CORYNEBACTERIUM

LABORATORY DIAGNOSIS

Course Outcomes

- On completion of this course, the students will be able to: On completion of this course, the students will be able to: Perform sample collection from bacterial infections area and their diagnosis.

Course Objectives

- History & Introduction
- Classification
- Pathogenesis
- Laboratory diagnosis
- Treatment.

INTRODUCTION

- Corynebacteria / “Coryneform bacteria” – a group of non-spore forming, gram- positive bacilli, tend to be clubbed or irregularly shaped; (*coryne* = club)
- *Corynebacterium diphtheriae* the causative agent of Diphtheria is the major pathogen in this group.
- Other pathogenic corynebacteria are:
- *C. Ulcerans:* Diphtheria like lessions.
- Corynebacteria Causing Superficial skin infections:
  C. minutissimum and *C. tenuis*.
- *Diphtheriods:* Normal commensals in throat, skin and conjunctiva

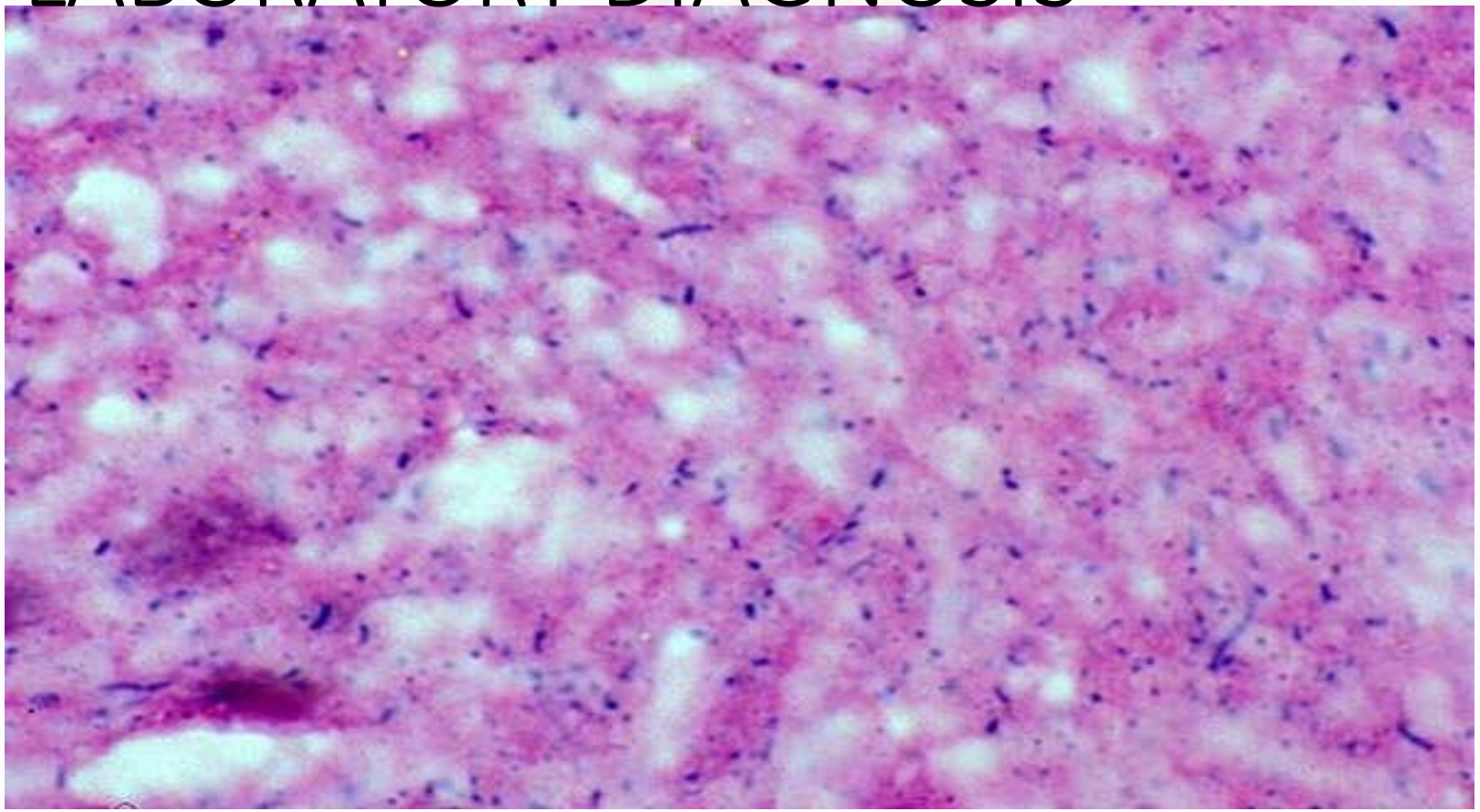
LABORATORY DIAGNOSIS

- This is to confirm the clinical impression and for epidemiological purpose;
- Specific treatment must never be delayed for laboratory reports, if the clinical picture is strongly suggestive of diphtheria;
- Any delay may be fatal…!
- Laboratory diagnosis consists of the isolation of the organism and demonstration of it's toxicity;

LABORATORY DIAGNOSIS

- Specimens :
  - Swabs from – nose, throat or other suspected lesions;
- Smear examination: Gram stain
  - shows beaded rods in typical arrangement;
  - Difficult to differentiate from some commensal corynebacteria normally found in throat;
  - Albert’s stain or Neisser’s stain is useful for demonstrating the granules;

LABORATORY DIAGNOSIS



Numbers of large-sized Gram-positive rods are embedded within the pseudomembrane (Gram).

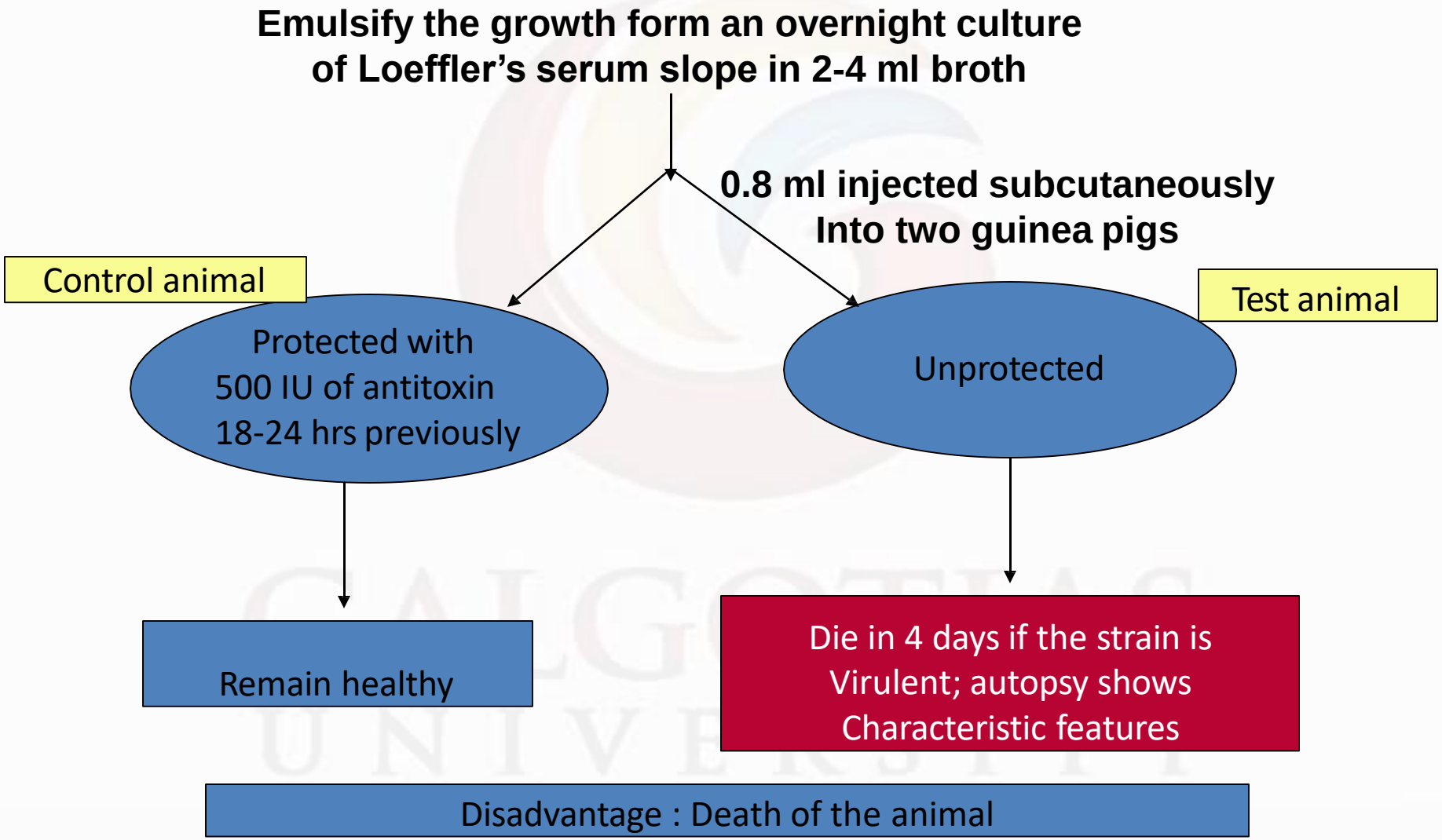
LABORATORY DIAGNOSIS : CULTURE

- If the swabs can not be inoculated promptly, they should be kept moistened with serum;
- Inoculate on :
  - Loeffler's serum slope
  - Tellurite blood agar or Tinsdale medium
  - Blood agar (for differentiating Staphylococcal or Streptococcal pharyngitis that simulate diphtheria);
- *Tellurite medium is particulary useful for isolating the organism from – convalescents, contacts or carriers;*

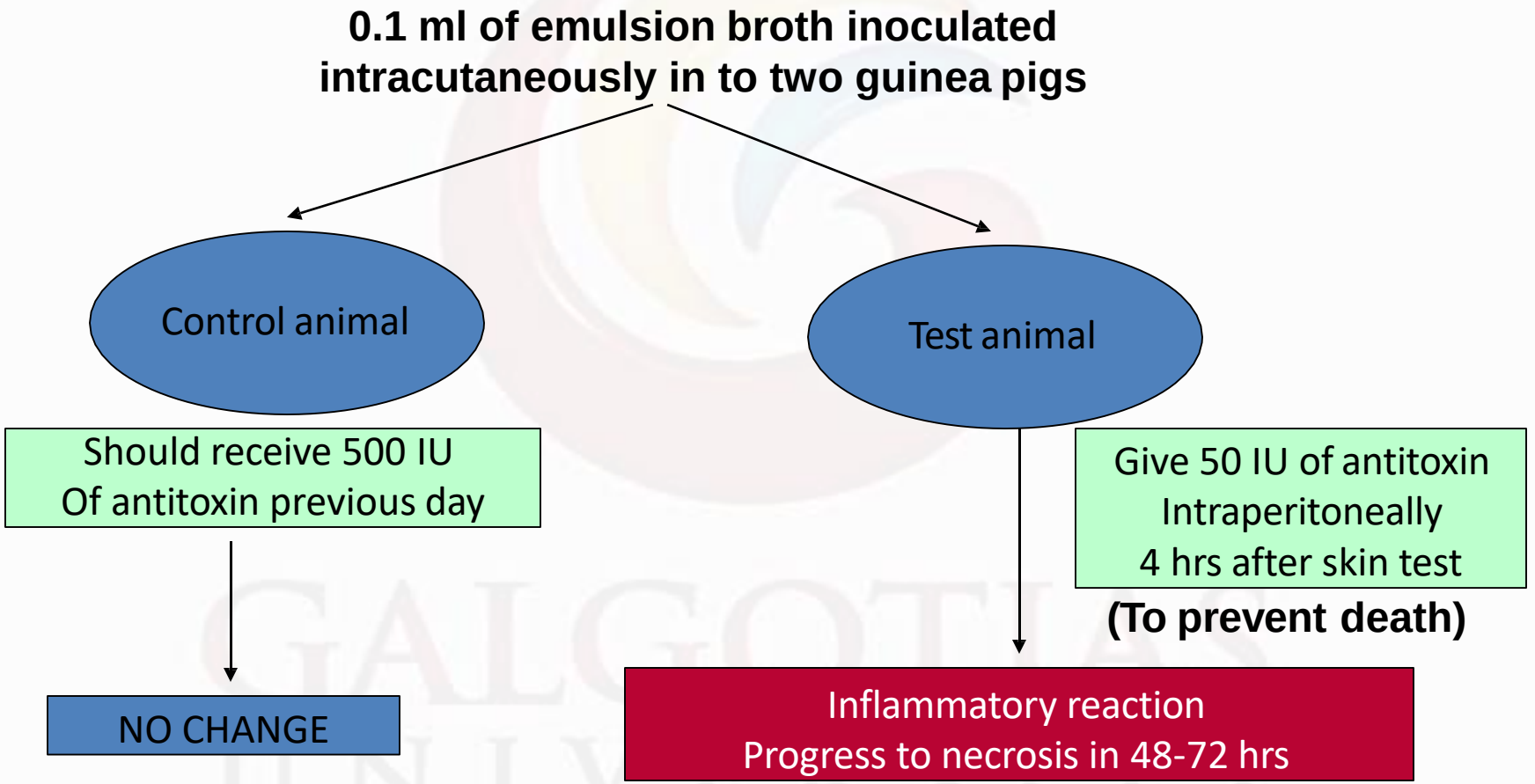
LABORATORY DIAGNOSIS : CULTURE

- Processing :
  - Serum slope may show growth in 4-8 hrs but if negative may need to be incubated for 24 hrs;
  - Smear may show ‘diphtheria-like’ organisms;
  - By about 48 hrs, Tellurite plates will yield growth;
  - The isolate must be submitted for – ‘Virulence tests’ or ‘Toxigenicity tests’ before the bacteriological diagnosis is complete;

SUBCUTANEOUS TEST



INTRACUTANEOUS TEST

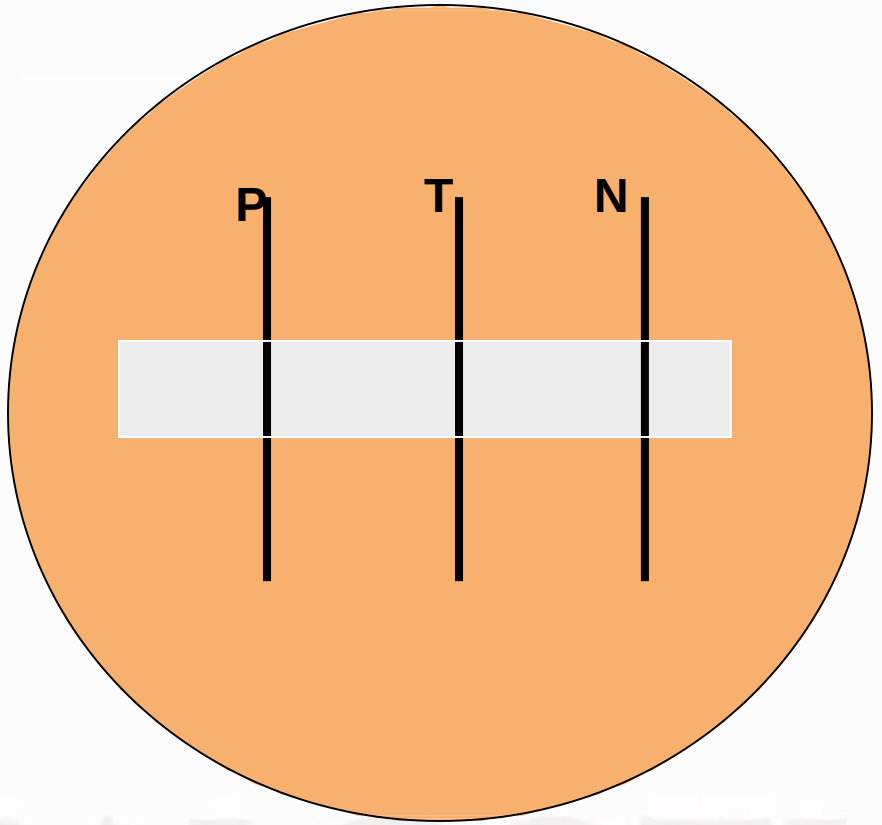


INTRACUTANEOUS TEST

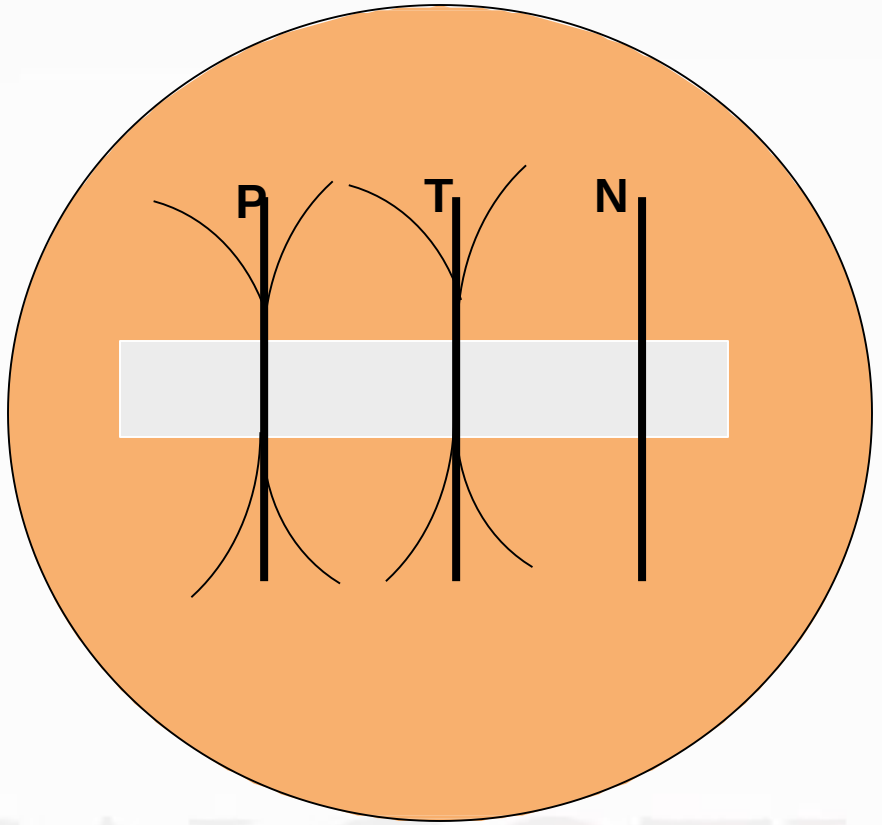
- Animal does not die;
- Rabbits may also be used;
- As many as 10 strains can be tested simultaneously;

Elek's gel precipitation test

- In vitro test;
- A rectangular strip of filter paper is saturated with the diphtheria antitoxin(1000 units/ml);
- This strip is placed on : agar plate with 20% horse serum, while the medium is setting;
- The cultures to be tested are streaked at right angles to the filter paper strip;
- A positive and negative control should be put;



Incubate the plate for 24-48 hrs at 37^0C



After incubation – line of precipitation can be observed
Where the toxin and antitoxin meet at optimum conc.
The lines of precipitation will indicate that
the test strain is toxigenic

EPIDEMIOLOGY

- Mainly a disease of childhood(pediatrics) in endemic areas – uncommon below 1st year; peaks at 5.
- In nature, *C.diphtheriae* occurs in the respiratory tract, in the wounds or in the skin of the infected persons or carriers;
- Transmission is by-
  - Droplet dissemination from cases or carriers
  - Direct contact
  - Occasionally, fomites;
- Nasopharyngeal or cutaneous carriage of toxigenic or nontoxigenic strains can persist for life in healthy people;

PROPHYLAXIS

- Diphtheria can be controlled by immunisation;
- Types of immunisation available for diphtheria:
  - Active
  - Passive
  - combined
- The objective of immunisation is to increase protective levels of antitoxin in circulation;

Active immunisation- schedules

- Primary immunisation:
  - 3 doses of DPT begening at 4^{th} week of age, 8^{th} and 12^{th} week under Routine Immunization schedule(Govt. of Tanzania)
- Booster (DPT) at 15-18 months of age;
  - Further booster, as ‘DT’ at – 5 years of age;
- Dosage : 0.5 ml
  - 10-25 Lf units of toxoid - recommended for children

Active immunisation- schedules

- Contraindications:
  - Acute febrile illness : postpone till recovery
  - Severe local or systemic reaction to pertusis component of DPT are likely; if they occur, immunise with DT; acellular pertusis vaccine can be added if the reaction is a local one;

Passive Immunisation

Historical engraving showing how the medicinal serum was obtained from immunized horses.

- Using antitoxin or ADS(Antidiphtheritic serum);
- As an emergency measure when susceptibles are exposed to infection;
- Subcutaneous administration of 500-1000 units of antitoxin or ADS(Antidiphtheritic serum);
- Risk of hypersensitivity as horse serum used;

Combined immunisation

- Administration of first dose of toxoid on one arm, while ADS is given on the other arm, to be continued by full course of active immunisation;
- All cases that received prophylactic ADS should receive combined immunisation;

TREATMENT

- Specific measure : prompt administration of antitoxin to neutralize the circulating toxin;
  - Dose: 20,000-1,00,000 units
  - Half the dose given IV
  - Antitoxin treatment is generally not indicated for cutaneous diphtheria
- Antibiotics : Penicillin or Erythromycin for 14 days;
- Complete bed rest;
- Supportive therapy and treatment of complications
- Erythromycin: for treatment of carriers.

Reference

1. Dr. C P. Baveja, Text book of Microbiology for MLT, Second Edition, Arya Publication, 2017.
2. Dir. Prof. C P Baveja, Textbook of Microbiology, 4th edition, Arya Publication, 2013.