C.diphtheriae

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Corynebacteria

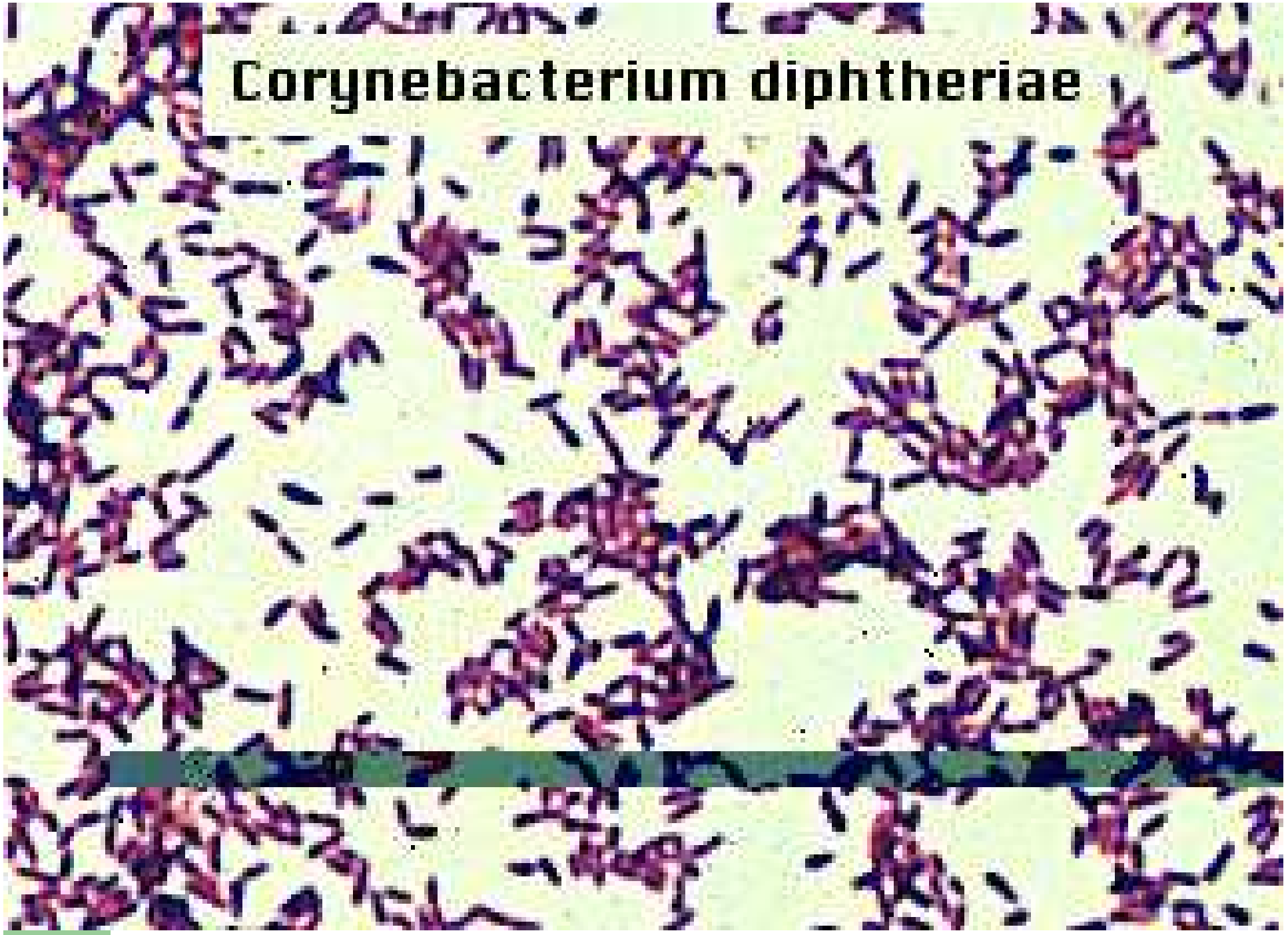
- Corynebacteria are closely related to mycobacteria and nocardiae.
- They are gram positive non sporing rods, club shaped, containing volutin granules, nonmotile, non-capsulate and non acid fast, aerobic or facultative anaerobic, catalase positive and Oxidase negative.
- Human pathogens include C.diptheriae, C.ulcerans and C.pseudotuberculosis.

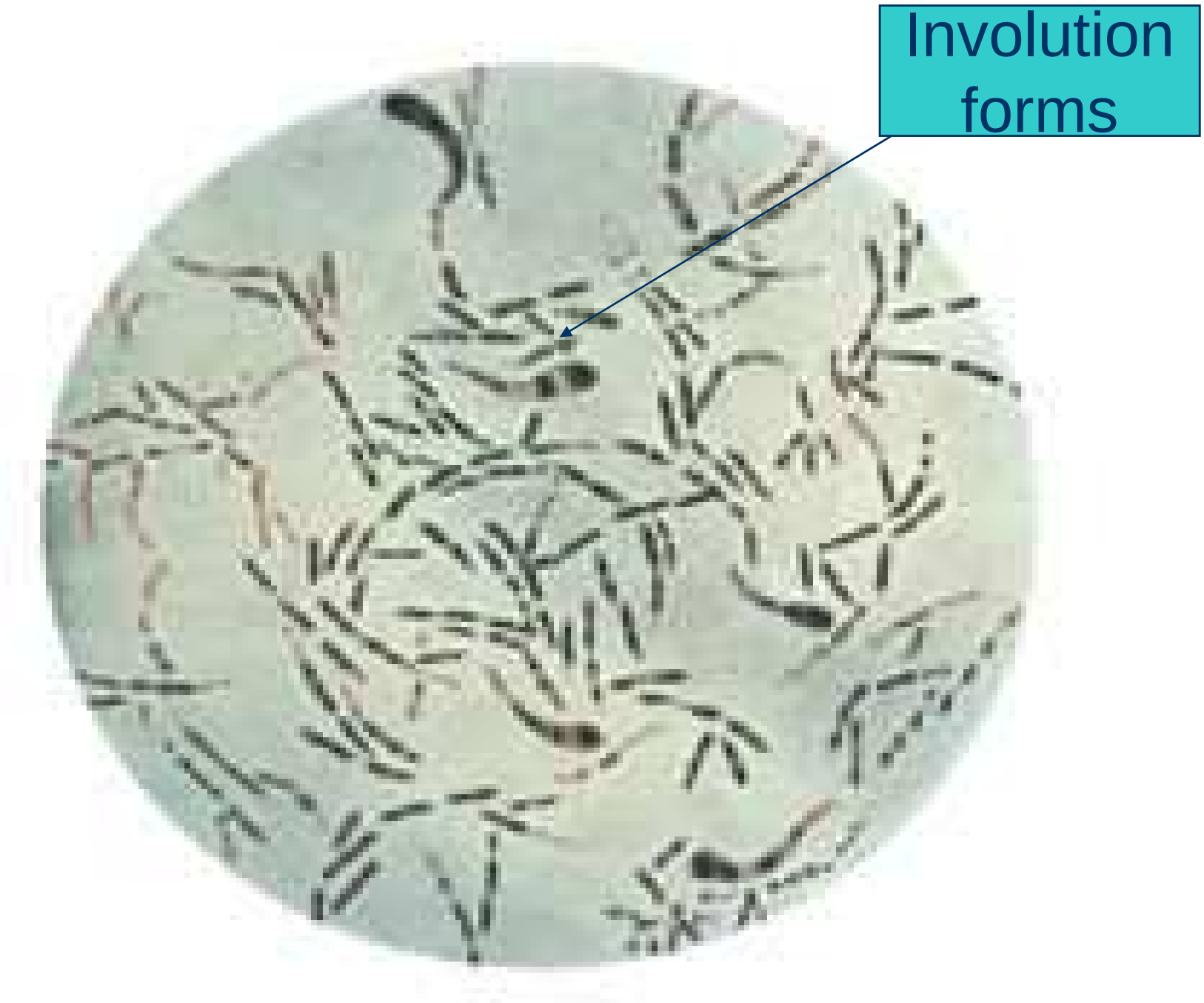
History

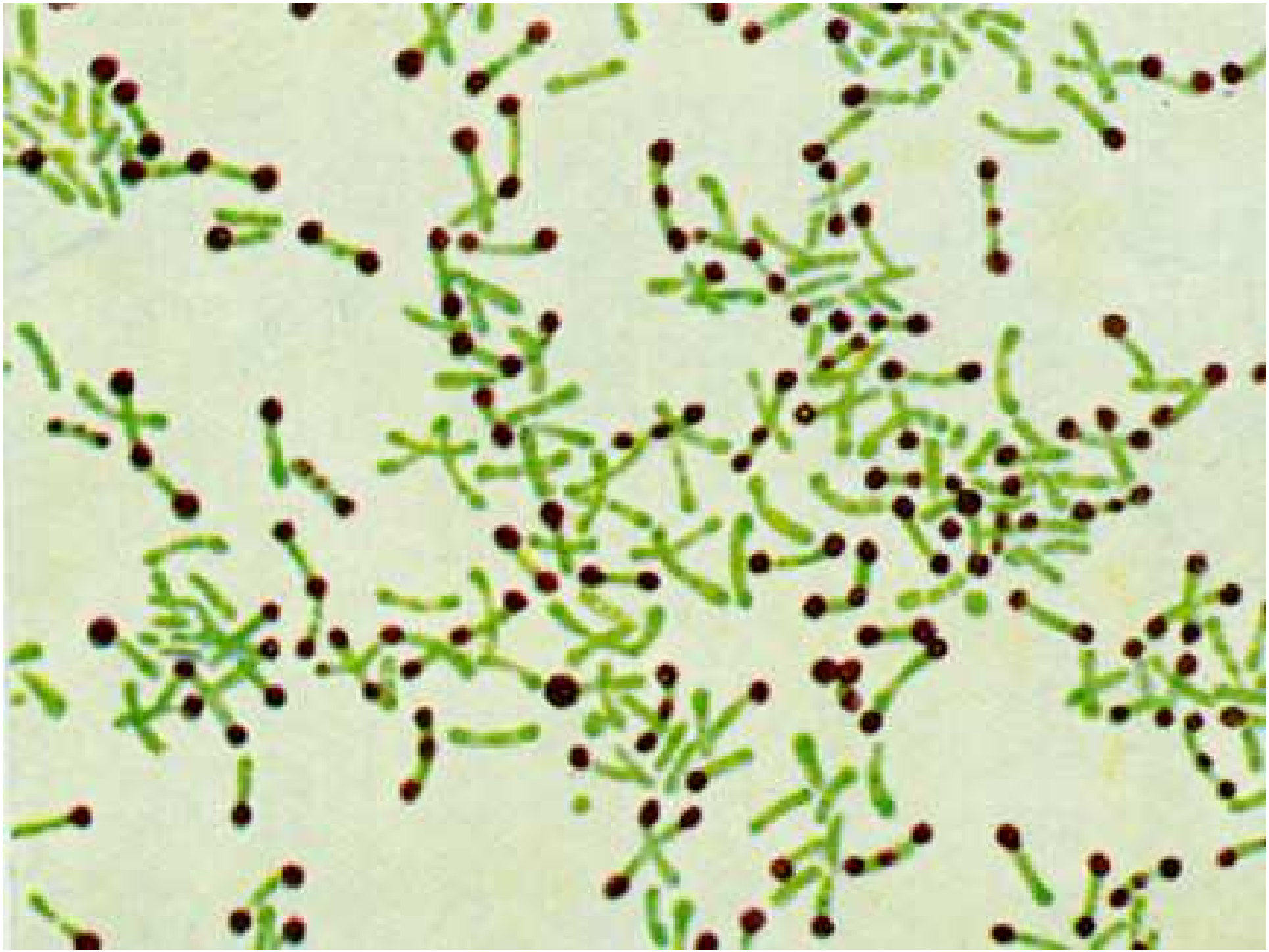
- Bretonneau(1826) : First recognized diphtheria as a clinical entity who called it `diptherite`(diphtheros, meaning leather).
- Klebs(1883) : First observed diphtheria bacillus.
- Loeffler(1884) :First cultivated
- Roux Yersin(1888) :Discovered diphtheria exotoxin and established its pathogenic effects.

Morphology

- Size : 3-6µm x 0.6-0.8µm.
- Slender rod with tendency to clubbing at one or both ends.
- Pleomorphic
- Nonsporing, non capsulated, nonmotile.
- Gram positive but tend to be decolorized easily.
- Contain metachromatic granules (volutin).
- Special stains : Albert's, Neisser`s and Ponder`s
- Arrangement : Chinese letter or cuneiform


Corynebacterium diphtheriae


Involution forms



Cultural characteristics

- Enrichment with blood, serum or egg is necessary for good growth.
- Optimum Temp. is 37^{o} C (range 15-40^{o}C)
- Aerobe and facultative anaerobe.
- Usual media for cultivation of diphtheriae bacilli are Loeffler's serum agar and Tellurite blood agar.
- On Loeffler's serum, grow very rapidly within 6-8 hrs with yellow tint.

Loeffler's Serum slope

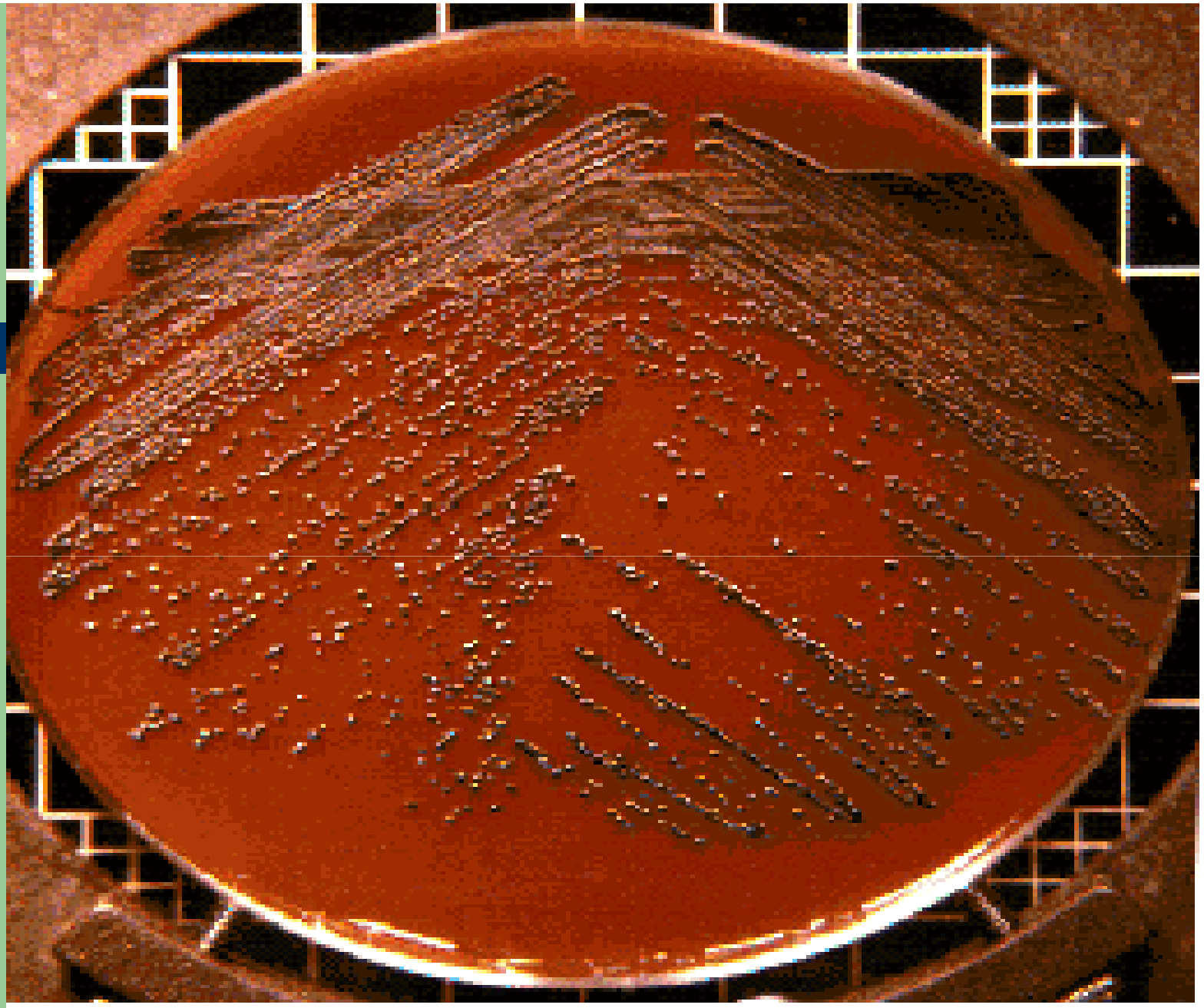
Cultural characteristics cont..

- On Tellurite blood agar, black colored colonies developed after 48hrs incubation.
- The addition of cystine to a tellurite-containing medium makes Tinsdale`s medium.
- McLeod classified diphtheriae bacilli in three types- gravis, intermedius and mitis.

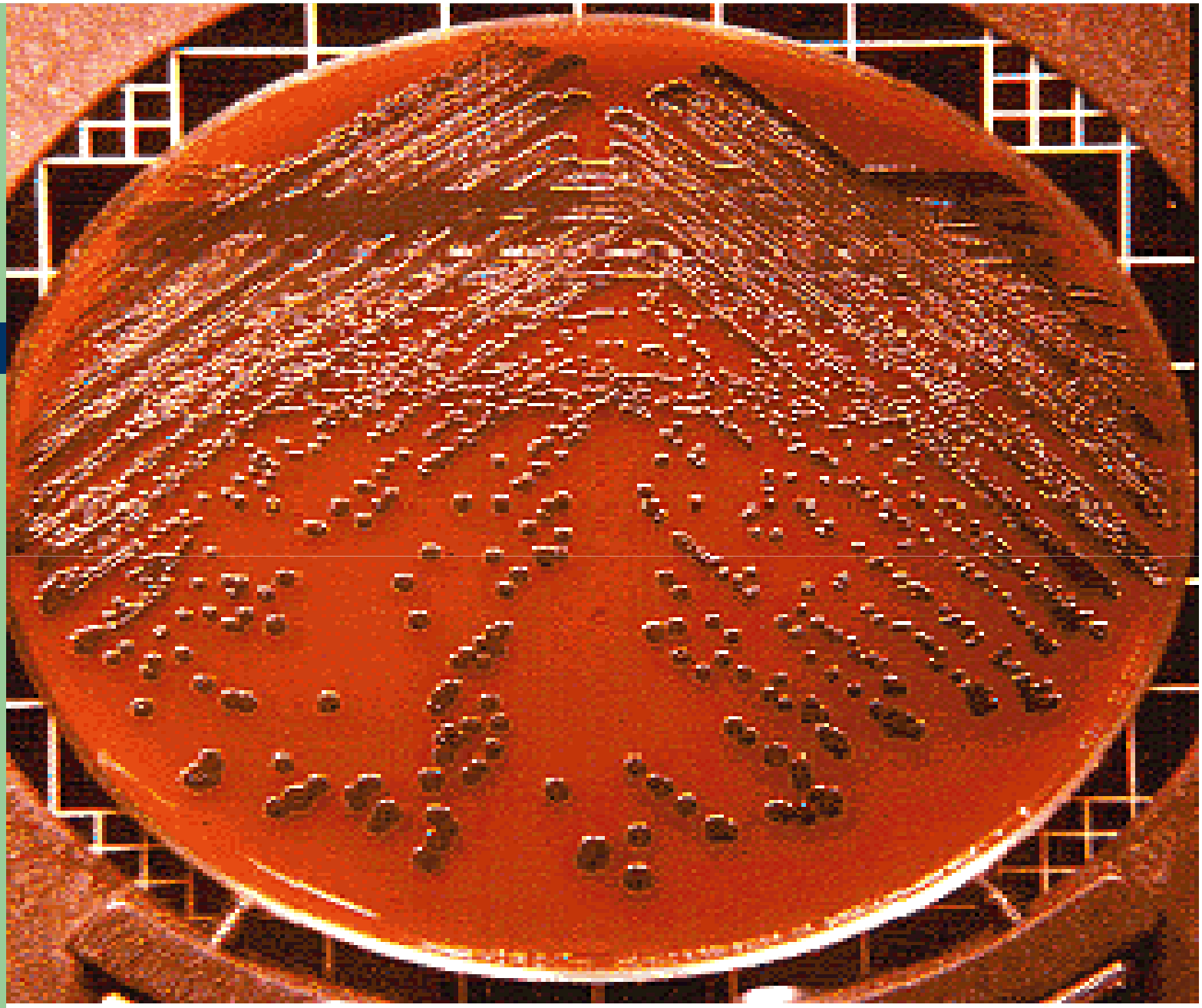

Black colour colony
Of C.diphtheriae

Cultural characteristics cont..

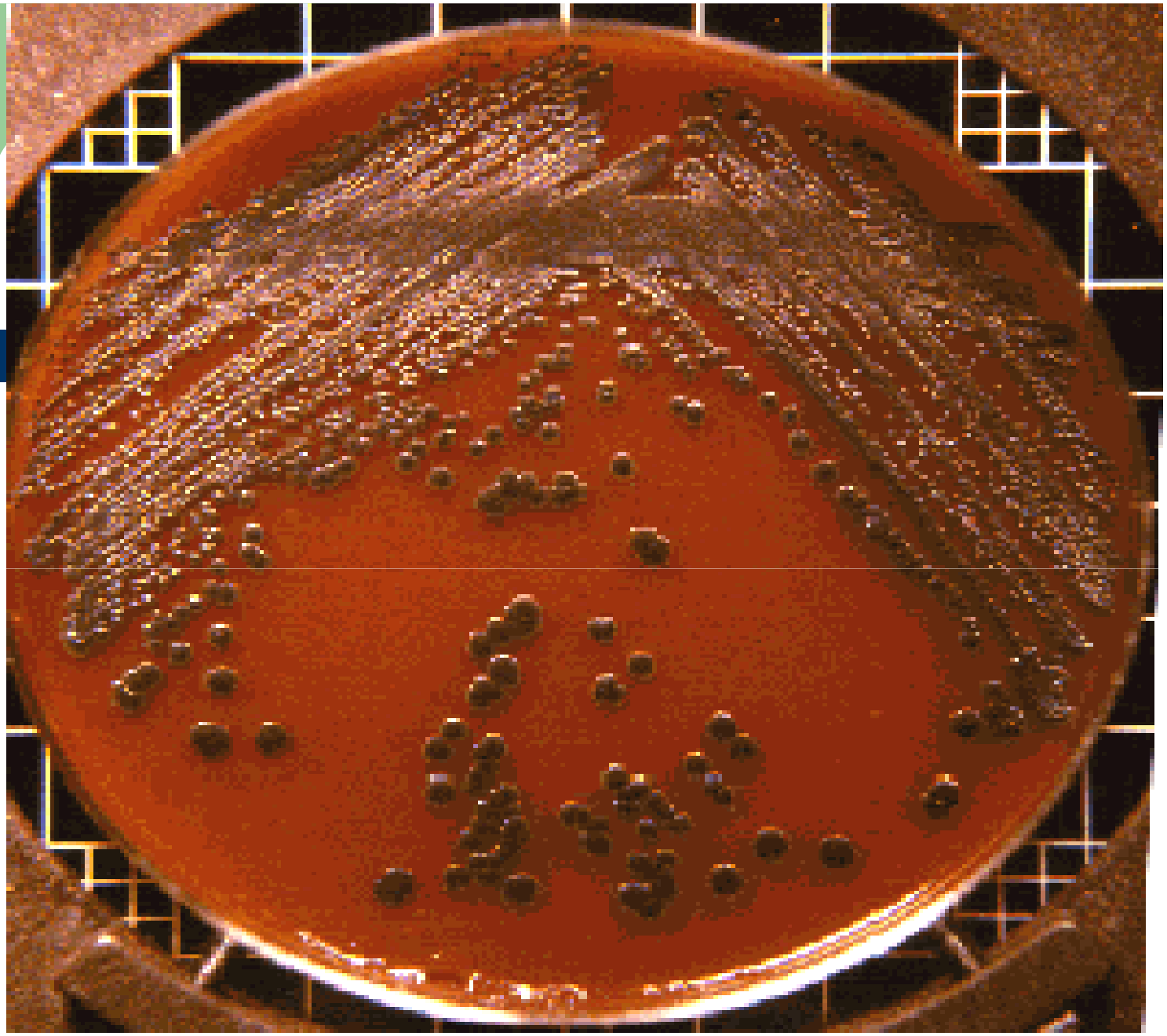
- Gravis : relatively large(2.5mm) colonies, grey black, radially striate (daisy head), which are hard and brittle,nonemulsifiable,
- Mitis : smaller convex grey black soft colonies, emulsifiable, 1-2 mm in diameter, `poached egg` colony
- Intermedius : the smallest, 0.5 mm, grey black, remarkably uniform colonies, `frog s egg`
- Mitis strains and some gravis strains form small zones of hemolysis.



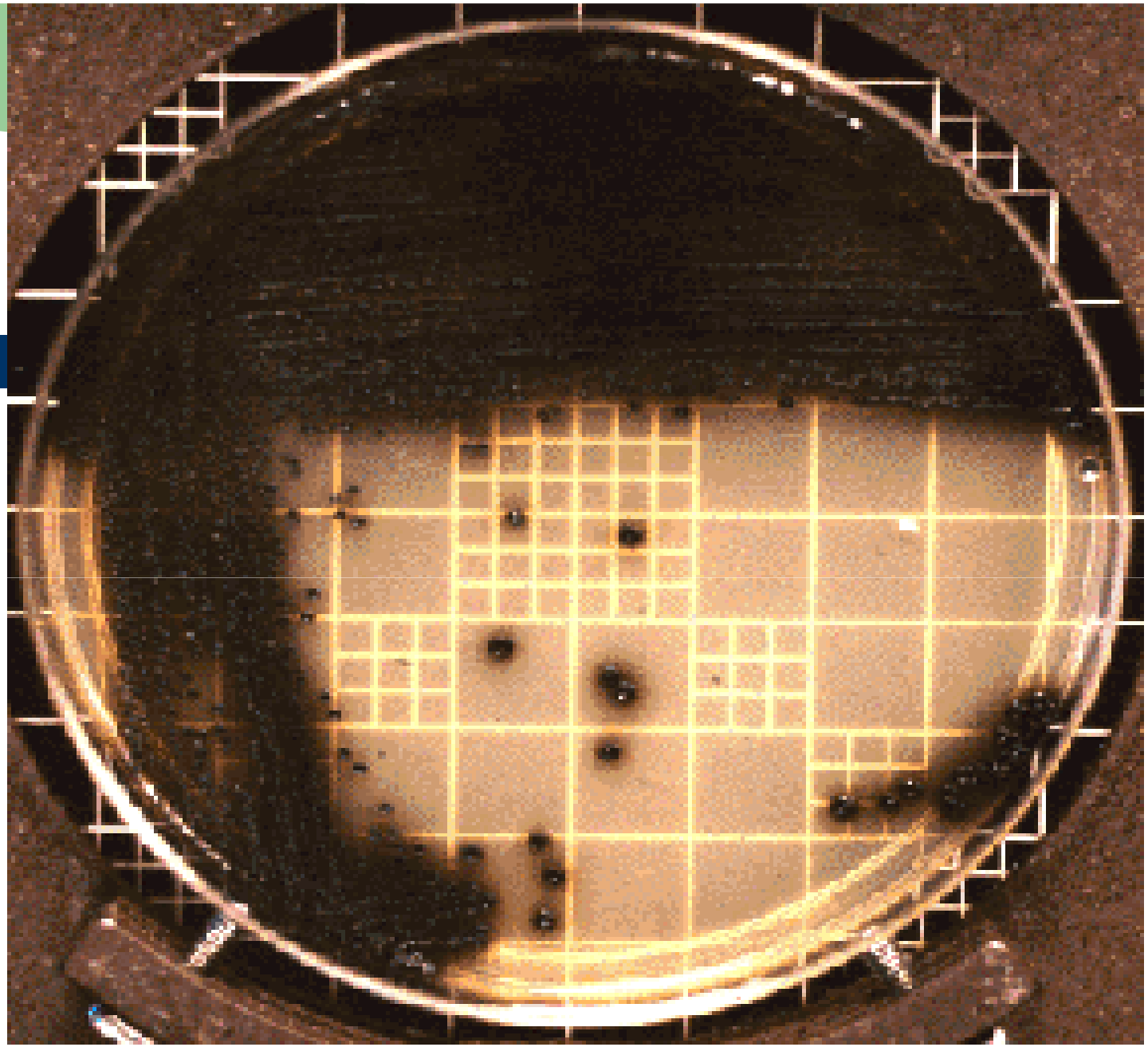
Corynebacterium diphtheriae, intermedius
Chocolate tellurite agar



Corynebacterium diphtheriae, mitis
Chocolate tellurite agar



Corynebacterium diphtheriae, gravis
Chocolate tellurite agar



Corynebacterium diphtheriae, mitis
Tinsdale agar

Biochemical reactions

- Ferment glucose and maltose but not lactose or mannitol in Hiss's serum peptone water medium.
- Do not hydrolyze urea.

Toxin

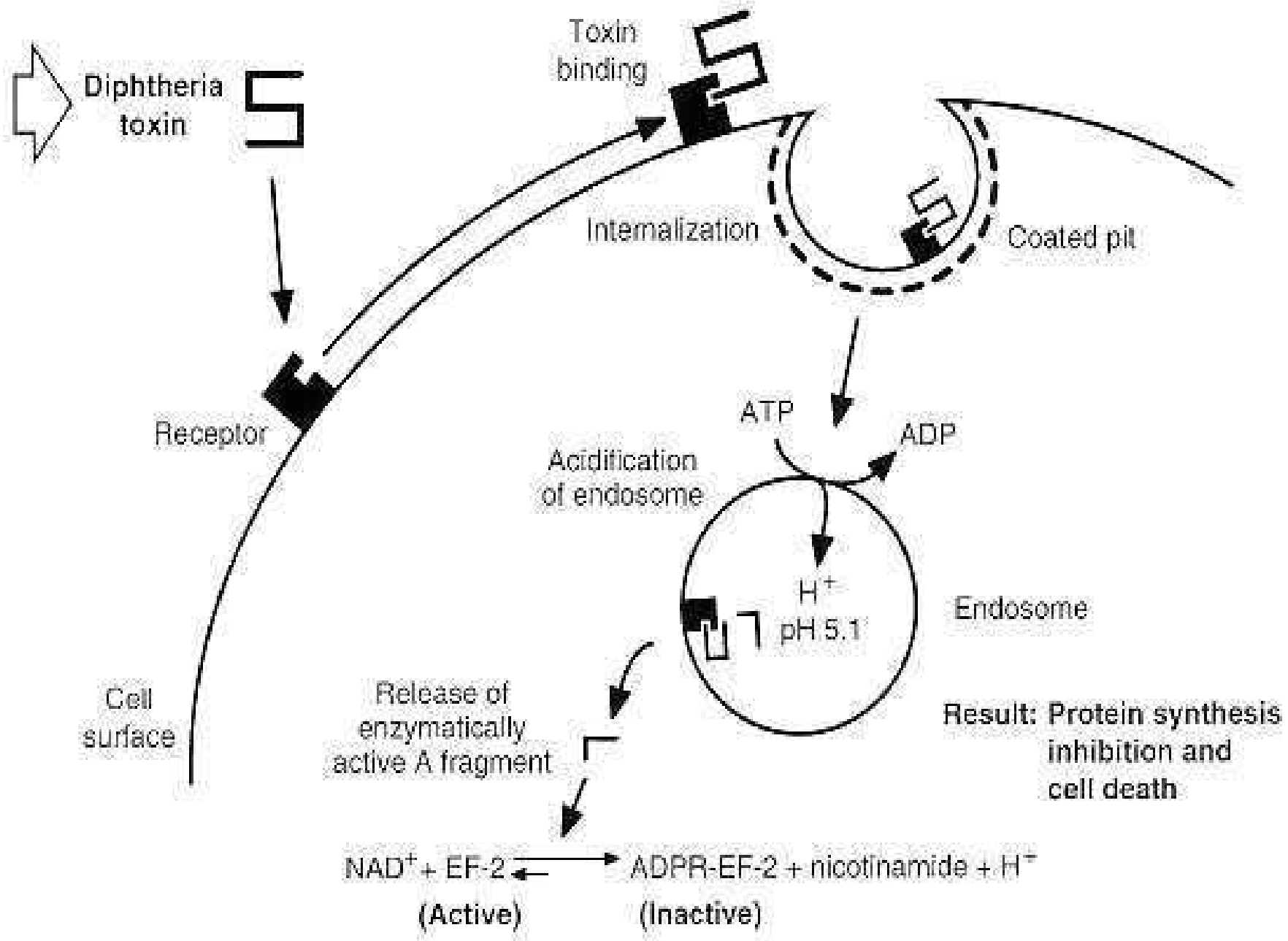
- Pathogenic effects are due to exotoxin
- The strain universally used for toxin production is the `Park Williams 8`strain
- It’s a protein with MW is 62000.
- Extremely potent and the lethal dose for a 250g guinea pig is 0.0001 mg.
- It consists of two fragments A (active) and B (binding).

Toxin cont…

- Prolonged storage, incubation at 37^{o}C for 4-6weeks, treatment with 0.2-0.4% formalin or acid pH converts it Toxoid.
- Toxoid is toxin that has lost its toxicity but not its antigenicity.
- Toxigenicity of diphtheriae bacillus depends on it corynephages (tox+), which act as a genetic determinant.
- Toxin production is influenced by iron concentration(0.1mg).

Toxin cont…

- Acts by inhibiting protein synthesis.
- Fragment A inhibits polypeptide chain elongation in the presence of nicotinamide adenine dinucleotide by inactivating the elongation factor EF-2.
- It has a special affinity for certain tissues like myocardium, adrenals and nerve endings.

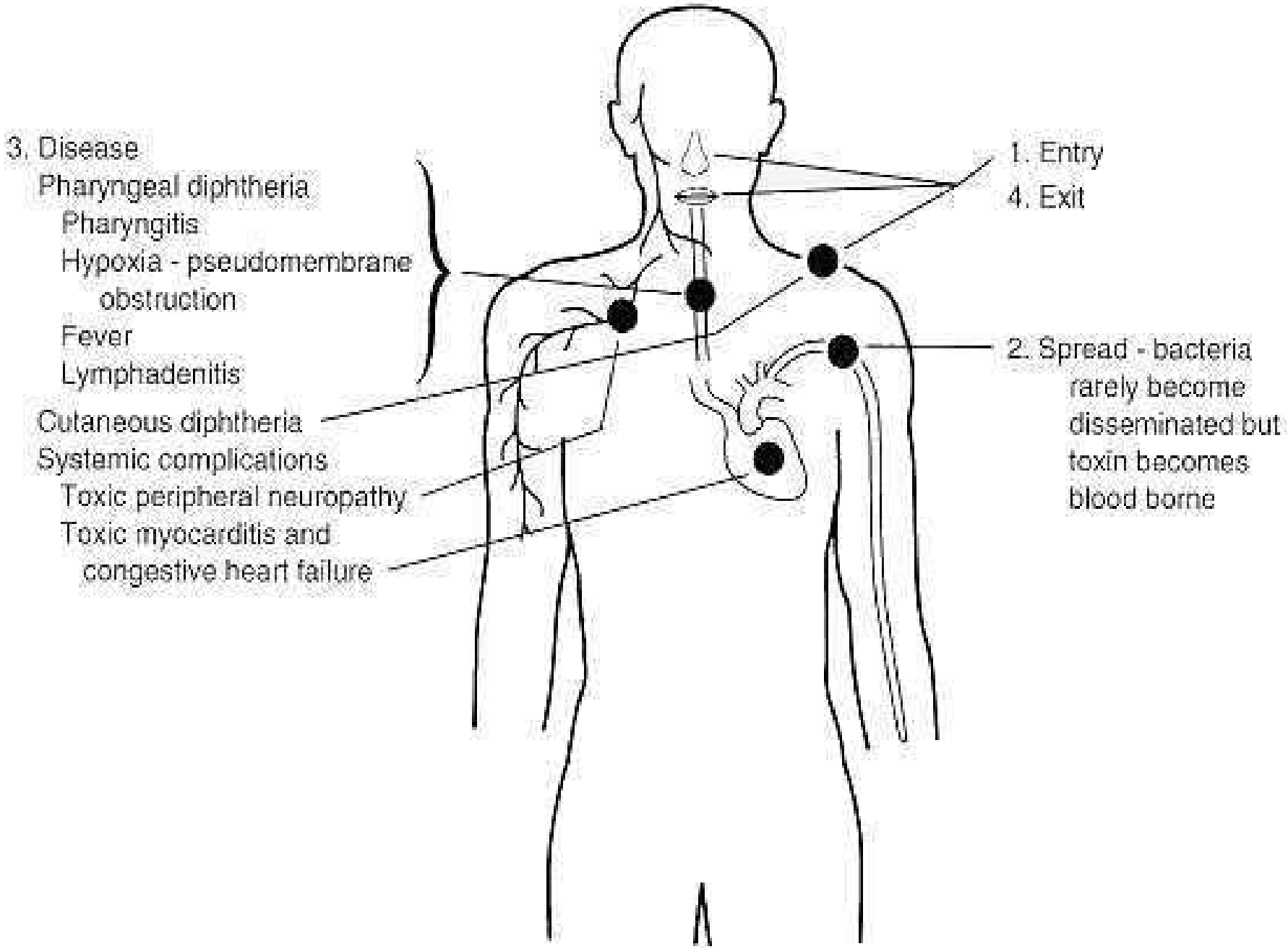

Diphtheria toxin
Toxin binding
Internalization
Coated pit
Receptor
ATP
ADP
Acidification of endosome
H+
pH 5.1
Endosome
Cell surface
Release of enzymatically active A fragment
Result: Protein synthesis inhibition and cell death
NAD+ + EF-2
(Active)
ADPR-EF-2 + nicotinamide + H+
(Inactive)

Typing

- Bacteriphage typing : 15 types, type I and III are Mitis, IV and VI intermedius, VII avirulent gravis and remainder virulent gravis.
- Bacteriocin (diphtheriocin) typing
- Bacterial polypeptide analysis
- DNA restriction patterns
- Hybridization with DNA probes

Pathogenicity

- Incubation period : 3-4 days
- Commonest site : 1) faucial 2) laryngeal
  3) Nasal 4) otitic 5) conjunctival
  6) genital 7) Cutaneous
- Faucial diphtheria is the commonest.
- Types of diphtheria according to the clinical severity:]
  1) Malignant
  2) Septic
  3) Hemorrhagic


3. Disease
Pharyngeal diphtheria
Pharyngitis
Hypoxia - pseudomembrane obstruction
Fever
Lymphadenitis
Cutaneous diphtheria
Systemic complications
Toxic peripheral neuropathy
Toxic myocarditis and congestive heart failure
1. Entry
4. Exit
2. Spread - bacteria rarely become disseminated but toxin becomes blood borne

Laboratory diagnosis

- The specimens to be collected depend on the type of the lesion.

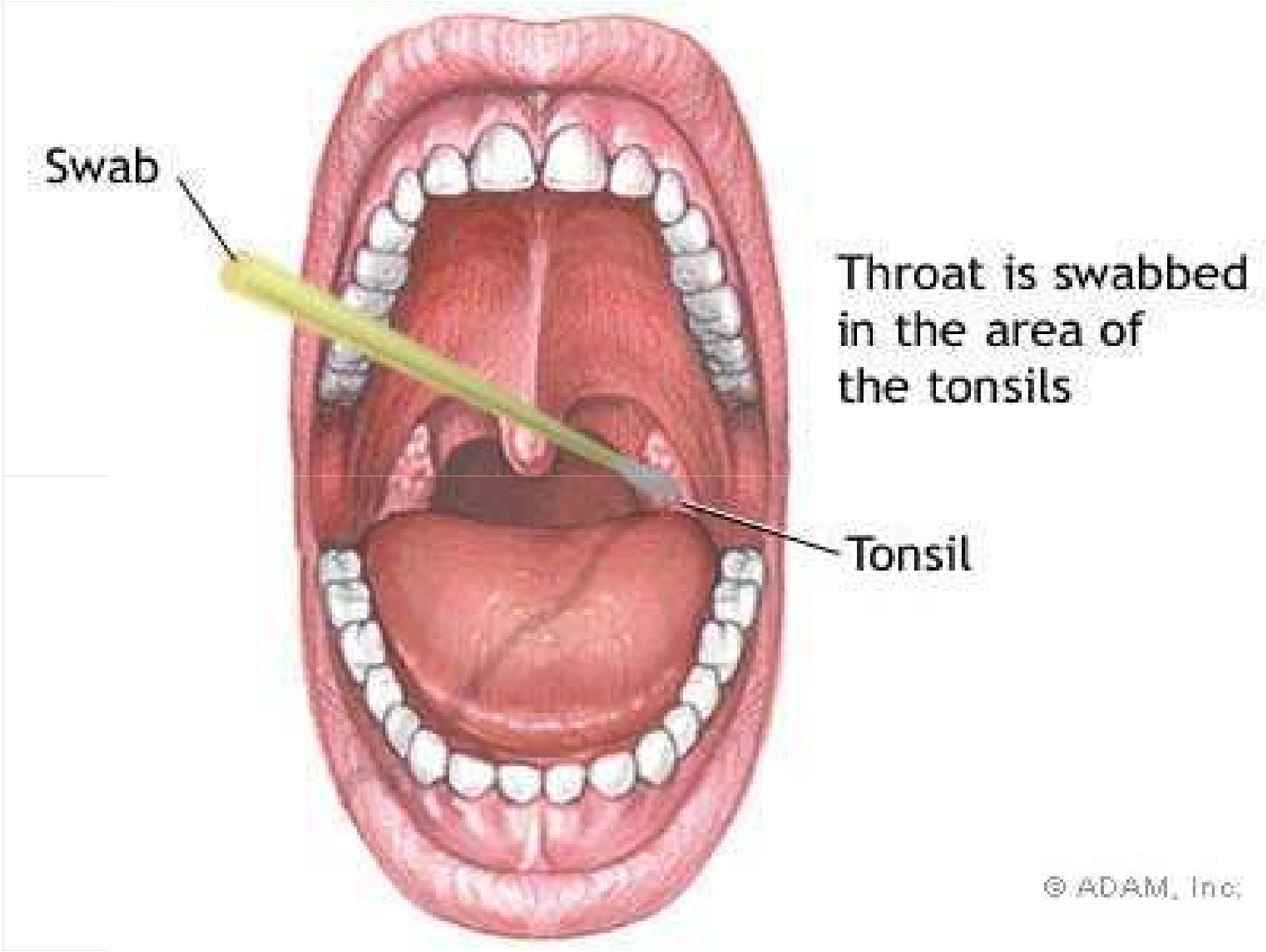
1 Culture confirmation

Direct microscopy → Culture on BA,TA,LS

↓

Antibiotic sensitivity ← Biochemical test

2 Virulence tests


Swab
Throat is swabbed in the area of the tonsils
Tonsil
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Virulence tests

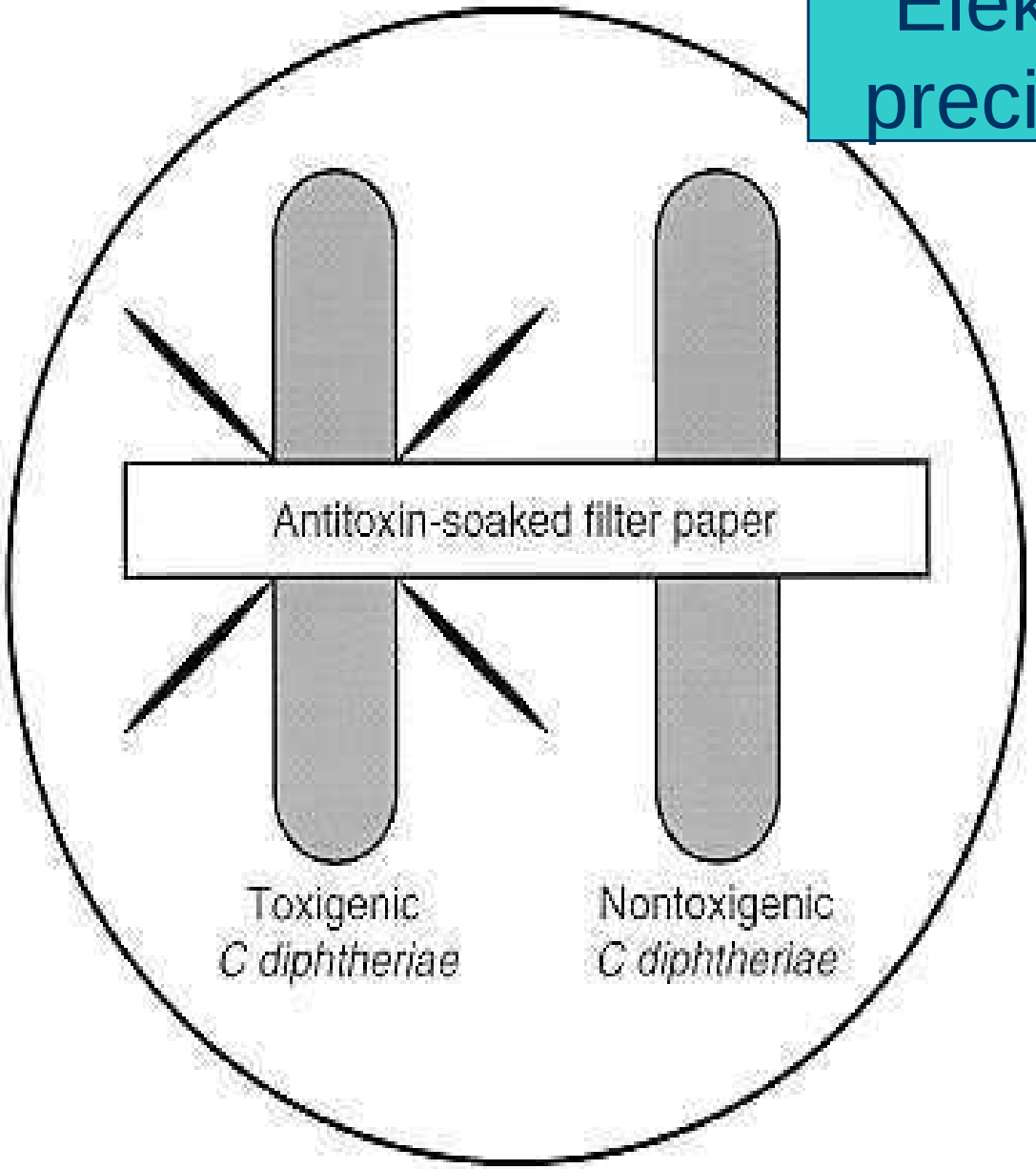
- **In vivo tests**

1) Subcutaneous test
2) Intracutaneous test

In vitro tests

1) Elek`s gel precipitation test
2) Tissue culture test

Elek`s gel precipitation



Prophylaxis

- Three methods :

1) Active
2) Passive
3) Combined

1) Two preparations for active immunization

a) Formol toxoid
b) Adsorbed toxoid

Prophylaxis cont…

- Passive immunization : consists of the subcutaneous injection of 500-1000 units of antitoxin (antidiphtheritic serum, ADS).

Treatment

- Consists of antitoxic and antibiotic therapy.

  Dose : 20000 to 100000 units depend upon the severity.