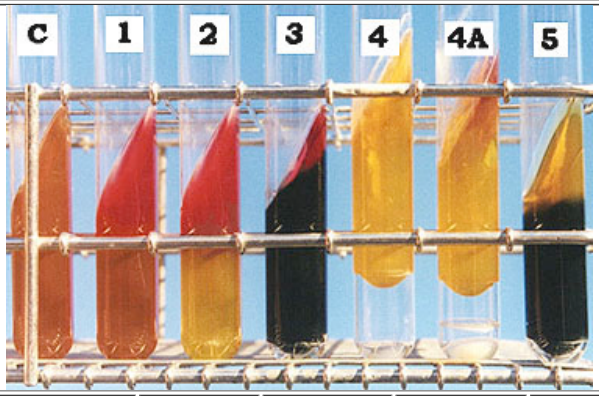
Kligler Iron Agar (KIA)

source of amino acids which may be deaminated (alkaline rx.)	peptone, proteose peptone, beef extract, yeast extract
amino acid added to note its decarboxylation (alkaline rx.)	none
fermentable sugar(s) (acid rx.)	lactose (**1%**) and glucose (**0.1%**)
pH indicator	**phenol red:** net acid = yellow, net alkaline = red
source from which H_2S may be produced	sodium thiosulfate
indicator of H_2S production	ferrous sulfate

Note the relative amounts of sugars in KIA according to the table seen above. By the degree of acid produced from fermentation, differentiation can be made between non-fermenters, glucose-fermenters (which produce a relatively small amount of acid) and those which ferment both glucose and lactose (producing a relatively large amount of acid which diffuses througout the medium and easily overneutralizes the aerobic deamination reaction in the slant). Organisms which produce hydrogen sulfide from the reduction of thiosulfate are easily detected; the H_2S reacts with the iron in the medium to produce ferrous sulfide, a black precipitate. The medium is inoculated with the needle, first stabbing down the center to the bottom of the tube and then streaking up the slant. Incubation is for one day at 37°C. The various combinations of reactions are explained and illustrated below. (Tube "C" is the uninoculated control tube which shows an orange (neutral) reaction throughout.)



corresponding tube no. above	1	2	3	4*	5
deamination of amino acids (aerobic alkaline rx.)	+	+	+	+	+
glucose fermentation (minor acid rx.)	−	+	+	+	+
lactose fermentation (major acid rx.)	−	−	−	+	+
H_2S production (black color)	−	−	+	−	+**
typical examples	*Pseudomonas* (a non-enteric)	*Morganella Providencia Shigella*	*Citrobacter Salmonella Proteus Edwardsiella*	*E. coli Enterobacter Klebsiella*	H_2S+ *E. coli* lactose+ *Salmonella*

* **Tube 4:** Much **gas** is often seen for this tube, evidenced by cracks in the medium. Also, lactose fermenters which are **methyl red-negative** may show a "reversion" toward an alkaline reaction as neutral products are formed from some of the acid. This appears as shown in **Tube 4A** where a slight reddening of the slant occurs as the alkaline deamination reaction becomes no longer over-neutralized by acid from fermentation. How might such a tube appear after two or more days of incubation? (Recall the methyl red test.)

** **Tube 5:** Enough acid can be produced to cause the black iron sulfide precipitate to break down and not be seen. In this case, the tube will look like no. 4.