Growth and Culturing of Bacteria

Jacquelyn G. Black, Microbiology, 9th Edition

Chapter 6 – Page 146

Mahmoud Alkawareek, PhD

Growth and Cell Division

- Microbial growth is defined as the increase in the number of cells, rather than in terms of cell size

  Nevertheless, the 'mother cell' usually doubles in size and duplicates its contents before it divides into two 'daughter cells'

- Cell division in bacteria usually occurs by **binary fission** or sometimes by **budding**
- In binary fission, the cell duplicates its components and a transverse **septum** grows in the middle of the cell dividing it into two independent daughter cells.
- In continuously dividing cells, DNA synthesis is continuous & replicates the bacterial chromosome shortly before the cell divides.

  The chromosome is attached to the cell membrane which grows & separates the replicated chromosomes.

Mahmoud Alkawareek, PhD

Growth and Cell Division

- In some species, incomplete separation of cells occurs which results in the formation of special cell arrangements, i.e. tetrads, sarcinae, sterptococci, etc
- In yeast & a few bacteria cell division occurs by **budding**, where a smaller new cell develops from the surface of an existing cell & then separates from the parent cell
- Budding vs binary fission

  Although both are asexual forms of reproduction where two genetically identical cells 'clones' are produced, in binary fission the parent cell is divided into two equally sized new cells, whereas budding produces a small new cell in addition to the existing parent cell.

Growth and Cell Division

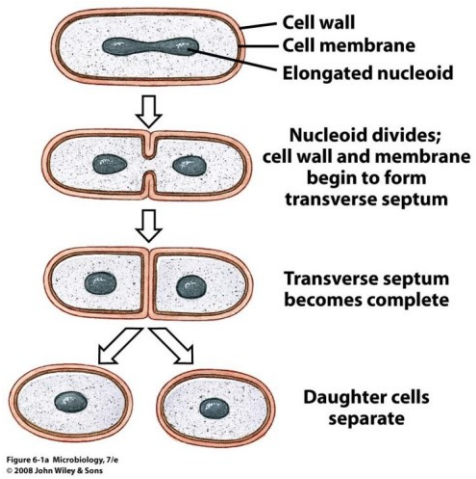


Figure 6-1a Microbiology, 7/e
© 2008 John Wiley & Sons

Binary fission

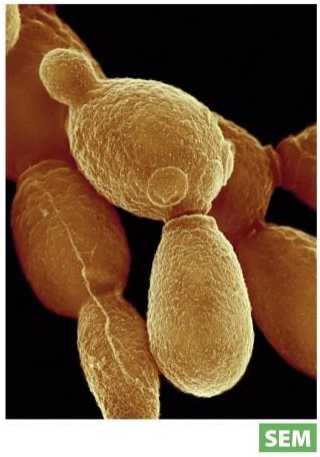
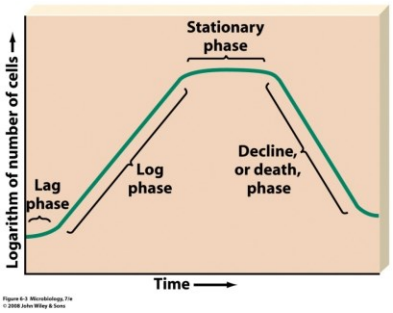


Figure 6-2 Microbiology, 7/e
SPL/Photo Researchers, Inc.

Budding in yeast

Phases of Growth

- When bacteria are introduced (inoculated) into a fresh nutrient medium, they show **four** major phases of growth:
  1. Lag phase
  2. Log (exponential) phase
  3. Stationary phase
  4. Decline (death) phase



  - These phases form the **standard bacterial growth curve**

Phases of Growth

- Lag phase
  - Cells don't increase in number, but are metabolically active

    i.e. the cells are increasing in size, incorporating various molecules from the medium, synthesizing enzymes & and producing large quantities of ATP (energy)
  - Length of lag phase depends on the characteristics of the bacterial species **and** the conditions in the growth media (both the old medium and the new one)

    Some species adapt to the new medium in 1-2hrs, others take several days

Phases of Growth

- Log (exponential) phase
  - Once bacteria are adapted to the new medium, growth (increase in number) occurs at exponential or logarithmic rate (straight line if plotted on log y-axis)
  - In log phase, organisms divide at their most rapid rate, a regular genetically determined interval called the **generation time**

    Generation time for most bacteria is between 20 min to 20 hrs; typically less than 1 hr
  - The population of m.o. doubles in each generation time

Phases of Growth

- Log (exponential) phase
  - But bacterial cells don't all divide precisely together, rather they show a situation called 'non-synchronous growth'
    - Synchronous growth: a hypothetical situation where all cells divide exactly together after each generation time → a stair-step curve.
    - Non-synchronous growth: a natural situation where each cell divides sometime during the generation time → smooth curve

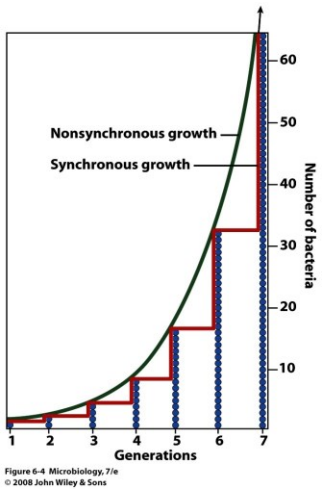


Figure 6-4 Microbiology, 7/e

Phases of Growth

- Log (exponential) phase
  - In a flask or a tube, log phase is limited in time; because as the number of cells increases nutrients & O_2 are used up, waste materials accumulate and living space is limited. This will reduce the ability of cells to produce ATP & **growth rate decreases**.
    - In this situation, the log phase levels off & will be followed by a stationary phase, unless fresh medium is added or the organisms are transferred to another fresh medium
    - Log bacterial growth can be maintained by using a device called '**chemostat**' which has a growth chamber where fresh medium is continuously added (from an attached reservoir) as old medium is withdrawn.

Phases of Growth

- Stationary phase
  - When cell division decreases to a rate equal to that of cell death, the number of cells remains constant, which appears as horizontal straight line on the bacterial growth curve
  - In this stage, the medium contains limited amount of nutrients & may contain toxic quantities of waste materials, O_2 is limited to aerobic organisms & damaging pH changes may occur.
- Decline (death) phase
  - Medium is less & less supportive to cell division, so cells lose their ability to divide & thus die. The no. of live cells decreases at logarithmic rate.
  - The duration of this phase is highly variable as the logarithmic phase, both depend on genetic characteristics of the organism. Some bacteria contain few bacteria that remain alive after months or years.

Phases of Growth

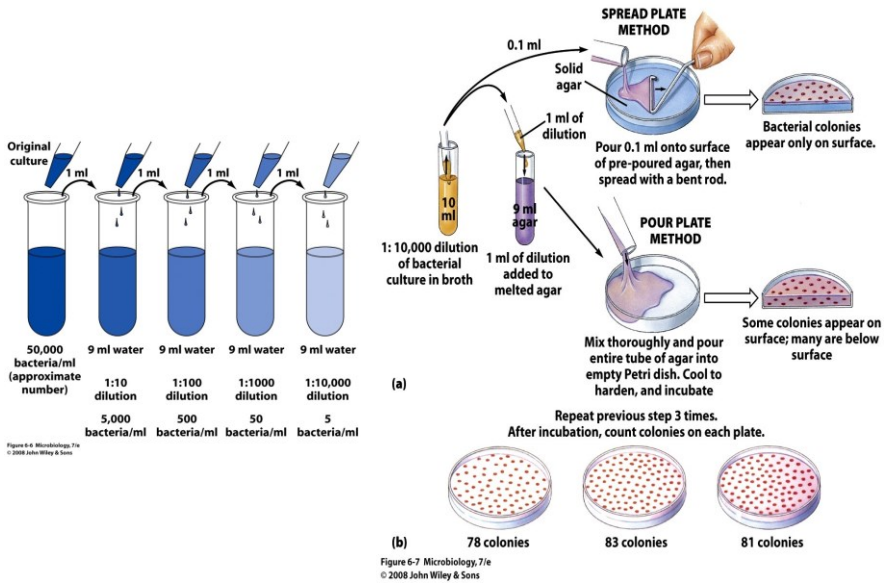
- Growth in colonies
  - When growing on a solid medium, a cell divides exponentially forming a small **colony** containing all the descendants of the original cell.
  - The colony grows rapidly at its edges whereas cells nearer the centre grow more slowly & begin to die. Thus all phases of growth occur simultaneously in a colony.
  - Each single living bacterial cell will divide to form a colony i.e. each bacterial cell represents a colony-forming unit (CFU).

Measuring Bacterial Growth – Enumeration of Bacteria

- It is measured by estimating the no. of cells that have arisen by binary fission during a growth phase. Expressed as number of viable (living) organism per unit volume (i.e. ml)

1. Serial dilution & plate count method
   - Principle: only living bacterium will divide & form visible colony on agar plate.

     Agar plate: Petri dish containing nutrient medium solidified with agar.
   - Serial dilution: series of dilutions e.g. 1/10 → 1/10 → 1/10 etc, then transfer 0.1ml to agar plate. The transfer is done either by
     a. Pour plate method or
     b. Spread plate method



Measuring Bacterial Growth – Enumeration of Bacteria

- Pour plate method: add 1ml diluted culture from serial dilutions to melted nutrient agar, mix, then pour in empty plate → agar cools down → solidified → incubated → colonies develop within medium & on medium surface

  Disadvantage: damage to colonies exposed to heated agar, smaller colonies inside agar compared to those on surface.

- The spread plate method: 0.1ml sample is placed on the surface of cool solidified agar medium. The sample is spread evenly → incubate → colonies on surface.
- Countable no. of colonies /plate (30-300 CFU)

  It is difficult to count more than 300 colonies on one plate whereas less than 30 is not statistically representative

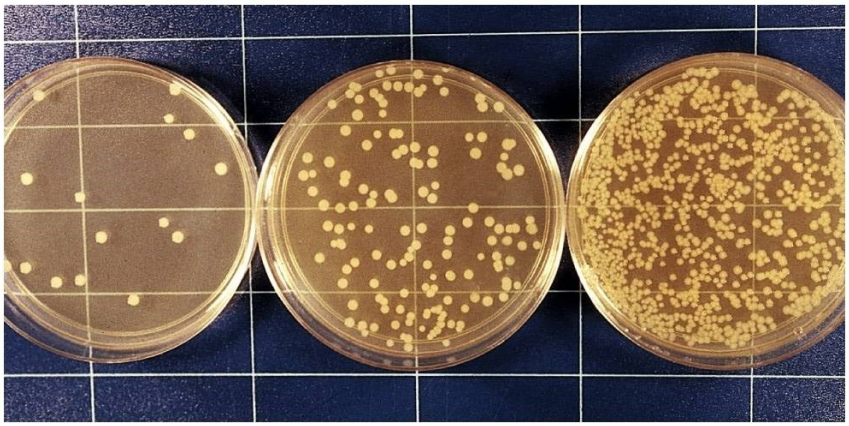


Figure 6-8c Microbiology, 7/e
M. Gabridge/Visuals Unlimited

Measuring Bacterial Growth – Enumeration of Bacteria

- The colonies are counted by the aid of **colony counter** (magnifying lens+ special electrical marker).
- Actual no. of colonies = no. of colonies on plate x dilution factor
- The concentration of bacterial cells in the original suspension (culture) is calculated from the number of colonies and is expressed as cfu/ml
- To improve accuracy: shake tubes before sampling & make several plates from each dilution.
- Weakness of the process:

  Doesn't count the cells that died by the time of plating & does not include m.o. that cannot grow on the utilized growth medium.

Measuring Bacterial Growth – Enumeration of Bacteria

2. Direct microscopic count
   - A known volume of medium is introduced into specially calibrated etched glass slide called **counting chamber** (similar to hemocytometer).
   - Cells are then counted, under the microscope, in specific areas and their number per unit volume is calculated.
   - Disadvantages:
     - Cannot distinguish between living & dead cells
     - Requires large no. of cells
     - The bacterial suspension should be homogeneous

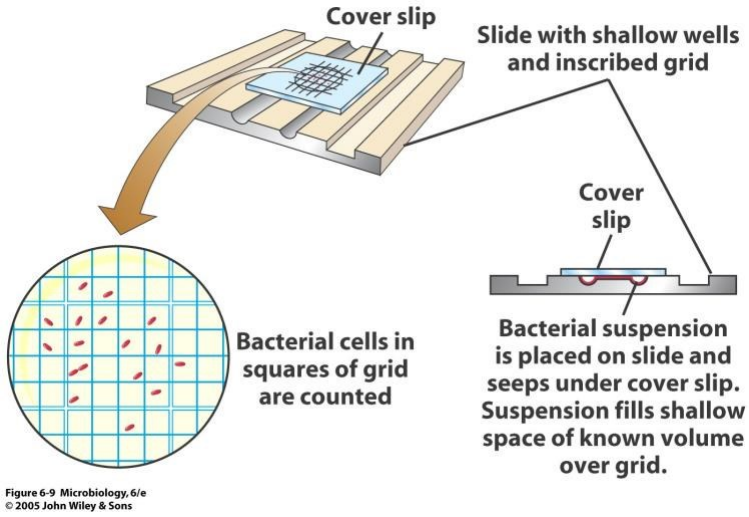


Figure 6-9 Microbiology, 6/e
© 2005 John Wiley & Sons

Counting chamber

Measuring Bacterial Growth – Enumeration of Bacteria

3. Filtration method
   - A known volume of fluid (i.e. water or air) is drawn through a filter with pores smaller than bacteria (e.g. 0.45µm) → filter is placed on solid medium → incubate → count the no. of cells in each plate → calculate the number of cells per unit volume (e.g. 100 ml or 1 L)
4. Other methods
   - Simple observation:
     - Gas production: can be detected by capturing the gas in small inverted tubes
     - Acid production: by incorporating pH indicators
     - Turbidity
   - By measurements
     - Turbidity can be measured by **spectrophotometer** or colorimeter: important to monitor rate of growth without disturbing the culture
     - No. of cells can be determined by **dry weight measurement**

Culturing Bacteria - Introduction

- To study bacteria, it is important to obtain pure culture
  - i.e. a culture that contains only a single species of organisms
- Pure culture is necessary to study nutritional needs, growth characteristics, pathogenicity and antimicrobial susceptibility of individual spp.
- Pure cultures are usually obtained using 'solid' growth media
- Agar is an ideal solidifying agent for microbiological media, why?
  - It doesn't melt below 95°C, and after melting it solidifies at ~40°C (hysteresis)
  - Inert substance: only very few organisms can digest it

Culturing Bacteria – Methods of Obtaining Pure Cultures

- The streak plate method
  - Procedures:
    - Pick bacteria on **sterile** wire loop
    - Move the wire along the agar surface depositing **streaks** of bacteria on surface
    - Loop is flamed
    - Pick bacteria from the bacteria deposited on agar & streak new regions on agar
    - Flame & repeat…
  - Individual organisms are deposited in the region streaked last
    - i.e. after incubation, isolated colonies usually appear on agar surface in that region
    - isolated colonies, that represent an individual m.o., can then be picked up and transferred to fresh medium for further studying

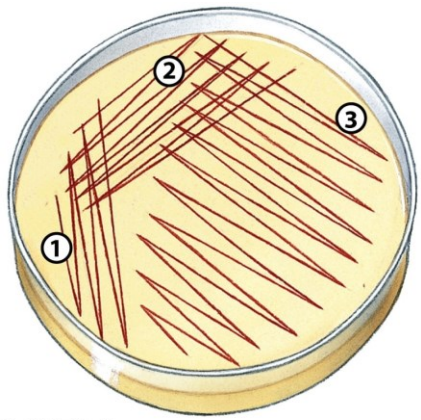
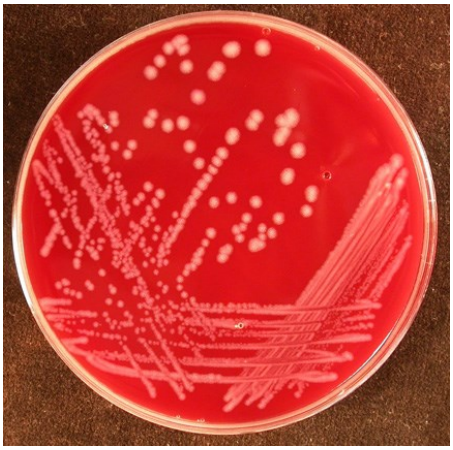


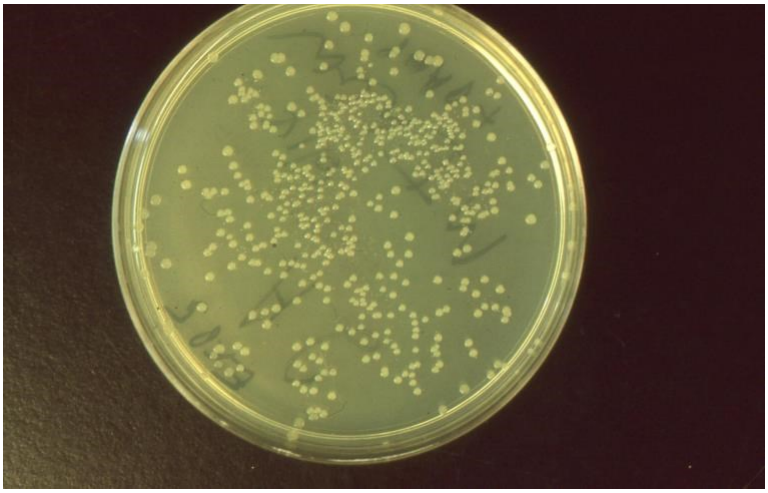
Figure 6-19a Microbiology, 7/e
© 2008 John Wiley & Sons



The streak plate method

Culturing Bacteria – Methods of Obtaining Pure Cultures

- The pour plate method
  - Makes use of serial dilutions so that the final dilution contains about 1000 organism
  - 1ml of this dilution is then placed in 9ml of melted agar medium (at 45°C) & the medium is quickly poured into a sterile plate
  - The resulting plate will contain small no. of bacteria some of which will form isolated colonies on the agar
  - Since some m.o. are embedded in agar medium, this method is useful for growing microaerophiles that cannot tolerate exposure to atmospheric levels of oxygen



The pour plate method

Culturing Bacteria - Culture Media

- Growing bacteria in the lab requires knowledge of their nutritional needs & the ability to provide these substances in a medium
- Although many bacteria can be grown in the lab nowadays, some m.o., such as those causing syphilis & leprosy, still cannot be cultured in lab media but rather need cultures containing living human or animal cells

Culturing Bacteria - Culture Media

- Types of media
  - Lab medium is a synthetic medium prepared from materials of precise or reasonably well-defined composition
  - **Defined synthetic medium**: synthetic medium that contains specific kind & amount of chemical substances
  - **Complex medium** (chemically nondefined medium): contains reasonably familiar materials but varies slightly in chemical composition from batch to batch
    - Complex media may contain peptone, blood or extracts from beef, yeasts, soybean, etc.
    - Peptone: a product of enzymatic digestion of proteins (from meat or fish) that provides small peptides that m.o. can use
  - Both liquid nutrient broth & solid agar medium are used to culture bacteria

A Defined Synthetic Medium for Growing *Proteus vulgaris*

Ingredient	Amount	Ingredient	Amount
Water	1 liter	K_2HPO_4	1 g
$MgSO_4 \cdot 7H_2O$	200 mg	$FeSO_4 \cdot 7H_2O$	10 mg
$CaCl_2$	10 mg	Glucose	5 g
NH_4Cl	1 g	Nicotinic acid	0.1 mg

Trace elements (Mn, Mo, Cu, Co, Zn as inorganic salts, known quantities of 0.02–0.5 mg each)

A Complex Medium Suitable for Many Heterotrophic Organisms

Nutrient Broth Ingredient	Amount
Water	1 liter
Peptone	5 g
Beef extract	3 g
NaCl	8 g
Solidified Medium	
Agar	15 g
Above ingredients in amounts specified	

Culturing Bacteria - Culture Media

- Commonly used media:
  - Most routine lab culture media contain peptone, such media can be enriched by:
    - Yeast extract: contains a number of vitamins, coenzymes and nucleosides
    - Casein hydrolysate: made from milk protein and contains many a.a.
    - Blood (or serum): contains many nutrients needed by fastidious pathogens

      Blood agar (usually sheep's blood) is also used to identify m.o. that cause hemolysis

Culturing Bacteria - Culture Media

- Selective, differential and enrichment media:

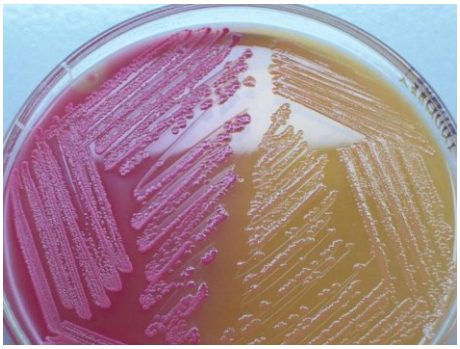
  These media are very important in diagnostic medicine

  - **Selective medium**: it encourages the growth of some m.o. but suppresses the growth of others
    - e.g. an antibiotic can be added to the growth medium so as only m.o. that are resistant to this antibiotic can grow
  - **Differential medium** (indicator media): has an indicator constituent that causes an observable change (colour change or pH change) in the medium when a biochemical reaction , that is characteristic to a certain m.o., occurs
    - This will allow to distinguish a certain type of m.o. (colony) from others growing on the same plate
    - E.g. blood agar can be used to distinguish hemolytic bacteria

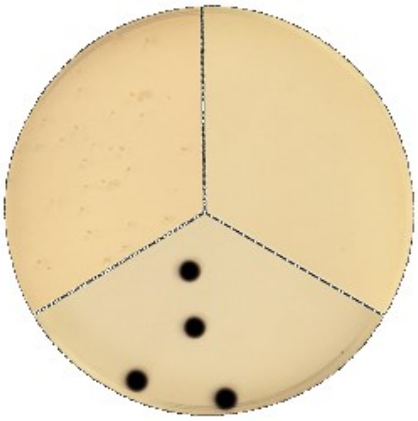
Culturing Bacteria - Culture Media

- Selective, differential and enrichment media:
  - Some media can be selective and differential at the same time, examples:
    - MacConkey Agar:
      - ✓ It has crystal violet & bile salts which inhibit the growth of G+ve bacteria but allows the growth of G-ve ones → selective
      - ✓ It also has sugar lactose and pH indicator that turns colonies of lactose-fermenters (Lac+) into red colonies & the non-lactose-fermenters (Lac-) into colorless colonies → differential
      - ✓ E.g. it can be used to differentiate between *E. coli* (Lac+) and *Salmonella* (Lac-)
    - Sulfite Polymyxin Sulfadiazine (SPS) Agar:
      - Used for the detection of *Clostridium botulinum*
      - The two antibiotics inhibit the growth of most m.o. other than *Clostridium* spp. → selective
      - Sulfite is reduced by *Clostridium botulinum* to sulfide which a **black** iron sulfide precipitate → differential

Culturing Bacteria - Culture Media



MacConkey agar with Lac+ colonies (left) and Lac- colonies (right)



SPS agar plate showing black *Clostridium* colonies

Culturing Bacteria - Culture Media

- Selective, differential and enrichment media:
  - **Enrichment medium**: it contains special nutrients that allow the growth of particular m.o. that might not otherwise be present in sufficient numbers to allow it to be isolated and identified
    - e.g. *Salmonella typhi* may be in very small no. in faecal samples, so it is cultured on a medium containing the trace element **selenium** which supports the growth of this m.o.
    - Unlike selective medium, it doesn't suppress the growth of other m.o.
    - Blood agar and chocolate (heat-treated blood) agar are also considered as enrichment media and are frequently used to grow fastidious m.o.

Culturing Bacteria – Controlling Oxygen Content of Media

- Obligate aerobes:
  - Usually obtain O_2 from nutrient broth or on the surface of solidified agar, but some may need more.
  - So O_2 can bubbled through medium (with filters to prevent contamination)
- Microaerophiles:
  - A broth tube or agar plate can be incubated in a jar in which a candle is lit before the jar is sealed
  - Burning candle uses O_2 & adds CO_2, when the candle extinguishes → suitable conditions
- Obligate anaerobes:
  - All molecular O_2 must be removed
  - Addition of oxygen-binding agents like thioglycolate, cysteine (a.a) or sodium sulfide prevent O_2 from exerting its toxic effects on anaerobes
  - If the culture is in plates, special jars are used where special bags containing a chemical substance are placed to remove O_2 & generate CO_2
  - Stab cultures: a culture of anaerobic bacteria can be made quickly by stabbing a straight wire coated with m.o. into a tube of agar-solidified medium

Culturing Bacteria – Methods of Performing Multiple Diagnostic Tests

- Many kits use culture systems that contain a large no. of differential & selective media to identify different m.o.
  - e.g. Analytical Profile Index (API) and Enterotube Multitest System
  - Advantages: use small amount of media, occupy little space, efficient & reliable means of identifying infectious organisms
- API kit:
  - Plastic tray with 20 microtubes containing different dehydrated media
  - The microtubes are rehydrated & inoculated with bacterial suspension from an isolated colony → incubate → write the no. → check the list for identification
- Other tests depend on immunological properties of the m.o.

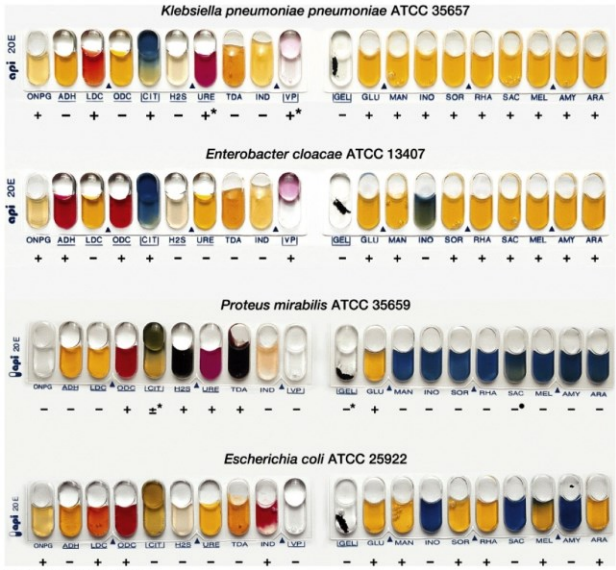


Figure 6-25 Microbiology, 7/e
Courtesy API/CounterPart Diagnostics/bioMerieux Vitek, Inc.

The API 20E system

Mahmoud Alkawareek, PhD