

A “VIRTUAL DEMONSTRATION” OF GLUCOSE O/F MEDIUM

A summary of Glucose O/F Medium is presented below, and a simplified demonstration will be given in lab during Exp. 7.

DETECTION OF GLUCOSE CATABOLISM IN MEDIA		<i>Alcaligenes faecalis</i>	<i>Pseudomonas fluorescens</i>	<i>Escherichia coli</i>
<p>Glucose O/F Medium and Glucose Fermentation Broth are each formulated with <u>peptone</u>, <u>glucose</u>, pH indicator, and other nutrients (all in aqueous solution). Amino acids in the peptone can be broken down by many organisms, resulting in release of ammonium (by aerobic deamination) which creates an alkaline pH. Glucose may be fermented and/or respired, resulting in acidic endproducts. <u>Fermentation</u> allows anaerobic growth and results in a very <u>large amount of acid</u> which is unlikely to be over-neutralized by deamination of the amino acids. <u>Respiration</u> results in a very <u>small amount of acid</u> which is detected when there is no fermentation present and also when deamination is minimal. Glucose O/F Medium is purposely formulated with relatively less amino acids, so there is less interference from the alkaline product (ammonium) from deamination.</p>		 <p>Each organism is shown in an “open tube” (without mineral oil) and a “closed tube” (with mineral oil which restricts oxygen).*</p>		
<p>REACTIONS IN GLUCOSE O/F MEDIUM pH indicator is brom thymol blue: blue = alkaline; green = neutral; yellow = acidic.</p> <p>AF: <i>Alcaligenes faecalis</i> is a strict aerobe and can respire, but it does not respire glucose. Amino acid deamination results in an alkaline reaction – detected by a blue color where the organism grows aerobically.</p> <p>PF: <i>Pseudomonas fluorescens</i> is a strict aerobe and can respire glucose. Associated with glucose respiration is a small amount of acid which will over-neutralize the alkaline reaction (from amino acid deamination), resulting in a net acidic reaction (yellow) where it grows aerobically.</p> <p>EC: <i>Escherichia coli</i> respire and ferments glucose. The large amount of acid from fermentation overneutralizes the alkaline reaction and permeates the entire tube with a strong yellow color resulting.</p>				
<u>Aerobic Reactions</u> (top 1/4 of open tube)	Growth (+ or –)	+	+	+
	Net pH observed	alkaline	acidic	strongly acidic
<u>Anaerobic Reactions</u> (closed tube and bottom 3/4 of the open tube)	Growth (+ or –)	–	–	+
	Net pH observed	neutral	neutral	strongly acidic
SUMMARIZED RESULT IN GLUCOSE O/F MEDIUM (O, F or –)		–	O **	F

* The “open tube” has access to air (oxygen) and will allow growth of all three organisms at the top of the medium. Respiration by these organisms uses up the oxygen in the medium such that the bottom 3/4 of the medium allows anaerobic growth by fermenters such as *E. coli*. The mineral oil in the “closed tube” more directly cuts off oxygen and allows anaerobic growth of fermenters.

** This small amount of acid produced with respiration is detectable only when fermentation is not present, and it is called the “O” (for “oxidative”) reaction. (In Glucose Fermentation Broth, the relative amount of amino acids is greater and that of glucose is lower. So, with more alkaline deamination, this acidic reaction would be overneutralized and therefore not seen in Glucose Fermentation Broth.)