

به نام خدا



MICROBIOLOGY LAB

# Litmus Milk Medium

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## ۶- لیتموس میلک

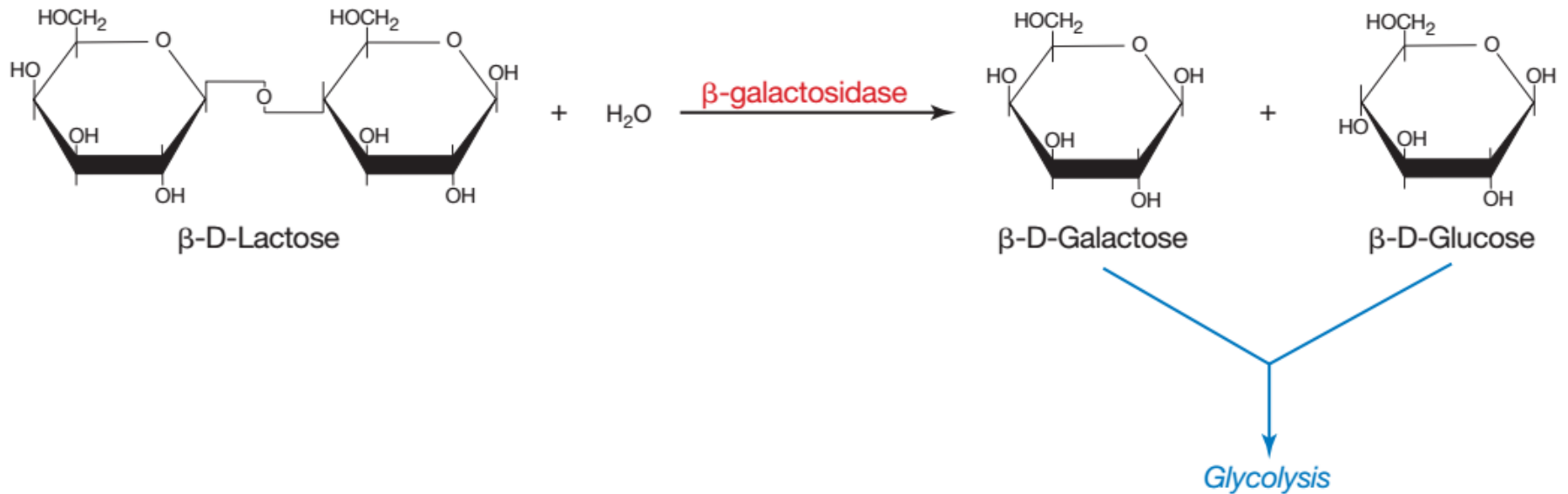
- محیط لیتموس میلک در ابتدا به منظور افتراق اعضای جنس کلستریدوم بکار گرفته می‌شد. این محیط بر اساس توانایی باکتری‌های رودهای در احیا لیتموس، خانواده‌ی انتروباکتریاسه را از سایر باسیل‌های گرم منفی متمایز می‌کند. این محیط همچنین به منظور کشت و نگه‌داری باکتری‌های لاکتیک اسید کاربرد دارد.
- چهار واکنش پایه در محیط لیتموس میلک رخ می‌دهد: تخمیر لاکتوز، احیا لیتموس، انعقاد کازئین و هیدرولیز کازئین. ترکیب این واکنش‌ها با هم تنوعی از نتایج را نشان می‌دهد که هر کدام از آن‌ها به منظور افتراق باکتری‌ها از هم مورد استفاده قرار می‌گیرند.

enzymatic complement. A variety of different biochemical changes result, as follows:

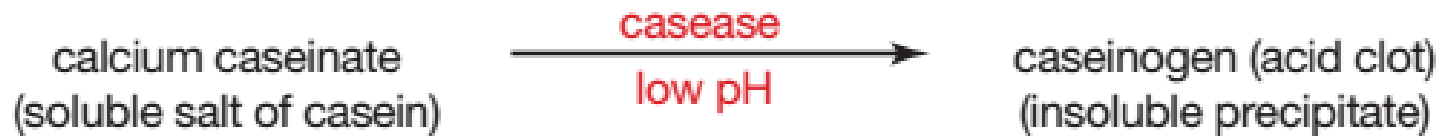


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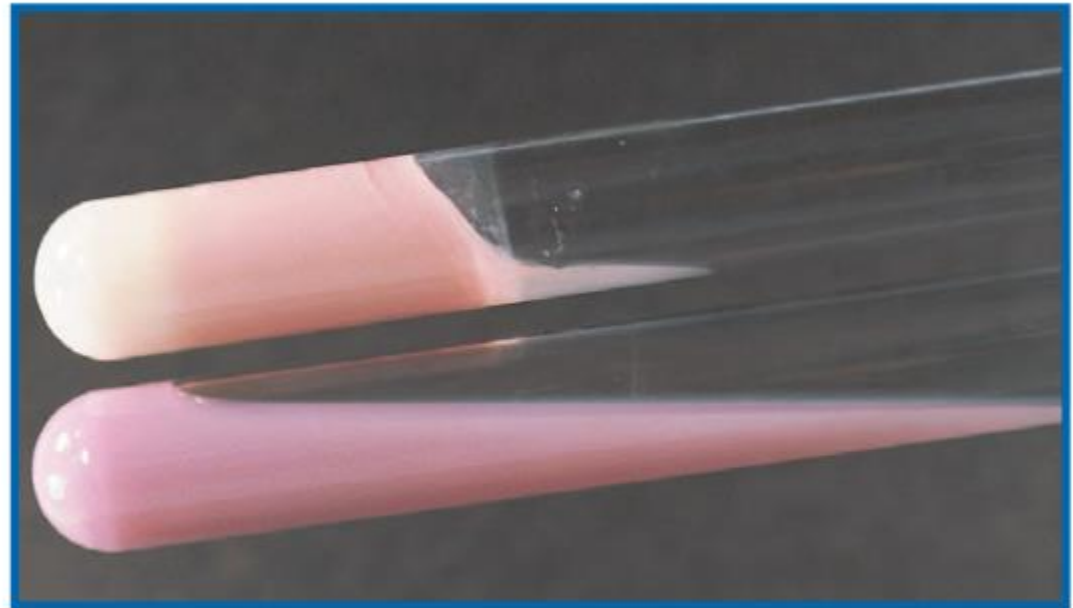




**5-72 LACTOSE HYDROLYSIS** ♦ Lactose hydrolysis requires the enzyme  $\beta$ -galactosidase and produces glucose and galactose—two fermentable sugars.

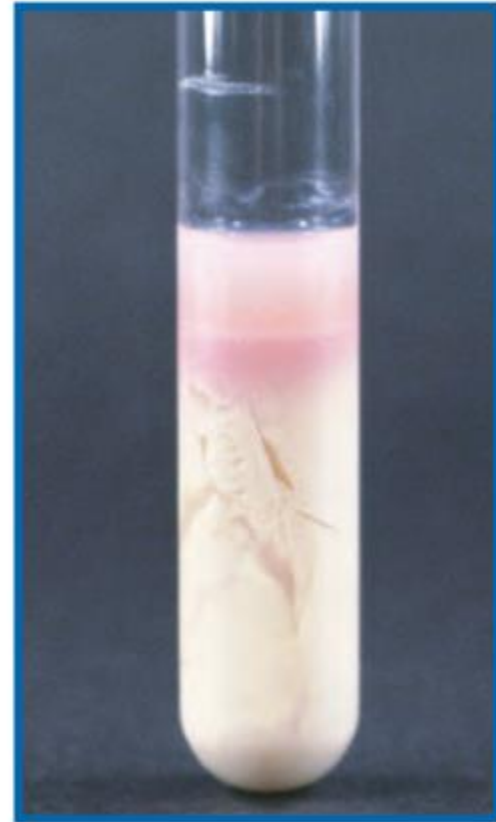


**5-73** ACID CLOT FORMATION ♦ An acid clot is the result of casease catalyzing the formation of caseinogen, an insoluble precipitate, under acidic conditions.



**5-74** ACID CLOT ♦ An acid clot appears in the top tube; an uninoculated control is below. Note the reduced litmus (white) at the bottom of the clot.

**5-75** GAS PRODUCTION FROM FERMENTATION ♦ This organism has produced gas fissures in the clot.



**5-76** CURD FORMATION ♦ Rennin converts casein to paracasein to form a curd.

## T A B L E O F R E S U L T S

Result	Interpretation	Symbol
Pink color	Acid reaction	A
Pink and solid (white in the lower portion if the litmus is reduced); clot not movable	Acid clot	AC
Fissures in clot	Gas	G
Clot broken apart	Stormy fermentation	S
White color (lower portion of medium)	Reduction of litmus	R
Semisolid and not pink; clear to gray fluid at top	Curd	C
Clarification of medium; loss of "body"	Digestion	D
Blue medium or blue band at top	Alkaline reaction	K
No change	None of the above reactions	NC

\*These results may appear together in a variety of combinations.



Species	RCM				Litmus milk	Acid from			
	Colour	Digestion	Odour	Gas		Glucose	Sucrose	Lactose	Salicin
<i>C. botulinum</i>	black	+	-	+	D	+	v	-	-
<i>C. perfringens</i>	black	+	+	+	CD	+	+	+	v
<i>C. tetani</i>	black	+	+	-	C	-	-	-	-
<i>C. novyi</i>	red	-	-	+	GC	+	-	-	-
<i>C. septicum</i>	red	-	-	+	AC	+	-	+	+
<i>C. fallax</i>	red	-	-	+	AC	+	+	+	+
<i>C. sordellii</i> <sup>a</sup>	black	+	+	+	CD	+	-	-	-
<i>C. bifermentans</i> <sup>a</sup>	black	+	+	+	CD	+	-	-	v
<i>C. histolyticum</i>	black	+	+	-	D	-	-	-	-
<i>C. sporogenes</i>	black	+	+	+	D	+	-	-	v
<i>C. tertium</i>		-	-	+	AC	+	+	+	+
<i>C. cochlearium</i>	red	-	-	+		-	-	-	-
<i>C. butyricum</i>		-	-	+	ACG	+	+	+	+
<i>C. nigrificans</i>		-	-	-		-	-	-	-
<i>C. thermosaccharolyticum</i>		-	-	+		+	+	+	+

RCM: Robertson's cooked meat medium

Litmus milk: A, acid; C, clot; D, digestion; G, gas

<sup>a</sup> *C. sordellii* also splits urea; *C. bifermentans* does not



the spectrum of litmus milk changes. Since some of the reactions take 4 to 5 days to occur, the cultures should be incubated for at least this period of time; they should be examined every 24 hours, however. Look for the following reactions:

**Acid Reaction** Litmus becomes pink. Typical of fermentative bacteria.

**Alkaline Reaction** Litmus turns blue or purple. Many proteolytic bacteria cause this reaction in the first 24 hours.

**Litmus Reduction** Culture becomes white; actively reproducing bacteria reduce the O/R potential of medium.

**Coagulation** Curd formation. Solidification is due to protein coagulation. Tilting tube at 45° will indicate whether or not this has occurred.

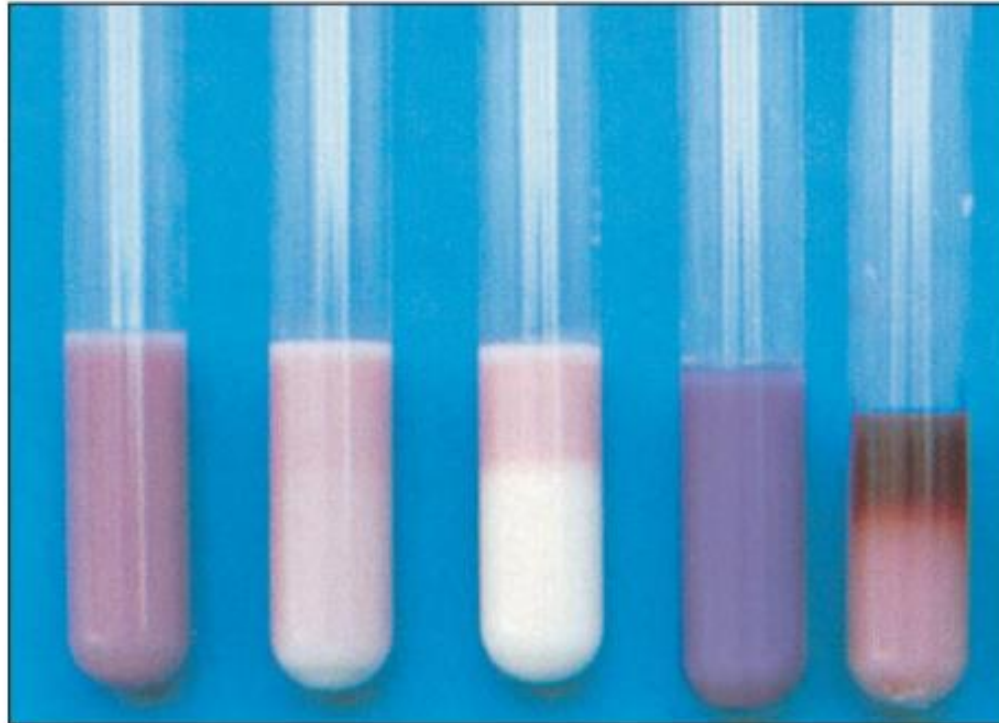
**Peptonization** Medium becomes translucent. It often turns brown at this stage. Caused by proteolytic bacteria.

**Ropiness** Thick, slimy residue in bottom of tube. Ropiness can be demonstrated with sterile loop.

Record the litmus milk reactions of your unknown on the Descriptive Chart.



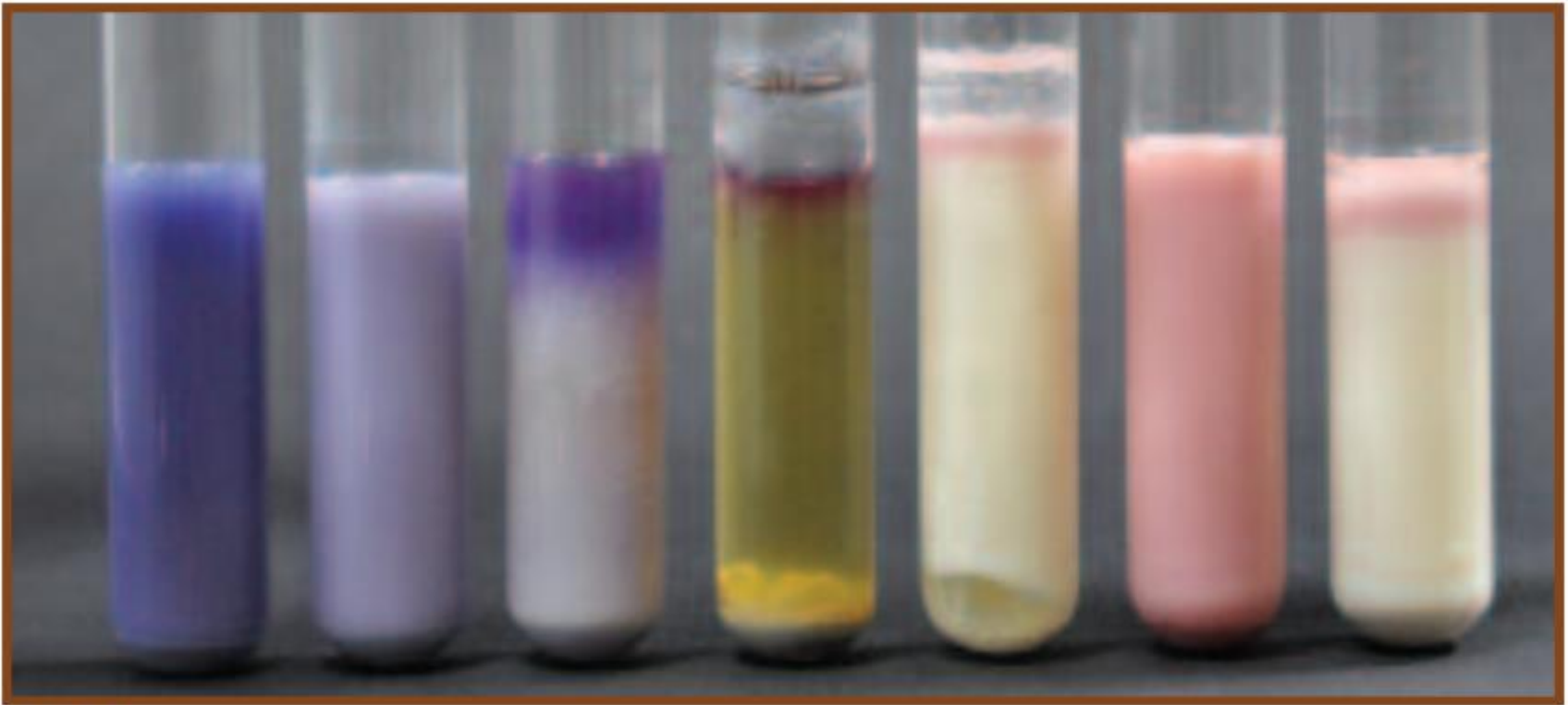




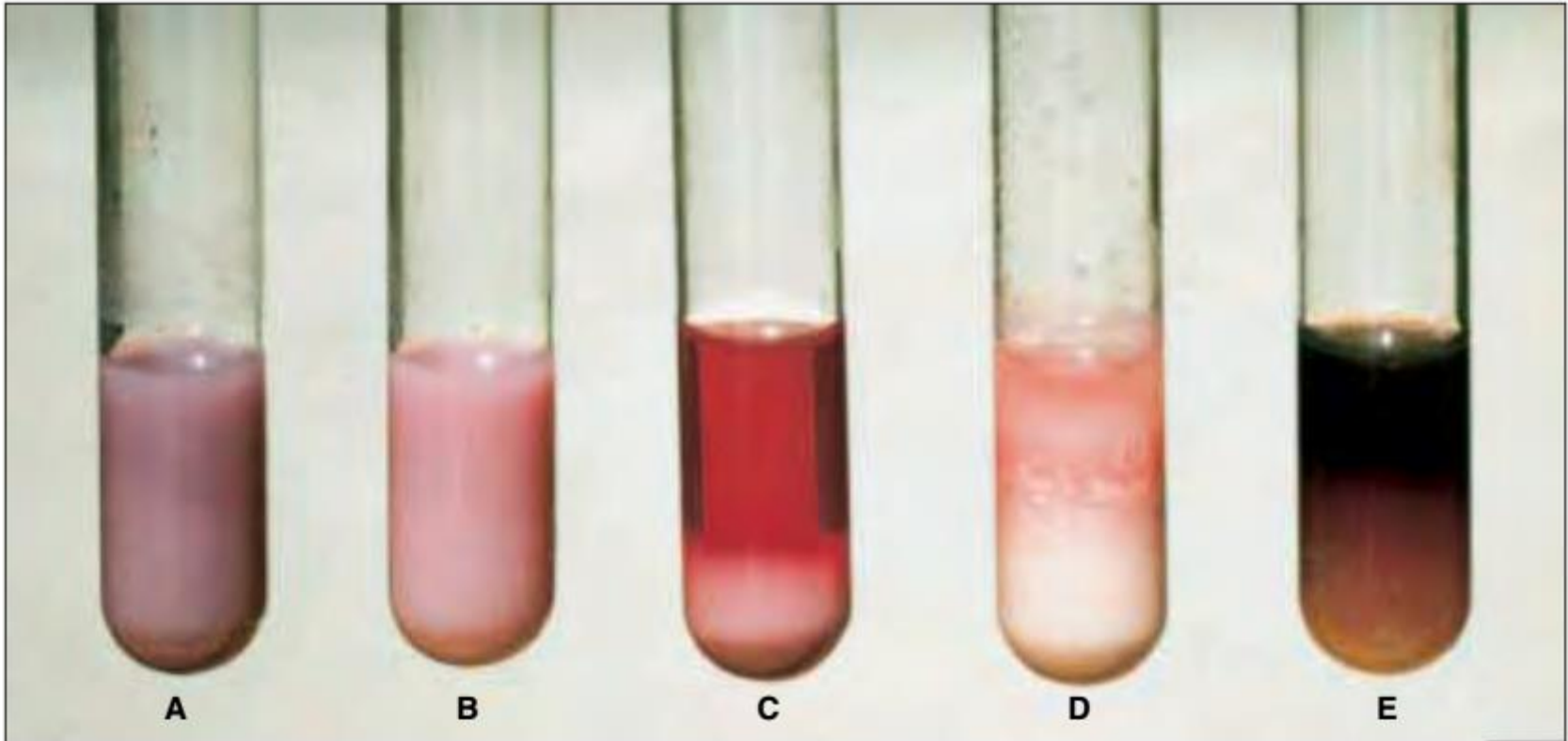
(a) (b) (c) (d) (e)

**Figure 28.1** Litmus milk reactions. (a) Uninoculated, (b) acid, (c) acid with reduction and curd, (d) alkaline, and (e) proteolysis.





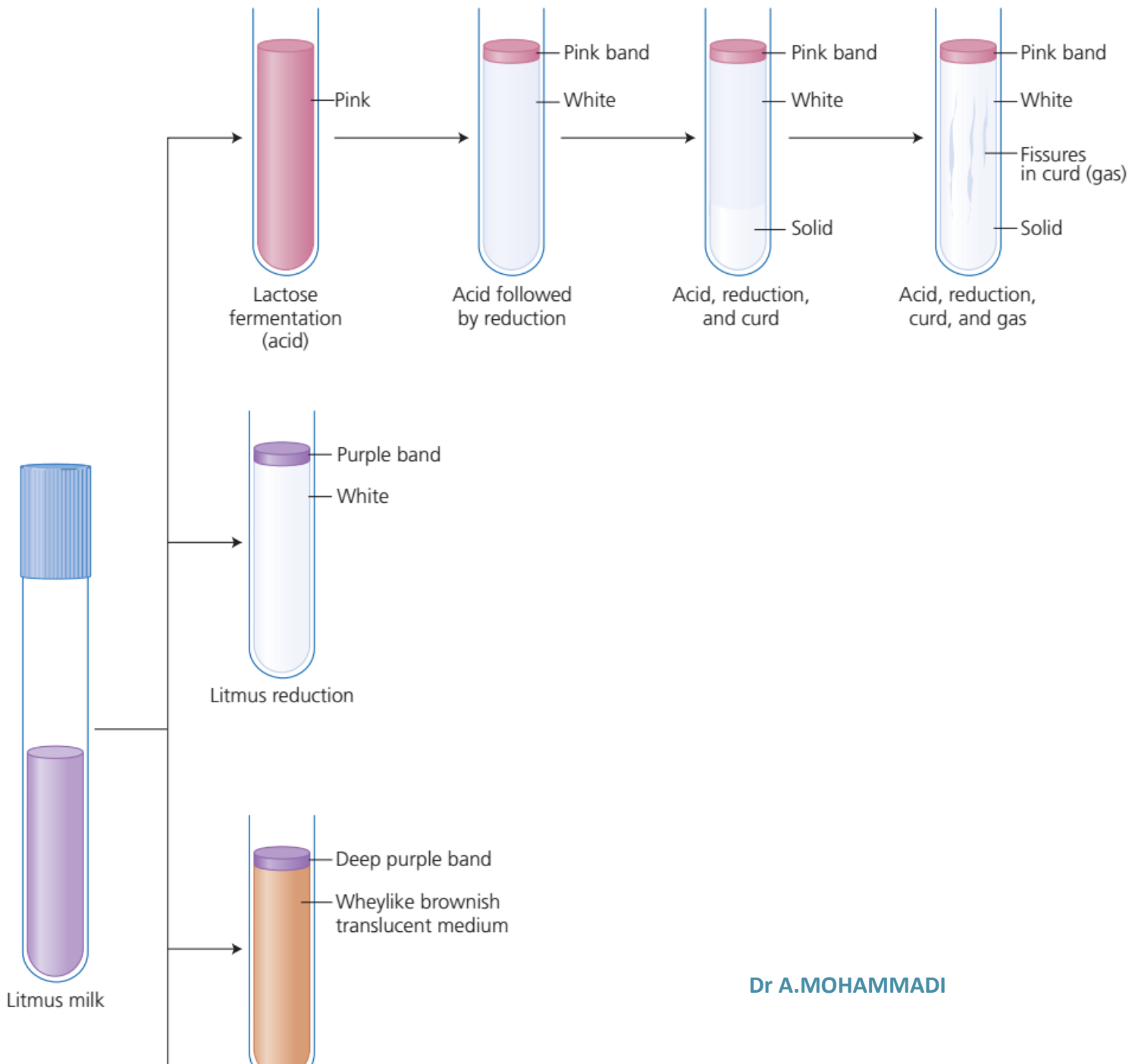
**7-54 REACTIONS IN LITMUS MILK** From left to right: *Alcaligenes faecalis* (K), uninoculated control, *Pseudomonas aeruginosa* (K), *Clostridium sporogenes* (D), *Clostridium acetobutylicum* (AGCR), *Escherichia coli* (A), and *Lactococcus lactis* (ACR). The clear fluid on the surface of the two *Clostridium* cultures is mineral oil used to make the medium anaerobic.



**Figure 50.2** Litmus milk reactions: (A) Alkaline. (B) Acid. (C) Upper transparent portion is peptonization; solid white portion in bottom is coagulation and litmus reduction; overall redness is interpreted as acid. (D) Coagulation and litmus reduction in lower half; some peptonization (transparency) and acid in top portion. (E) Litmus indicator is masked by production of soluble pigment (*Pseudomonas*); some peptonization is present but difficult to see in photo.

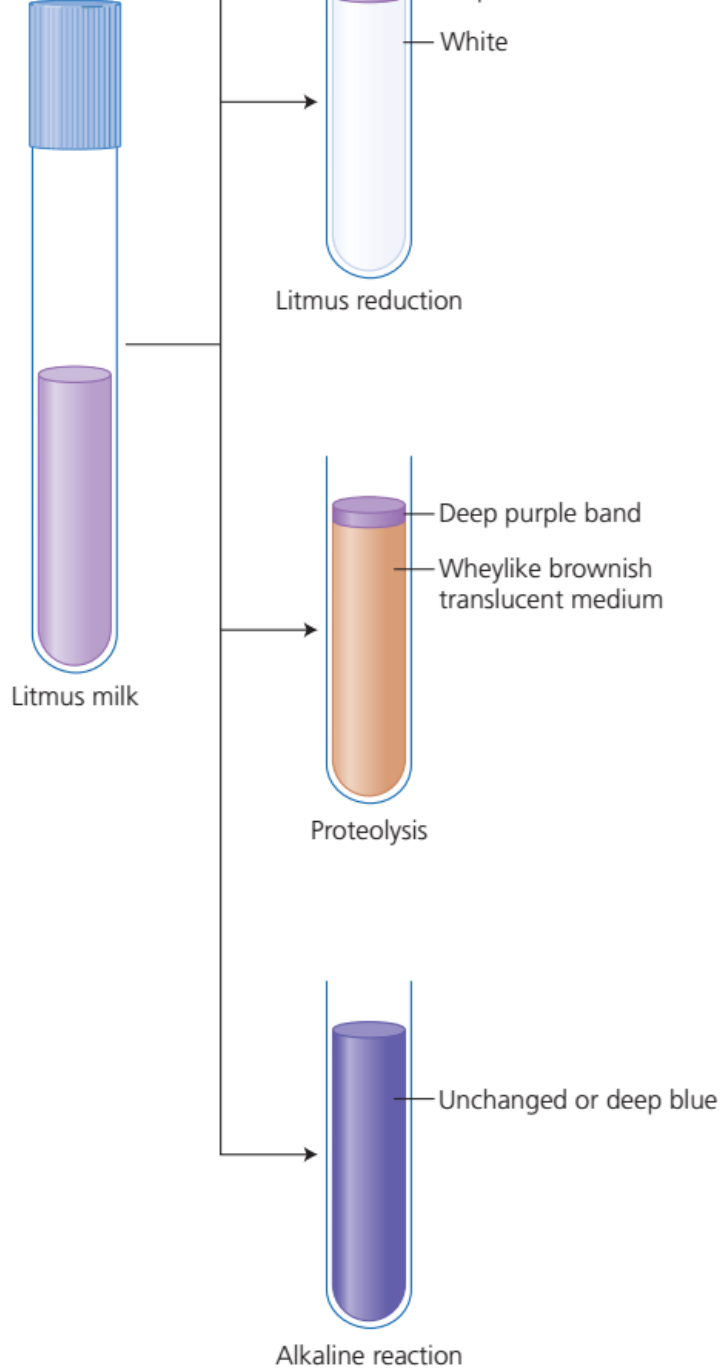


شکل) واکنش‌ها در محیط لیتموس میلک. از چپ به راست: هضم، واکنش قلیایی (DK)؛ تشکیل لخته‌ی اسید، احیا لیتموس (ACR)؛ تشکیل لخته‌ی اسید، احیاء لیتموس، تولید گاز به واسطه تخمیر (ACRG) - توجه به شکاف کوچک گاز در لخته داشته باشید؛ تشکیل دلمه، احیا لیتموس (CR)؛ لوله‌ی کنترل بدون تلقیح؛ واکنش اسیدی (A)؛ و واکنش قلیایی (K).



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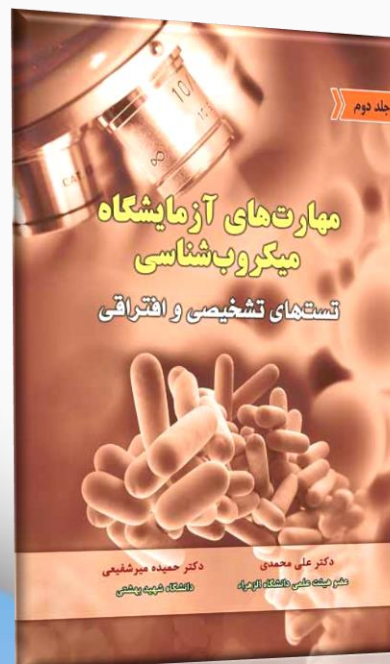
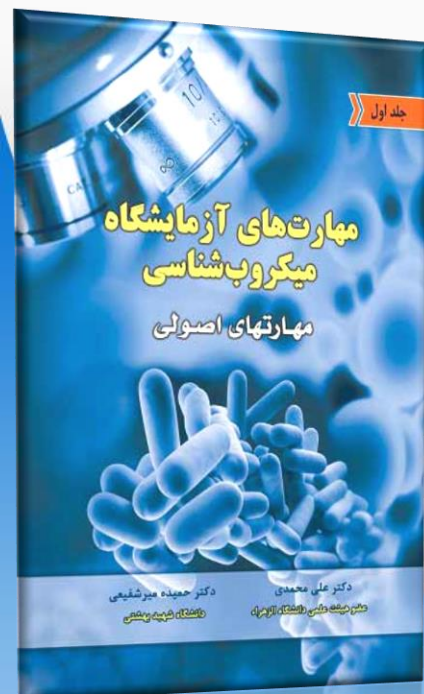
**Figure 28.2** Summary of possible litmus milk reactions

# منبع:

- **مهارت های آزمایشگاه میکروب شناسی ، جلد ۱- ۳ ،**

نگارش:

- دکتر علی محمدی-عضو هیئت علمی دانشگاه الزهرا (س)
- دکتر حمیده میرشفیعی - دانشگاه شهید بهشتی



از توجه شما سپاسگزارم

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