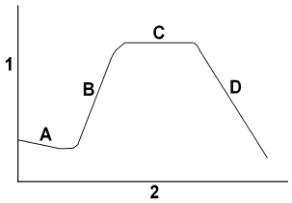
Cultivation of infectious agents

CULTIVATION - DEFINITION

Cultivation is one of the direct detection methods. **Goal** of the cultivation is to isolate an infectious agent in pure culture for its further identification or analysis.

CULTIVATION OF BACTERIAL, VIRAL & PARASITICAL AGENTS

Bacteria are reproduce by **binary fission** so one mother cell is divided into two identical daugther cells. The time required for the population to double is called the **generation time.** If a liquid medium is inoculated with a microbial culture and viable cells are plotted periodically a curve known as **the growth curve** is obtained (fig. below)



1. cell concentration [log]
2. time

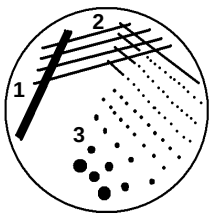
A. lag phase
B. exponential growth phase
C. stationary phase
D. death phase

Clinically important **bacteria** are **heterotrophic** (need organic carbon for growth), **mesophilic** (optimal temperature 30-37˚C) and **aerobic** (use oxygen as hydrogen acceptor), **facultative anaerobic** or **anarerobic organisms** (another hydrogen acceptor than oxygen e.g. *nitrates*).
Yeasts, molds and **some protozoal parasites** could be also cultivated on the same artificial media as bacteria are (e.g. Candida, Aspergillus, pathogenic ameboae).
Viruses and some intracellular bacteria require for their cultivation living host cells because they dont have own metabolism and are not able to reproduce extracelluarly (e.g. they need a tissue culture).

MEDIA FOR BACTERIAL CULTIVATION

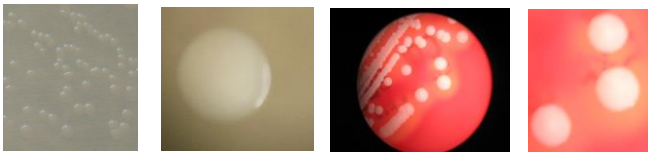
Liquid media – the nutrient broth: contains a variety of nutrients(source of amino acids and nitrogen), is made by boiling meat or vegetables. Arguments to use the broth could be diluting substances including material which are toxic for bacteria (e.g. antibiotics), or if too small number of damaged bacterial cells are in a specimen as a result of a previous therapy or an attack of innate or specific host immunity the cells could be resuscitated by the broth.
Solid media – the nutrient agar: broth media combined with agar (usually 2%). The agar does not contain any nutrients; it only keeps bacterial cells in the same position during cultivation. Eventually **distinct bacterial colonies** grow from one original bacterial cell, which could be seen by unaided eye. The shape & color of the colonies (*colony morphology*) could by typical for a particular bacterial group or genera. The colony could be isolated on a new agar plate to receive **pure bacterial culture**.
General media: described above (nutrient broth & agar).
Enrichment media: general media fortified with e.g. blood, yeast extract. Useful in growing fastidious microbes.
Selective & diagnostic media: used for cultivation of selected pathogenic bacteria (by supressing growth of others, e.g. via antibiotics as a component) and visualising some of their biochemical properties (color change dependent on enzymatic properties of the bacterium), respectively. The advantages of both are combined together (e.g. *MacConkey agar* – inhibits growth of Gram-positive bacteria, supports the growth of Enterobacteriae; detects microbes able to metabolize lactose).

Nutrient broth. Tube with sterile nutrient broth before inoculation of a bacterial culture (left) and turbid overnight culture of *Escherichia coli* (right).



Schema of growing cultures on agar media
Specimen was inoculated and diluted by a bacteriological loop. A sterile loop was used for each diluted step (1-3) to spread the inoculum over the agar. Confluent growth (1,2) or distinct colonies (3) appear after overnight cultivation. The size of the last colonies are the largest because of higher amount of nutrients per colony.

EXAMPLES OF BACTERIAL CULTURES

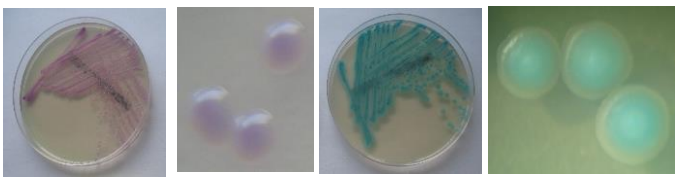


A B C D

Colonies of *Staphylococcus aureus* on a nutrient agar (A), detail (B), enrichment media – blood agar (C), detail (D)

A B C

Colonies of Enterobacteriae on a nutrient agar (e.g. Salmonella sp., *Escherichia coli*) (A), diagnostic media - Endo agar and lactose metabolizing culture of *Escherichia coli* (B) and lactose non-metabolizing culture of Salmonella species (C)



A B C D

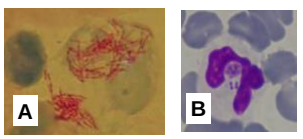
Chromogenic diagnostic media and colonies of *Candida albicans* (A),detail (B), *Candida krusei* grown on the medium (C) and detail (D)

CONDITIONS FOR CULTIVATION

Usually the **optimal temperature** and **time** for growing medically improtant bacteria, viruses and parasites are **37˚C** and **overnight**, respectively. In some special pathogens (e.g. slow-growing Mycobacteria, molds) the temperature and/or the time of cultivation can differ.
Atmosphere: **normal** for **aerobes** and **facultative anaerobes**.
Microaerophilic bacteria are cultivated in higher pressure of CO_2 (5%). **Anaerobes** are cultivated in a special thermostat **without oxygen – anaerostat.** Oxygen is toxic for them because they do not produse scavenging enzymes (e.g. catalase).

SPECIAL STAINING PROCEDURES

These staining procedures stain specifically unconventional bacteria
Acid fast bacteria & Ziehl-Neelsen staining (excercise 4)
e.g. *Mycobacterium* species (fig.A), Nocardia species
Capsules & India ink staining (Burri staining) (excercise 4)
e.g. encapsulated *Streptococcus pneumoniae, Klebsiella pneumoniae* **Bacterial spores &** (excercise 4) **Wirtz-Conklin staining**:
e.g. Bacillus species
Bacterial granules & Albert staining (excercise 4):
e.g. Corynebacterium species
Giemsa staining & intracellular bacterial pathogens: e.g. Anaplasma (fig.B), Rickettsia, Ehrlichia and Chlamydia species



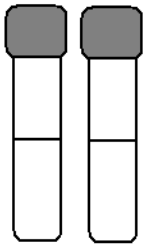
Red stained acid-fast bacilli – Mycobacterium sp. in patient sputum (A); morula of *Anaplasma phagocytophilum* inside patient neutrophil (B)

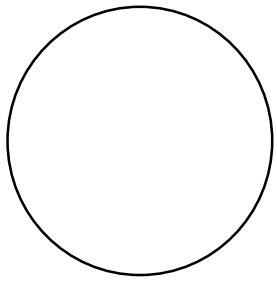
PRACTICAL PART – SPECIAL STAININGS & CULTIVATION OF INFECTIOUS AGENTS

1. INOCULATE A BROTH AND A SOLID CULTURE MEDIA WITH A CLINICAL MATERIAL. Inoculate conjunctival swab from a patient with suspected bacterial conjunctivitis in a nutrient broth (tube) and place it also onto an enrichment solid media (e.g. blood and chocolate agar) and streak it as suggested on the schema. Cultivate overnight at 37°C and read the result in the tube and on the plate. Interpret it. Schematically draw the results and add some notes you assume are the most significant.

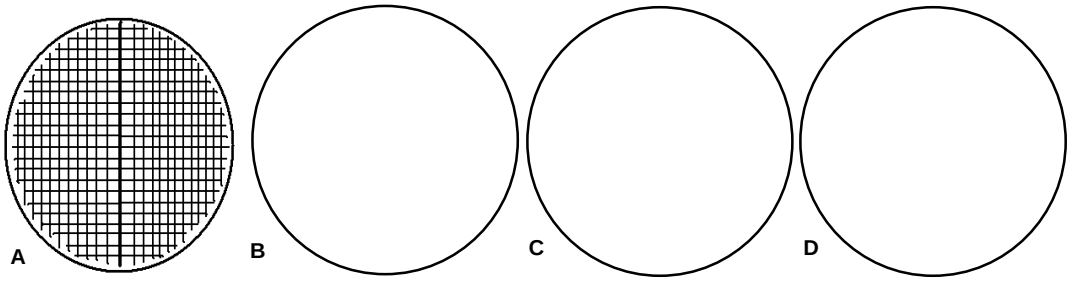
nutrient broth before inoculation (left tube)

nutrient broth after overnight cultivation (left tube)

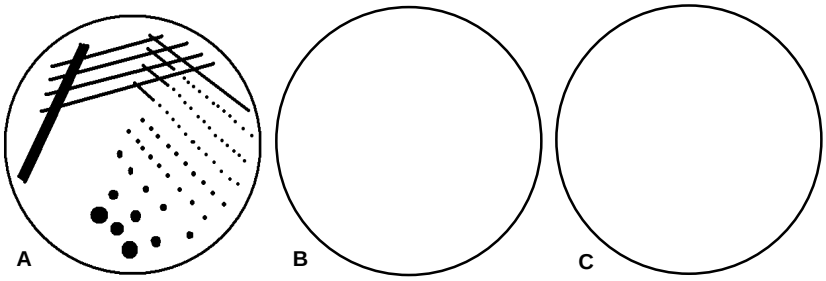




2. PLACE MIDDLE STREAM URINE FROM A PATIENT WITH SUSPECTED URINARY TRACT INFECTION ON BLOOD AGAR (B) AND ON DIAGNOSTIC MEDIA (e.g. Endo agar(C), CLED agar(C)). Use a sterile disposable loop to inoculate the urine onto the solid media (streaking the urine on all the surface by loop horinzontaly and then verticically as suggested on the figure A. Cultivate overnight at 37°C. Count grown colonies (colony forming units-CFU) per ml of the urine, try to (at least preliminary) specify the bacterial agent and interpret the results of CFU. Add some notes you assume are significant.



3. ISOLATE A SINGLE BACTERIAL COLONY AND STREAK ONTO AN AGAR MEDIA TO CULTURE A PURE CULTURE. Follow the schema below(A) to streak bacterial inoculums of Staphylococci (A) and Enterobacteriaceae (B) onto appropriate agar media. Specify the types of the media and reason why you have chosen them for the purpose. Cultivate overnight, draw the pictures, write notes.



4. Stain acid fast bacteria (*Mycobacterium* species) by Ziehl-Neelsen staining (A); capsules (e.g. *Streptococcus pneumoniae, Klebsiella pneumoniae*) by Burri staining (B), granules (Corynebacterium sp.) by Albert staining (C) and bacterial spores (e.g. Bacillus) by Wirtz-Conklin staining (D) following steps for the particular method (instructions on your bench). Draw the results and describe the principle.

LAB QUIZ

1. **How you could distinguish a sterile nutrient broth from one with a grown culture?**

2. **Dilution of a bacterial inoculum by bacteriological loop is essential to cultivate ……colonies which are essential for further ……of bacterial species.**

3. **Diagnostic media are based usually on two of their properties. They contain some ingredients which interfere with growth of some bacterial species so they ……growth of the other species, and because the latter species metabolize some of ingredients the species could be prelimnary diagnosed on basis of the ……of their colonies.**

4. **Could viruses or intracellular bacteria be cultivated on axenic (artificial) media? Why?**

5. **Describe the principle of Ziehl-Neelsen staining procedure.**