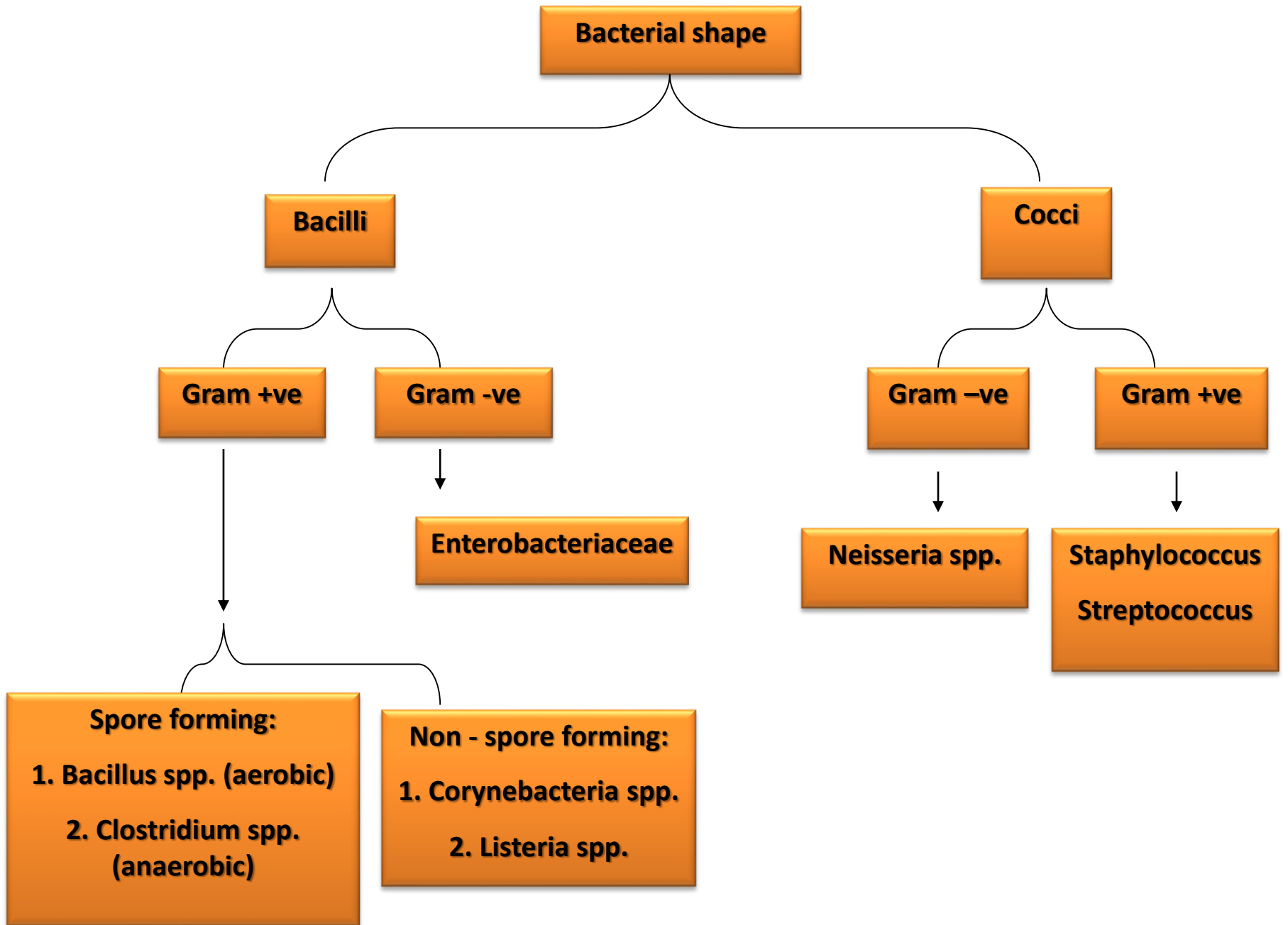


Corynebacteria





Corynebacteria

```
graph TD; A["Corynebacteria"] --> B["Pathogenic spp."]; A --> C["Non-pathogenic spp."]; B --> D["C. diphtheriae"]; C --> E["Diphtheriod  
(Normal URT)  
C. ulcerans & C. urealyticum"]; style A fill:#d3d3d3,stroke:#000,stroke-width:1px; style B fill:#d3d3d3,stroke:#000,stroke-width:1px; style C fill:#d3d3d3,stroke:#000,stroke-width:1px; style D fill:#d3d3d3,stroke:#000,stroke-width:1px; style E fill:#d3d3d3,stroke:#000,stroke-width:1px;
```

A hierarchical flowchart showing the classification of Corynebacteria. The root node is 'Corynebacteria' (underlined and italicized). It branches into two categories: 'Pathogenic spp.' and 'Non-pathogenic spp.'. 'Pathogenic spp.' leads to '*C. diphtheriae*'. 'Non-pathogenic spp.' leads to 'Diphtheriod', which is noted as '(Normal URT)' and includes '*C. ulcerans* & *C. urealyticum*'.

**Pathogenic
spp.**

C. diphtheriae

**Non-
pathogenic
spp.**

Diphtheriod

(Normal URT)

C. ulcerans & *C. urealyticum*

Corynebacterium: Habitat

- ◆ Skin
- ◆ upper respiratory tract
- ◆ GI tract
- ◆ Urogenital tract of humans

Corynebacterium: Pathogens

- ◆ *C. diphtheriae*
Diphtheria
- ◆ *C. pseudotuberculosis*
humans sheep, cattle,
suppurative
lymphadenitis
- ◆ *C. ulcerans* humans
pharyngitis
cattle -mastitis
- ◆ *C. haemolyticum*
pharyngitis cutaneous
infection
- ◆ *C. pyogenes* cattle,
sheep, swine
suppurative infection
- ◆ *C. pseudodiphtheriticum*
endocarditis

Related Organisms

Listeria monocytogenes

– **Listeriosis**

◆ *Erysipelothrix rhusiopathiae*

– **Erysipeloid**

Diphtheria

Corynebacterium

- ◆ Aerobic gram + rods
- ◆ -pleomorphic: club-shaped
- ◆ -pallisades
- ◆ -snapping cell division
- ◆ -metachromatic granules
 - methylene blue stain
 - volutin: polyphosphate

C. diphtheriae

Also called Kleb's Loeffler Bacilli (KLB).

Aerobic

non-spore

non – motile

Gram +ve bacilli.

lose Gram's stain easily .

Morphology

- Gram positive bacilli. 3-6 μ x 0.5-0.8 μ .
- V or K or L shape.
- Chinese letter pattern, angular ['æŋgjələər] arrangement



Chinese-letter morphology in Gram stain

Morphology

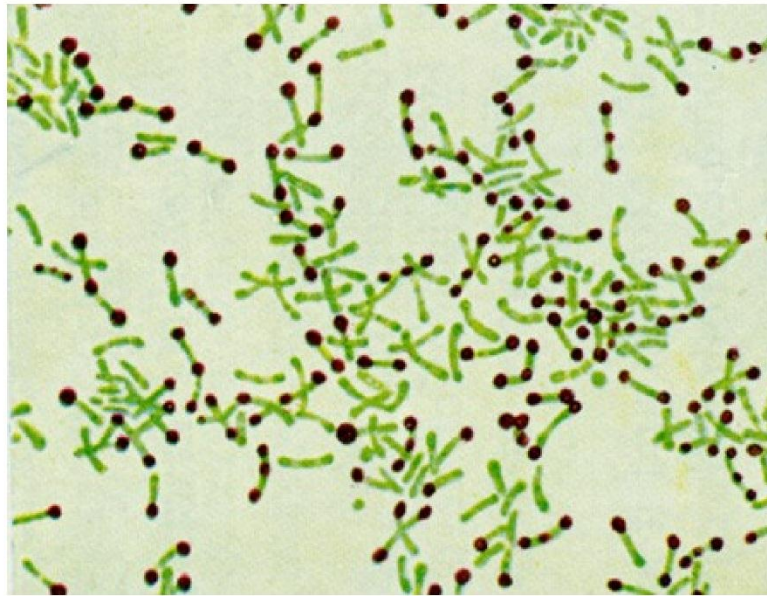
- Metachromatic 因温变色的 granules. volutin ['vɒljətɪn] 迂回体 granules, polymetaphosphate energy storage depots ['di:pəʊz]



methylene blue staining

Morphology

- Alberts stain – green and bluish black
- Nonmotile noncapsulated, nonsporing
- pleomorphic



Cellular Morphology

- ◆ Gram positive rods
- ◆ “Snapping division”
- ◆ Palisade cells
- ◆ “Chinese letters”





Corynebacterium diphtheriae cells stained by Albert's technique The barred appearance is due to the presence of volutin. Note also the characteristic Chinese-letter arrangement

Specialized media

Tellurite:

black colonies

Not diagnostically
significant

tellurite inhibits many
organisms but not *C.
diphtheriae*

Loeffler

best colonial morphology

Dextrose horse serum
(1887)

now Dextrose beef serum

Special stains:

Albert stain → DARK granules, GREEN bacilli

Neisser stain → redish- purple granules, colorless bacilli.

Biotypes

1) gravis(13 types)-most serious disease

Colonies: large, irregular, gray

2) mitis(40 types)-mild illness

colonies: small, round, convex, black

3) intermedius(4 types)-intermediate severity

Colonies: small, flat and gray

Classification

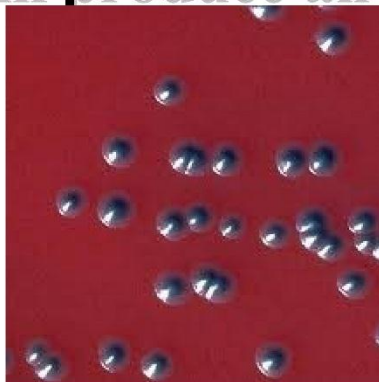
3 morphological types of *C. diphtheriae* are found on tellurite containing media:

△ **Mitis** ['maitis]– black colonies with a gray periphery

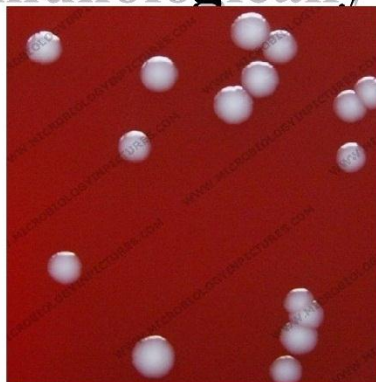
△ **Gravis** – large, gray colonies

△ **Intermedius** – small, dull gray to black.

All produce an immunologically identical toxin



Mitis



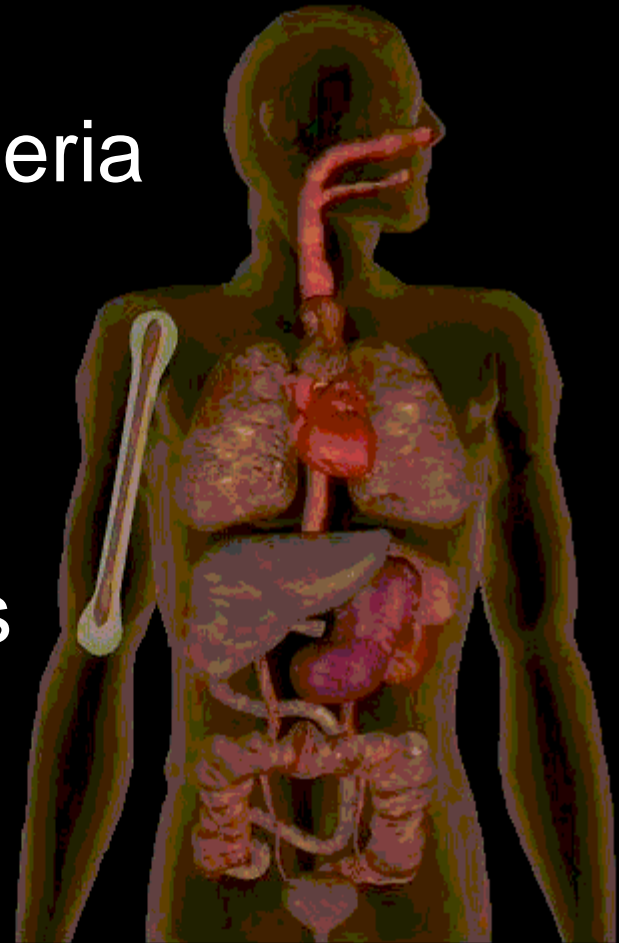
Gravis



Intermedius

Diphtheria

- ◆ Nasopharyngeal diphtheria
 - Pharyngeal
 - Laryngeal
- ◆ Cutaneous diphtheria
- ◆ Systemic complications



DISEASES

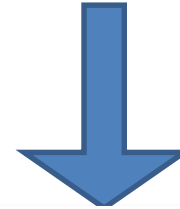
LOCAL INFECTIONS:



Respiratory diphtheria
(production of exotoxin)

Cutaneous diphtheria

SYSTEMIC



Necrosis of organs,
Nerve damage.

Pharyngeal diphtheria

- ◆ Inflammation
 - similar to strept throat
- ◆ Leucocytes
 - infiltrated
 - killed
 - embedded in fibrin clot
- ◆ TOXIN !!



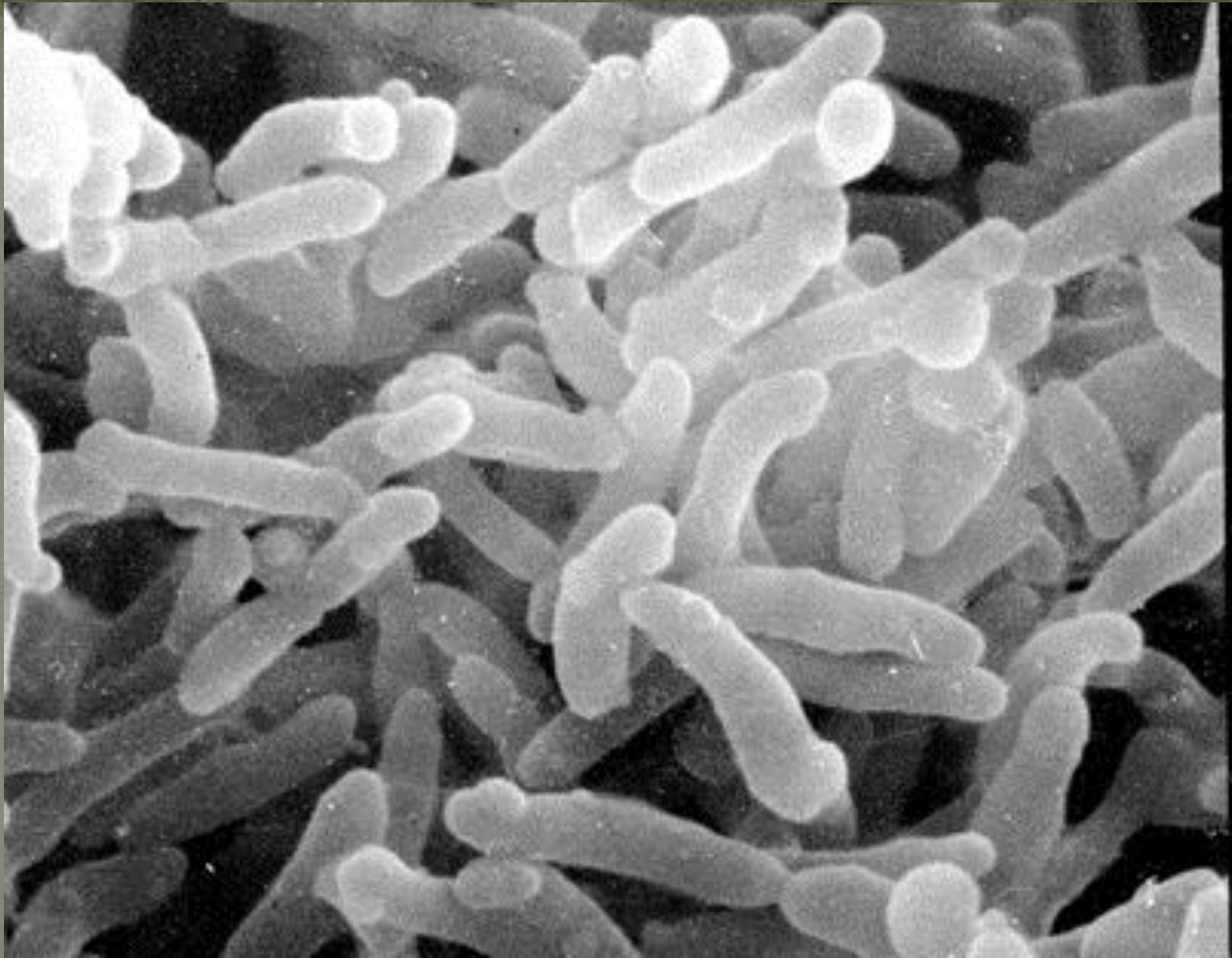


**10 y/o boy with
severe diphtheria**

- ◆ conjunctivitis
- ◆ pharyngeal membrane
- ◆ bull neck
- ◆ severe myocarditis
- ◆ all vaccines contraindicated



Diphtheria



EM of Corynebacterium

Diphtheria Symptoms

- ◆ Pharyngitis
- ◆ Hypoxia
 - Choking
 - “Garitillo”
- ◆ Fever (103 F)
- ◆ Lymphadenitis



ALL SIGNS & SYMPTOMS CAUSED BY TOXIN

Bacterial

**Come to the
Health Center**

Swollen
uvula

Whitish
spots

Red swollen
tonsils

Throat
redness

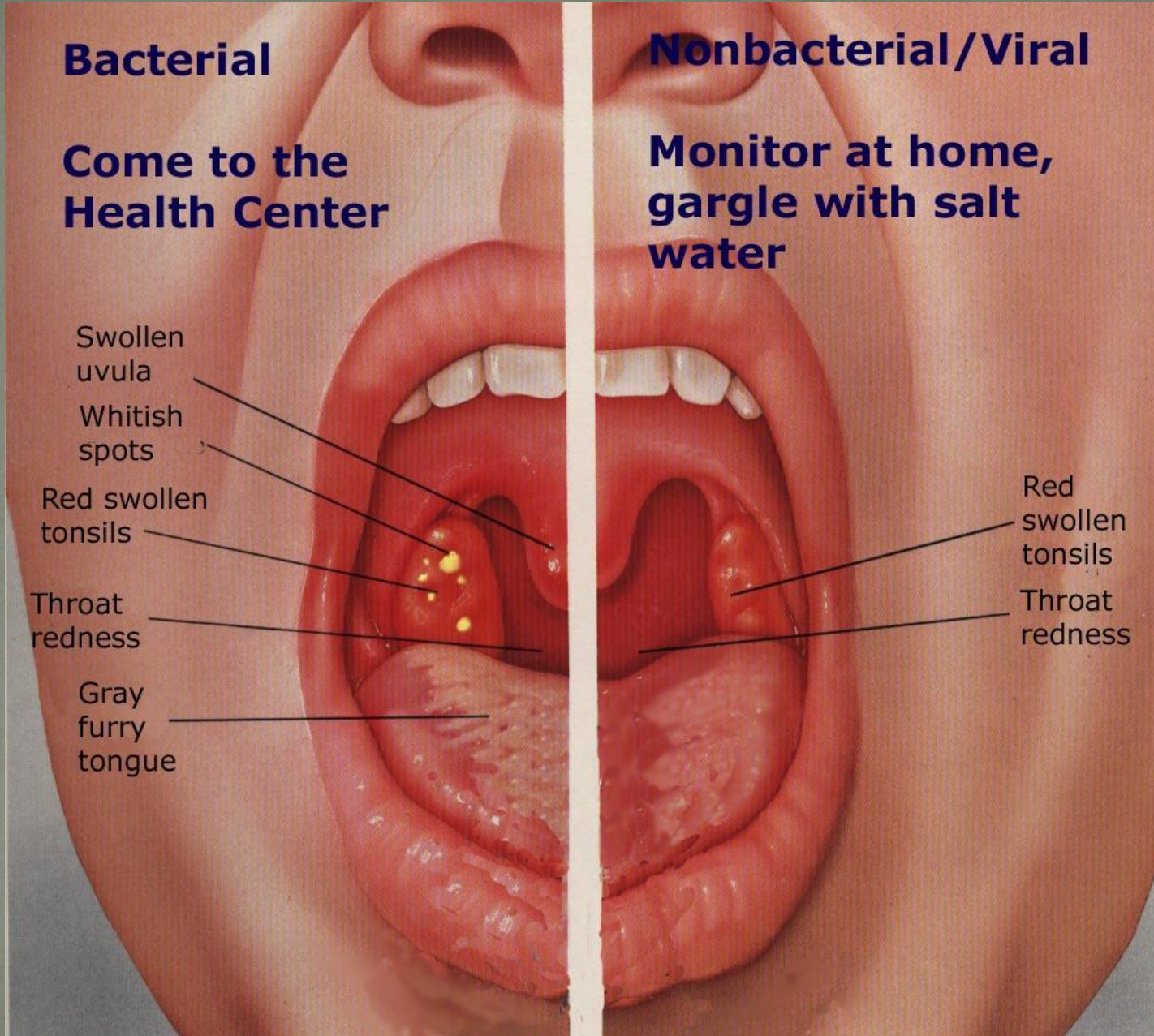
Gray
furry
tongue

Nonbacterial/Viral

**Monitor at home,
gargle with salt
water**

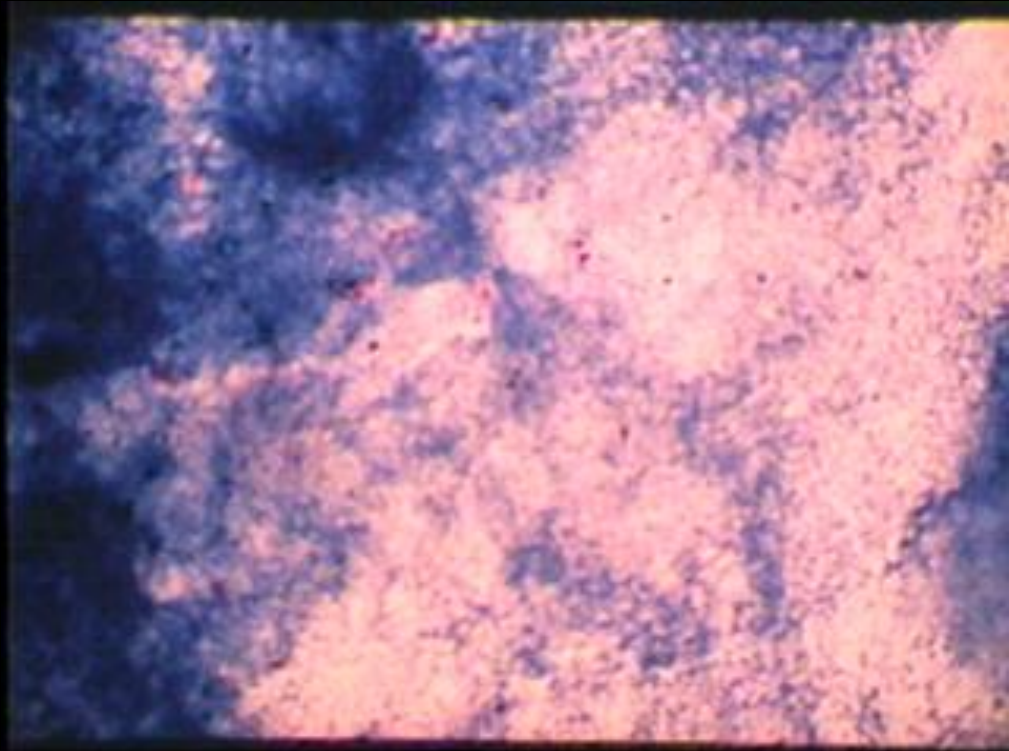
Red
swollen
tonsils

Throat
redness



Diphtheria Pseudomembrane

- ◆ No True membrane
- ◆ Very few live cells
- ◆ Deposit of dead cells and protein



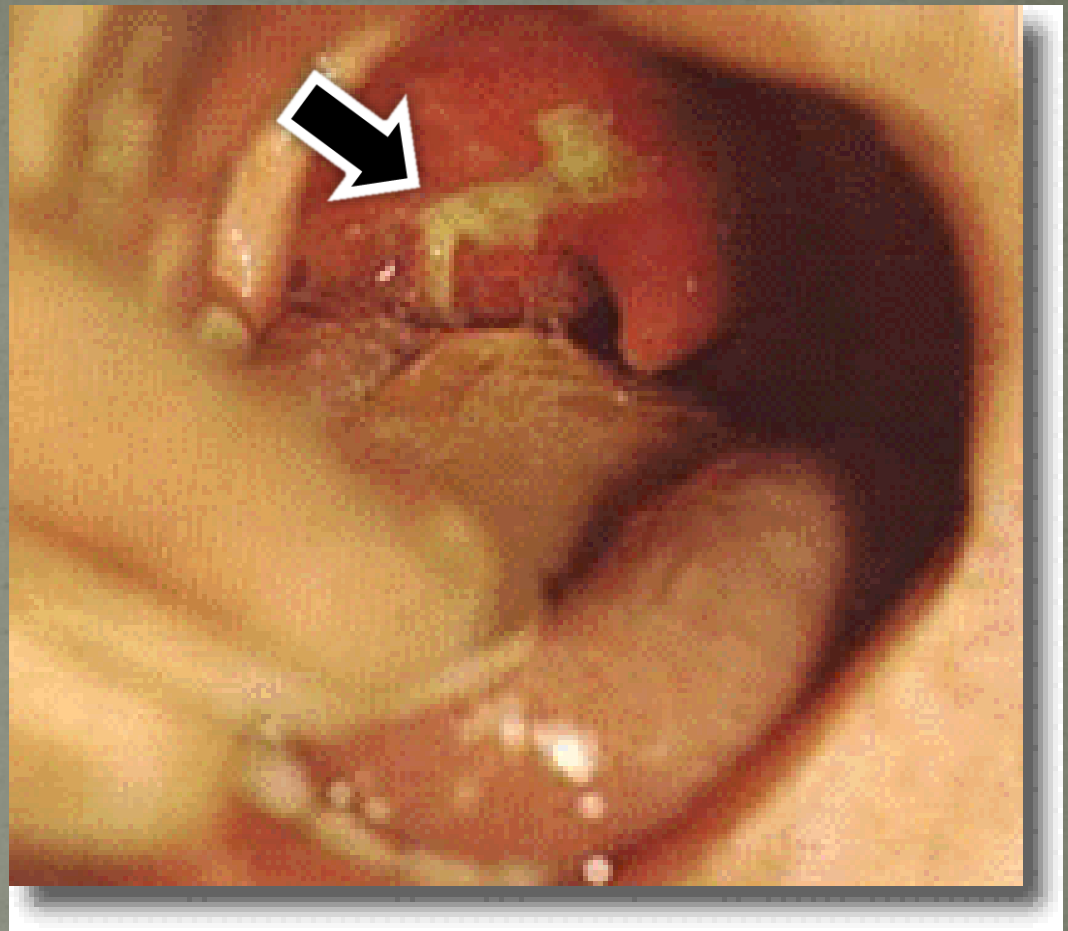
Pseudomembrane

◆ CONTAINS

- bacteria
- lymphocytes
- plasma cells
- fibrin
- dead cells

◆ COVERS

- tonsils,
- uvula,
- palate
- nasopharynx
- larynx.



Pseudomembrane of diphtheria



Pseudomembrane of diphtheria

Diphtheria

Systemic complications

◆ Nerves

- toxic peripheral neuropathy
- paralysis of short nerves
- mouth, eye, facial extremities

◆ Cardiac

- Congestive heart failure
- high amount of toxin 48-72 hours
- Low amount of toxin 2-6 weeks

Diagnostics criteria:

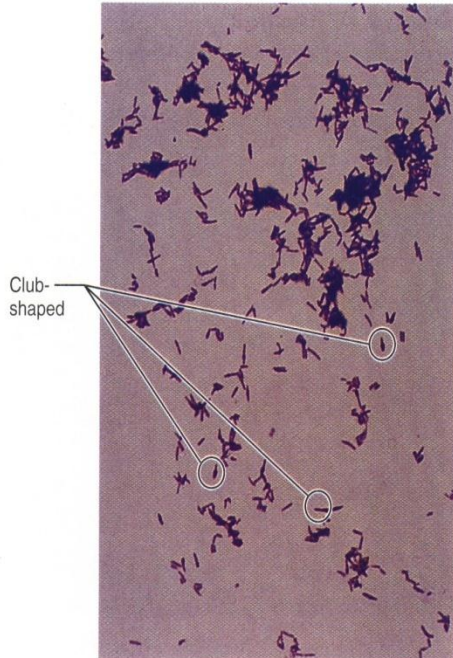
- 1- Metachromatic granules (volutin).**
- 2- Club – shaped (swallow at one end)**
- 3- Arrange in parallel or in acute angle, so called Chinese letters according to its appearance.**

Laboratory Diagnosis

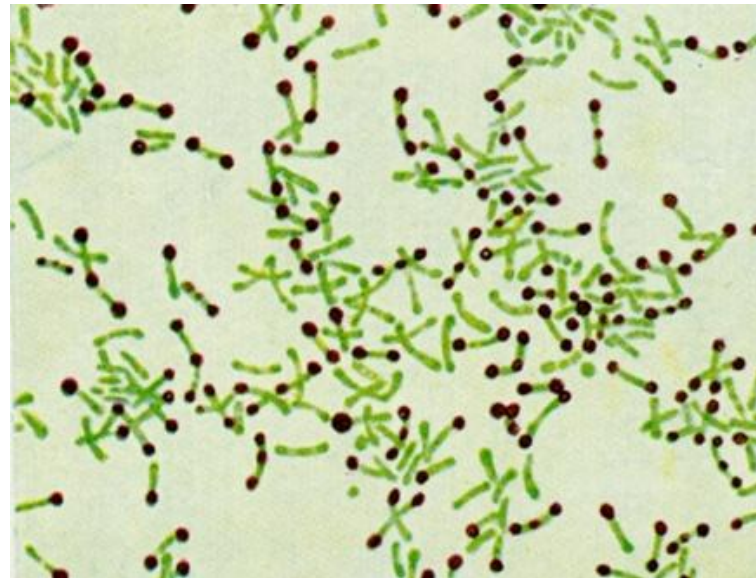
1. **Specimens :** Throat swab.
Skin swab.

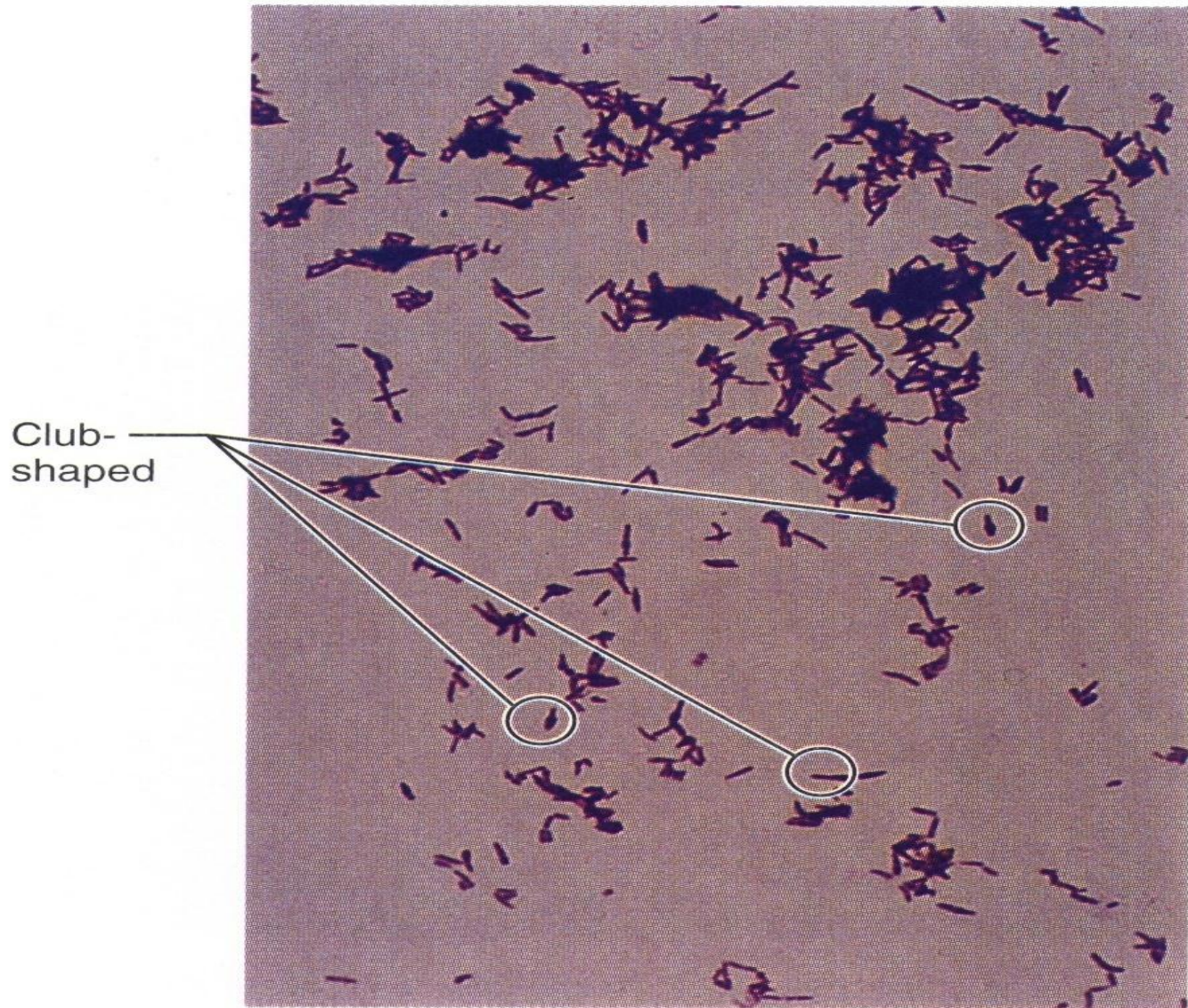
2. **Staining:**

Gram's stain → G + ve
bacilli, Chinese letter.



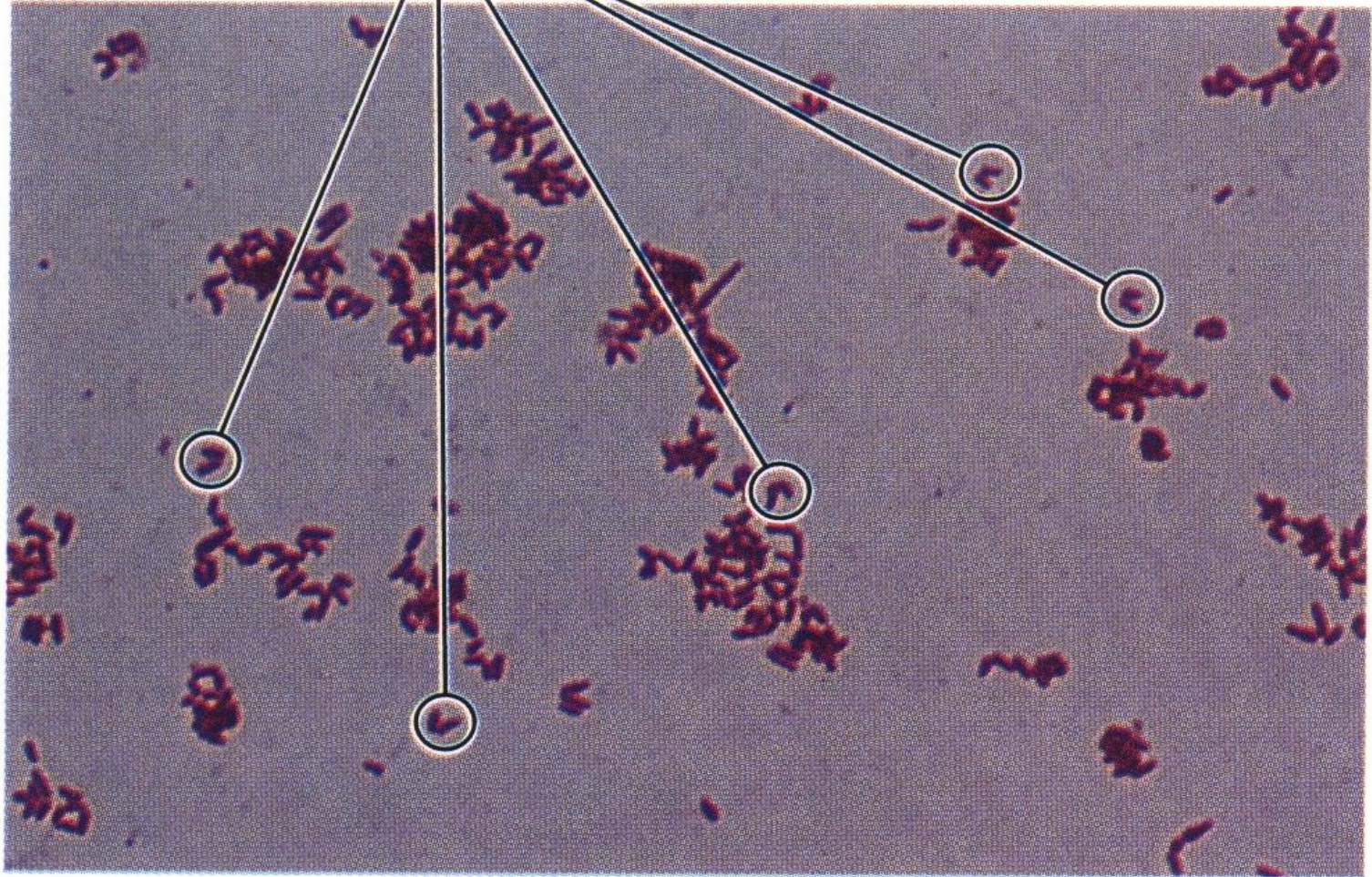
Albert's stain → Metachromatic
granules (dark), bacilli (green).





Gram's staining for Corynebacterium

V-shapes



Gram's staining for Corynebacterium



Gram's staining for Corynebacterium

3. Culture:

a. Loeffler's agar. (Enriched media only)
(12-18hr): contains serum or egg



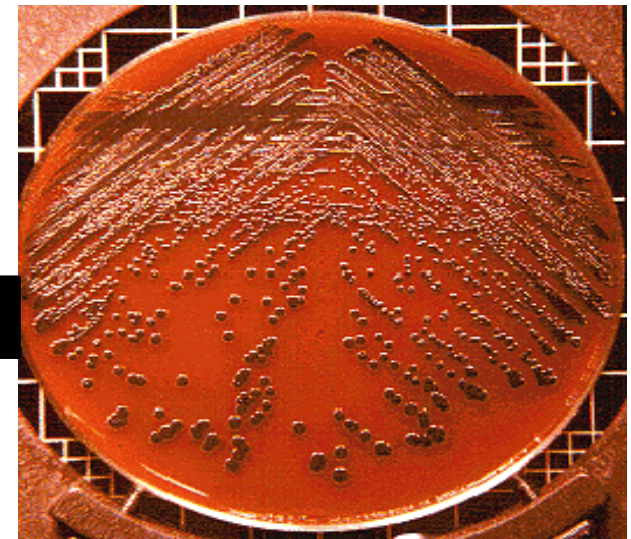
b. K⁺ tellurite blood agar (selective & enrichment media). 48hr

Black colonies

Colonies appear gray-black due to tellurite reduction



telluride



Corynebacterium diphtheriae, mitis
Chocolate tellurite agar

C. Can grow on Blood or chocolate agar:



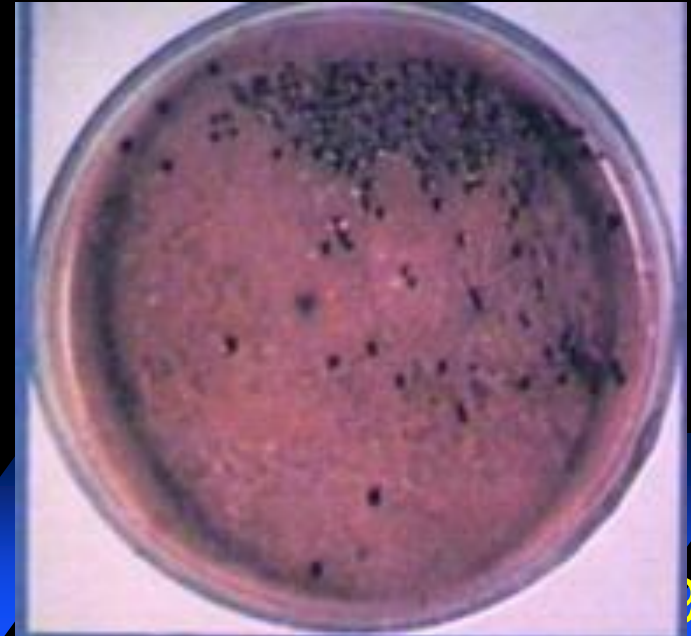
Corynebacteria on blood agar The bacteria grow into convex and semi-opaque colonies.



Loeffler medium

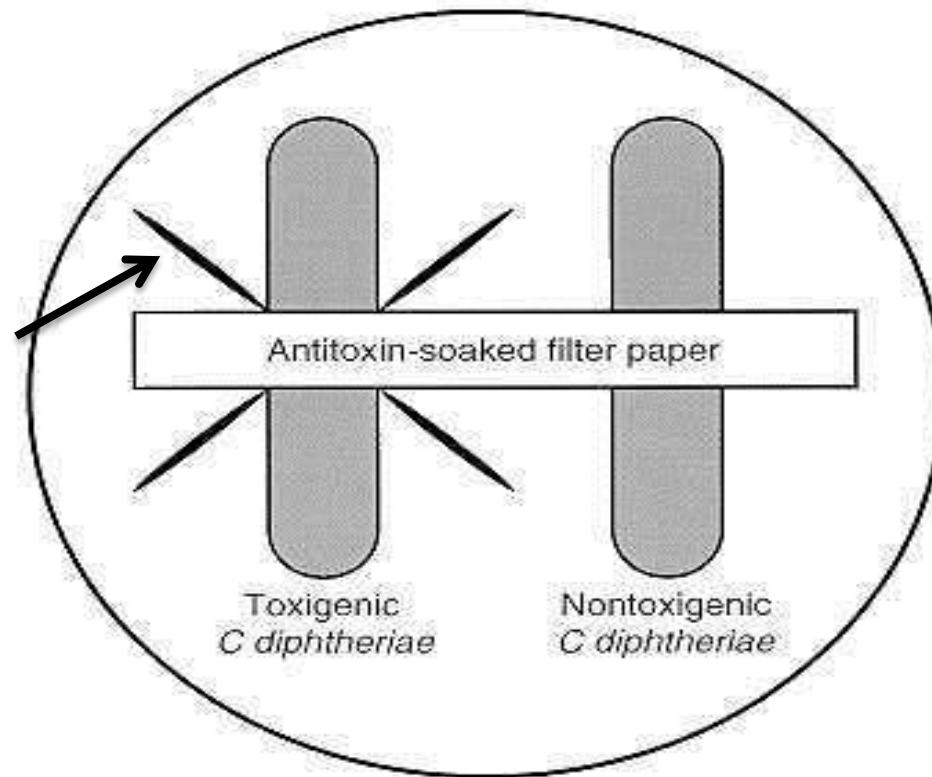
Blood tellurite

- ◆ Selective & differential medium
- ◆ Corynebacteria are resistant to tellurite
 - Reduced to tellurium
- ◆ Forms deposit in colonies
 - Colonies appear dark
- ◆ Biotypes
 - gravis, intermedius, mitis



4. Virulence test (Toxin production test):

- a. Guinea pigs lethality
- b. Gel-diffusion test (ELICK test):





Gel-diffusion test (ELICK test):

c. (PCR).

d. (ELISA).

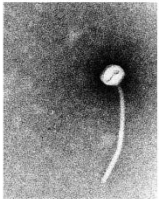
e. Immunoblot (immunochromogenic method).

Pathogenicity

- Produces exotoxin
- Lysogenic conversion with beta phage
- Toxin – heat labile protein
- A and B fraction
- Toxicity- disease
- Antigenicity- immunity
- Toxoid – toxin without toxicity

Pathogenesis

For *C. diphtherias* to cause diphtheria an exotoxin must be produced.



△ Is a heat-labile polypeptide produced during lysogeny [laɪ'sɒdʒəni:] of a β phage that carries the "tox" gene.

△ Alkaline pH of 7.8- 8.0, aerobic conditions, and a low environmental iron level are essential for toxin production (occurs late in the growth of the organism).

△ The toxin inhibits protein synthesis by ADP-ribosylating elongation factor 2[EF-2].

Virulence Factors

◆ Diphtheria toxin !!!

- blocks protein synthesis

◆ Dermonecrotic toxin

- sphingomyelinase
- increases vascular permeability

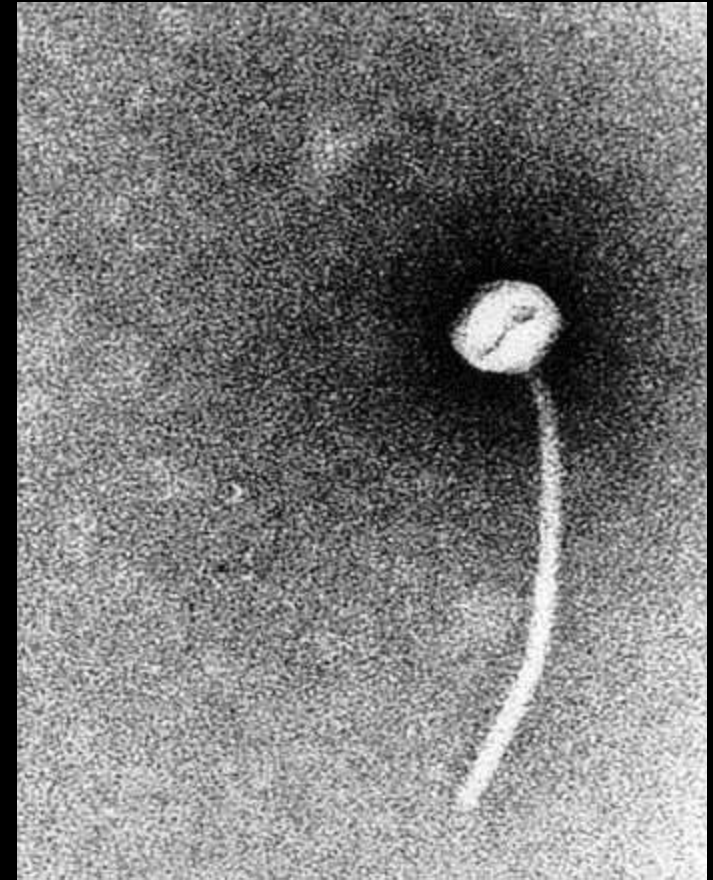
◆ Hemolysin

◆ Cord factor -Toxic trehalose

- corynemycolic acid, corynemyolenic acid
- 6,6'-di-O-mycoloyl- α,α' -D-trehalose

Diphtheria Toxin

- ◆ Blocks protein synthesis
- ◆ Protein 63Kd
- ◆ controlled by Tox gene
- ◆ lysogenic phage Beta-corynephage
- ◆ expressed if [iron] low
- ◆ 2 components A-B



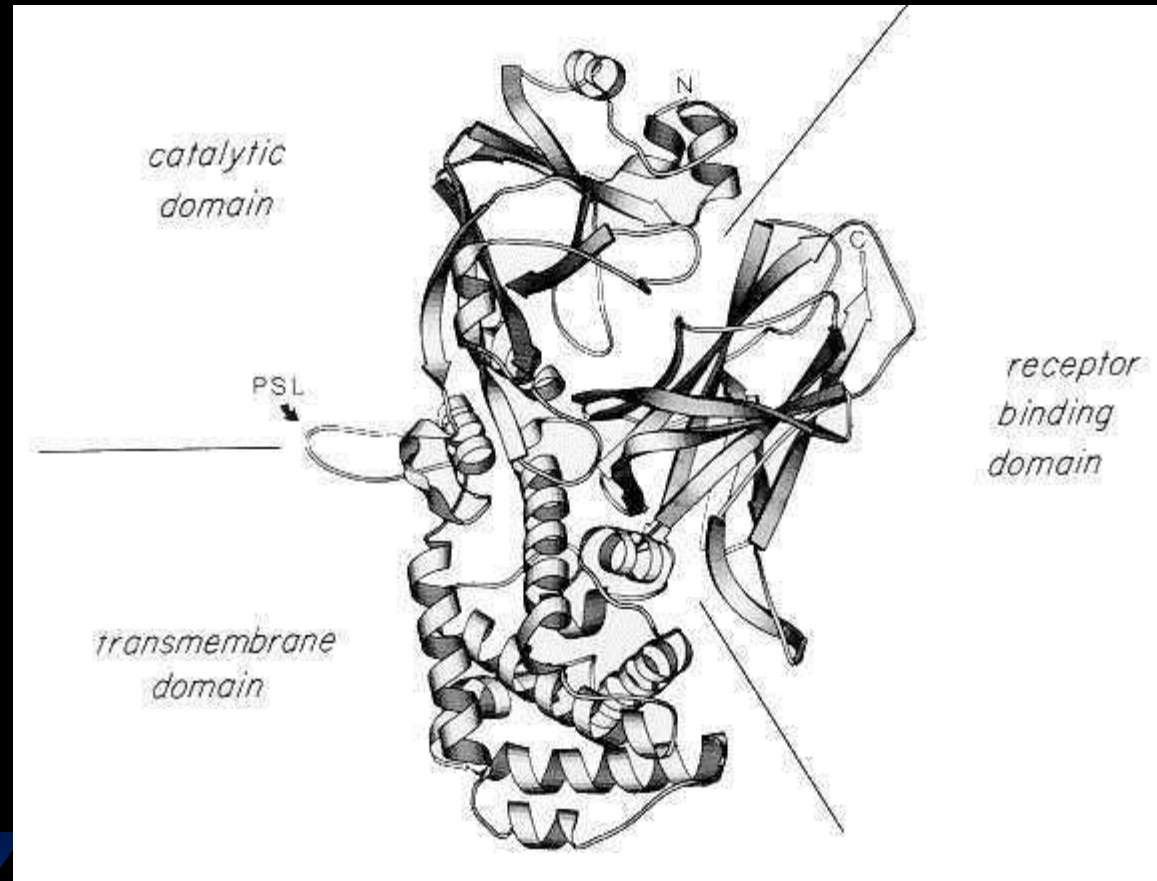
Toxin

◆ Part A

- Active site
- N terminal
- Enzyme

◆ Part B

- Binding site
- Binds to membrane receptor
- Transmembrane



Toxigenicity Tests

In Vitro Elek test

In Vivo Animal inoculation

rabbit skin test-necrosis

guinea pig challenge test- lethal

Toxigenicity test (virulence test)

i) Animal inoculation

-bacteria culture emulsified [ɪ'mʌlsɪfaɪ] 使乳化 in water and 0.8 ml injected into 2 guinea pigs

GP A-has diphtheria antitoxin (injected 2 hours before)

GP B-Doesn't have antitoxin

Result: Guinea pig B dies.

ii) Elek's gel precipitation test

-filter paper saturated with antitoxin is placed on agar plate with 20% horse serum

-bacterial culture streaked [stri:k] 条痕 at right angles to filter paper

iii) tissue culture test

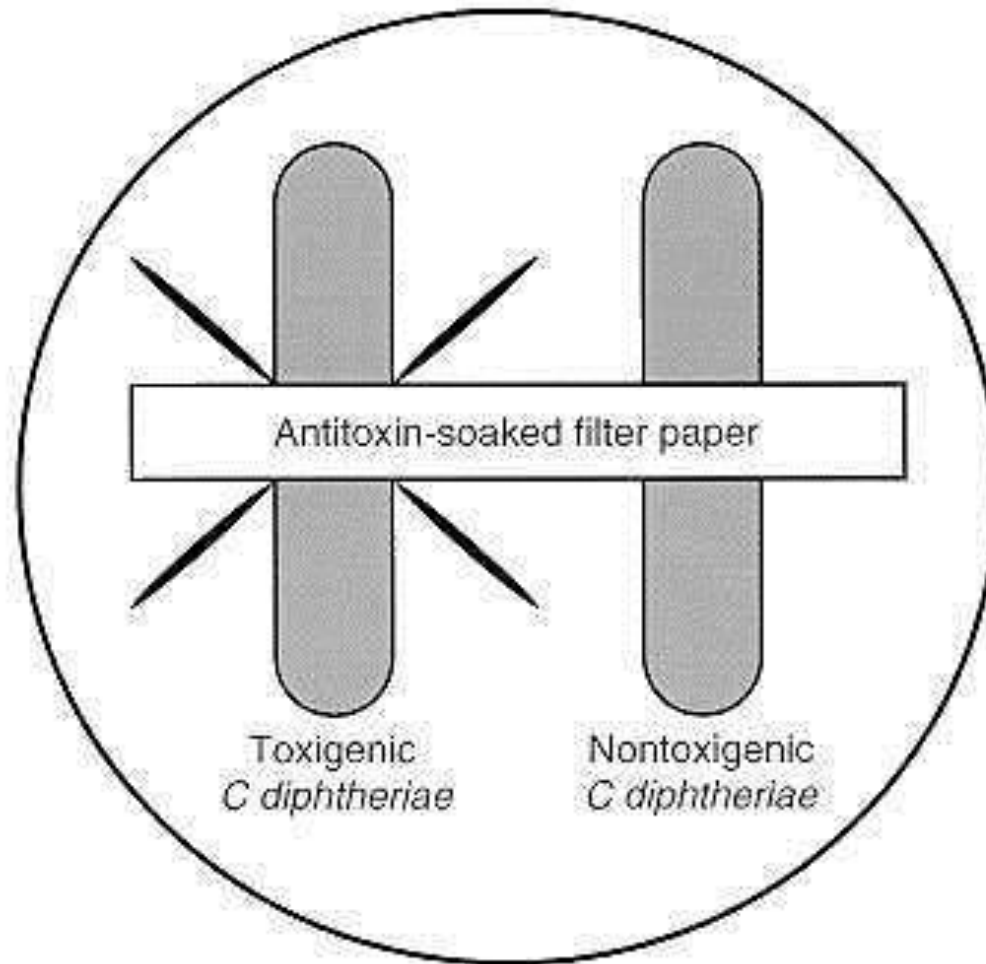
-incorporation of bacteria into agar overlay of eukaryotic cell culture monolayers.

Result: toxin diffuses into cells and kills them

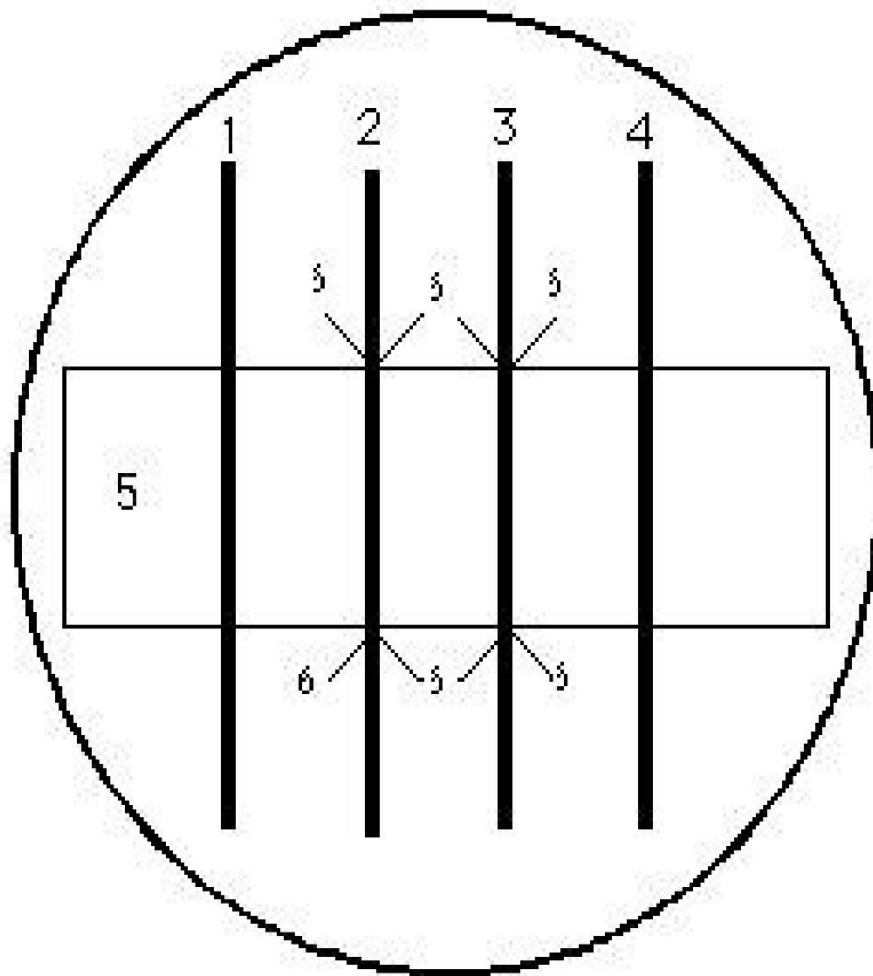
iv) PCR assays

-test presence of specific bacteriophage gene (tox)

Elek test



Elek's gel precipitation test



1 = *C. diphtheriae* (non-toxigenic)
negative control

2 = *C. diphtheriae* test organism

3 = *C. diphtheriae* (toxigenic)
positive control

4 = *C. diphtheriae* test organism

5 = ANTITOXIN STRIP

6 = lines of precipitate

Animal inoculation

Inject 2 mice with 5ml *C.diphtheria* cells
one mouse protected with 1000 units
C.diphtheriae antitoxin

Autopsy - adrenals hemorrhagic

Schick Test for Diphtheria



Complications

- 1)Asphyxia [æs'fɪksɪə] 窒息 -obstruction of resp tract
- 2)Acute circulatory failure
- 3)kidney failure
- 4)paralysis-soft palate, eye muscles, extremities (3rd-4th week)
- 5)septic sequelae-pneumonia, otitis media

Transmission:-

Respiratory air droplets, contact with cutaneous lesion and /or contaminated objects.

Treatment:-

1. Antitoxin ----- Neutralize toxin.
2. Antibiotics ----- Erythromycin to eliminate bacteria.

Protection:-

Vaccines (DPT), booster dose DT after 10 years.

Control

- ◆ Immunization diphtheria toxoid
- ◆ Schick test
 - check for antibodies
- ◆ Passive immunity
 - Antibodies
- ◆ Antibiotics
 - Penicillin & erythromycin

Epidemics

- ◆ Immune individuals
 - may be carriers
 - antibiotics
- ◆ Non immune individuals
 - Exposed
 - ❖ passive immunity antibodies
 - Not exposed
 - ❖ immunize with toxoid

The End

