Genus: Listeria
L. monocytogenes

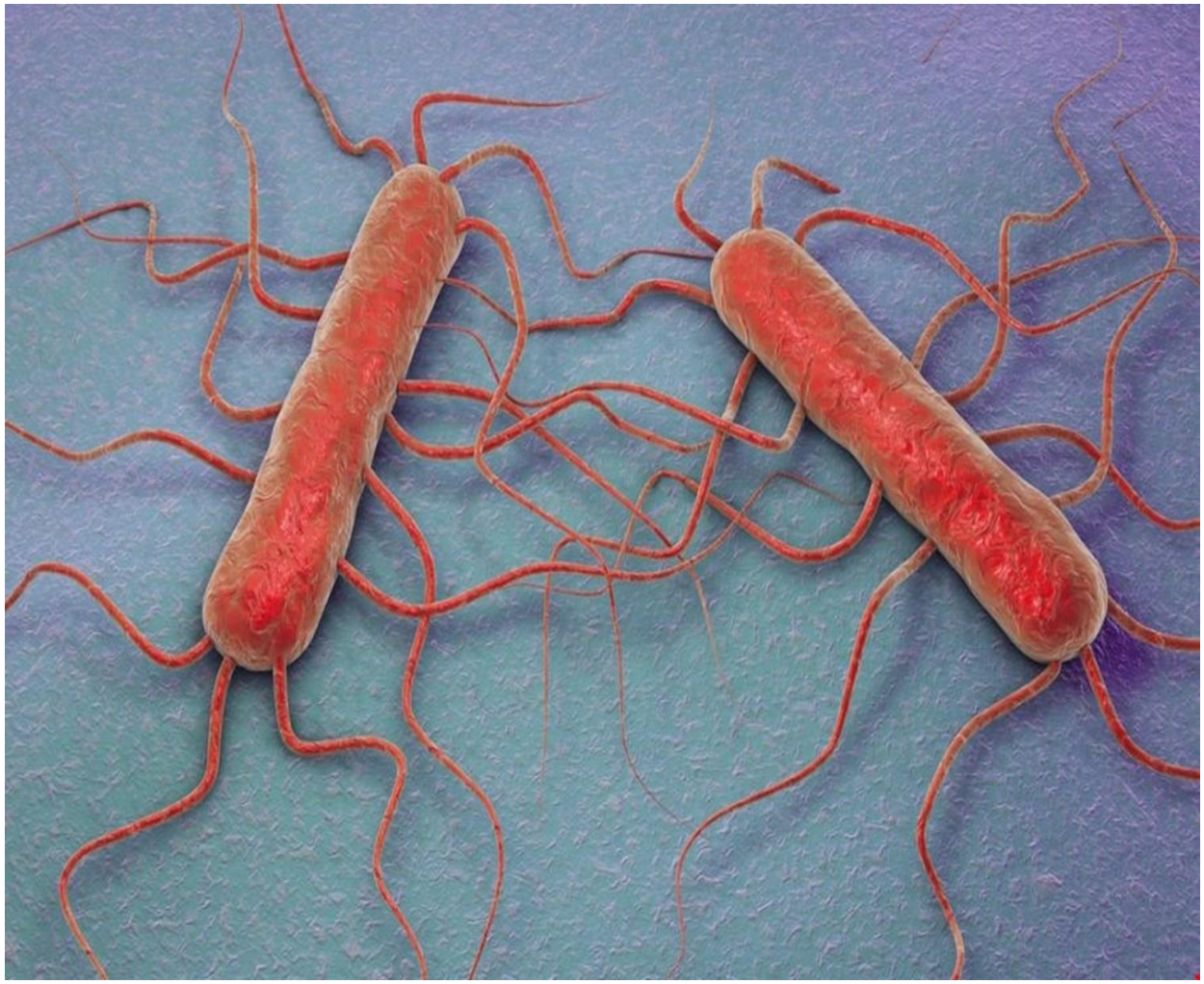
Assist. Prof.

Dr. Ali Aldeewan

General characteristics

- Gram positive rods
- Non-spore-forming
- Non-acid fast
- Medium sized (0.4-0.5µm in diameter) and (0.5-25µm length)
- Facultative anaerobes (growing enhanced by 10% co2)
- Catalase positive
- Oxidase negative

Listeria



General characteristics

- Hydrolyse aesculin
- Tolerate 10% Nacl2
- Motile by 1-5 peritrichous flagella
- They grow on nutrient agar and blood agar but not on macConkey agar

History and taxonomy

Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Listeriaceae
Genus	Listeria
Species	Listeria monocytogenes (Murray et al., 1926) Pirie, 1940

History and taxonomy

- **Listeria divided in to seven species with two distinct group the most**
- *L. monocytogenes,*
- *L. innocua,*
- *L. welshimeri,*
- *L. seeligeri,*
- *L. grayi*
- *L. ivanovii subsp. ivanovii and*
- *L. ivanovii subsp. Londoniensis.*
- Only *L. monocytogenes* causes disease in both animals and humans.
- *L. ivanovii* is known to cause spontaneous abortions in sheep.

Classification

- Listeria divided in to seven species with two distinct group the most important species in veterinary medicine is *Listeria monocytogenes*

Natural habitat

- Listeria spp. are widely distributed in the environment
- can be isolated from soil, plants, decaying vegetation and silage
- Asymptomatic fecal carriers occur in man and animal species.
- L .monocytogenes can be extracted in bovine milk

Natural habitat

- They can grow temperature range 3-45C
- Silage is commonly implicated

in outbreaks

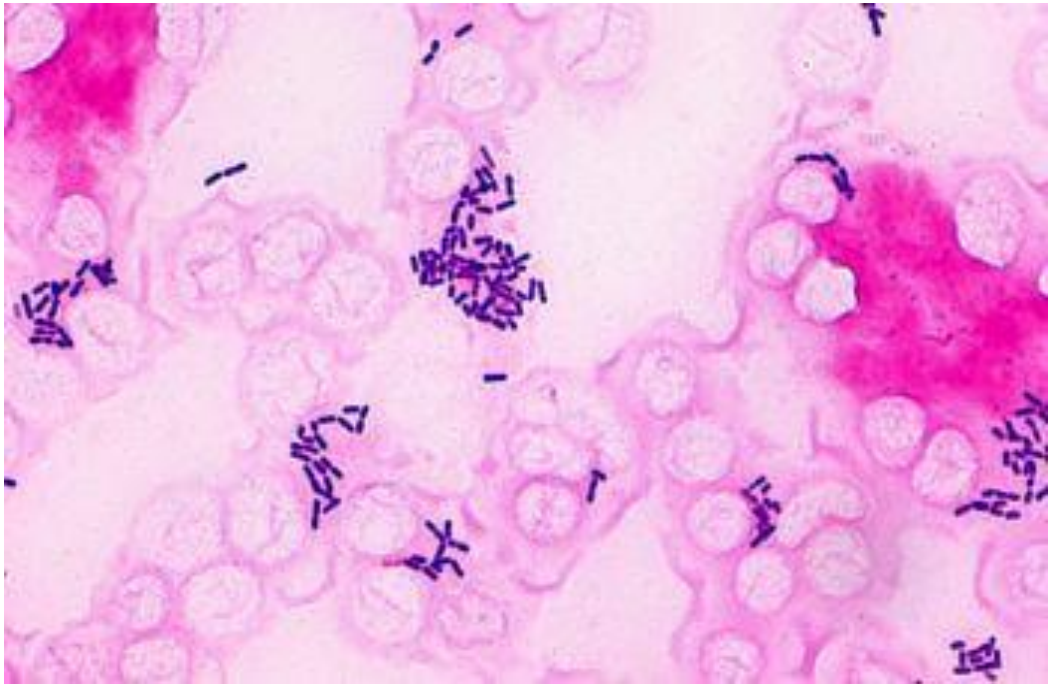
of Listeriosis

in cattle

and sheep

- Human foods associated with Listeriosis in man include coleslaw ,soft cheeses, milk and poultry meat

Veterinarians, medical doctors and people involved in food science know listeriosis by various names (circling disease, silage sickness, leukocytosis, cheese sickness, tiger river disease)



Pathogenesis

- It is thought that the pathogenic listeria spp. can penetrate the epithelia barrier in the intestine and multiply in hepatic and splenic macrophages aided by the haemolysin named listerolysin O.
- An alternative route may be through damaged mucosal surfaces to the central nervous system ,via the neural sheath of peripheral nerve ending of the trigeminal nerve.

Pathogenesis

- Most pathogenic bacteria require the availability of iron in the host for metabolic activities.
- High iron levels in silage that lead to elevated tissue concentrations of iron may be predispose cattle and sheep, fed on silage, to Listeriosis

Pathogenesis

- *L .monocytogenes* in human includes an influenza-like syndrome in pregnant women that may be result in infection of the foetus with abortion or premature birth. The neural form of the disease can occur in neonates and in adults with lowered cell-mediated immunity.
- Veterinarians and abattoir workers can acquire a primary cutaneous Listeriosis and this infrequently leads to a generalized form of the disease

Laboratory diagnosis

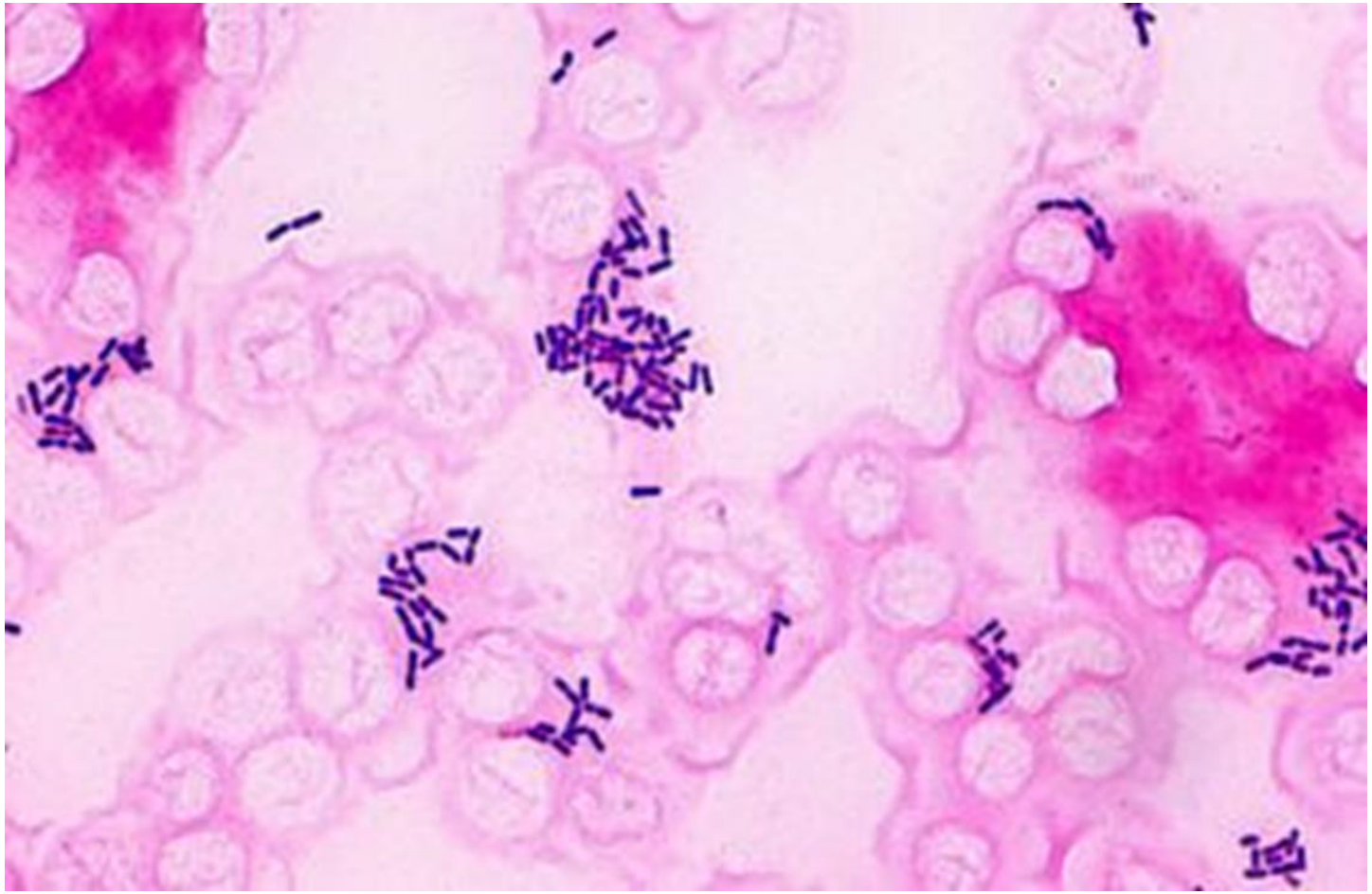
Samples collection

- Visceral form: samples from lesions in liver, kidney or spleen
- Neural form: spinal fluid, brain stem, tissue from several sites in the medulla oblongata
- Abortion form : placenta, foetal abomasal contents and or uterine discharges

Microscopic examination

- Stained smear are not as useful in Listeriosis as they in other diseases
- Smear from lesions may be reveals Gram positive rods(often coccobacillary)
- Histopathological examination of fixed 10% formalin from brain tissue can give presumptive diagnosis of neural listeriosis.

Microscopic examination



Isolation

- The routine media for isolation is ox and sheep blood agar and macConkey agar plate to detect any Gram negative pathogene or contaminants.
- Selective media include blood agar supplement with antibiotic
- Or blood agar containing 0.05% potassium telllurite (inhibitory for G- bacteria)
- Commercial selective agar such as listeria selective agar and these are designed mainly for the isolation of listeria from human foodstuffs.

Isolation

- A cold –enrichment procedure is necessary for brain tissue from neural listeriosis:
- Small pieces of spinal cord and medulla are homogenized and 10% suspension is placed in the refrigerator at 4C and subculture on blood agar once weekly for up to 12 weeks. This method select for *L.monocytogenes* which able to grow at refrigerator temperature

Identification

1.Colonial appearance

- Small transparent colonies with smooth borders appear on blood agar in 24 hours, becoming greyish-white and 0.5-2.0 mm in diameter in 48hrs.
- *L.ivanovii* produced a comparative wide zone haemolysis and is very similar in appearance to beta-haemolytic streptococcus
- L.monocytogenes and non-pathogenic *L.seeligeri* have narrow zones of beta-haemolysis, only under the colony itself.

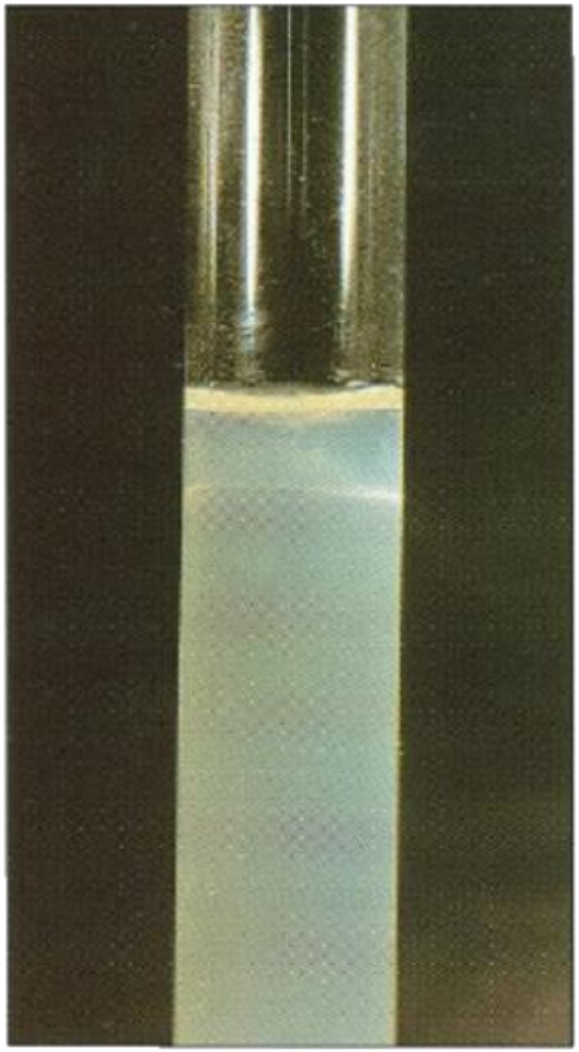
Identification

2. Microscopic appearance

- Short Gram positive rods or coccobacilli are seen at 24hrs with a tendency for cells from older cultures to decolorize.
- Singly, arranged in Y or V forms or short chains. In broth culture longer bacilli with palisade formation are seen.
- There are often many coccal forms in smears from young rapidly growing colonies, this and the colonial appearance on blood agar, can lead to confusion between the pathogenic listeria and beta-haemolytic streptococci.

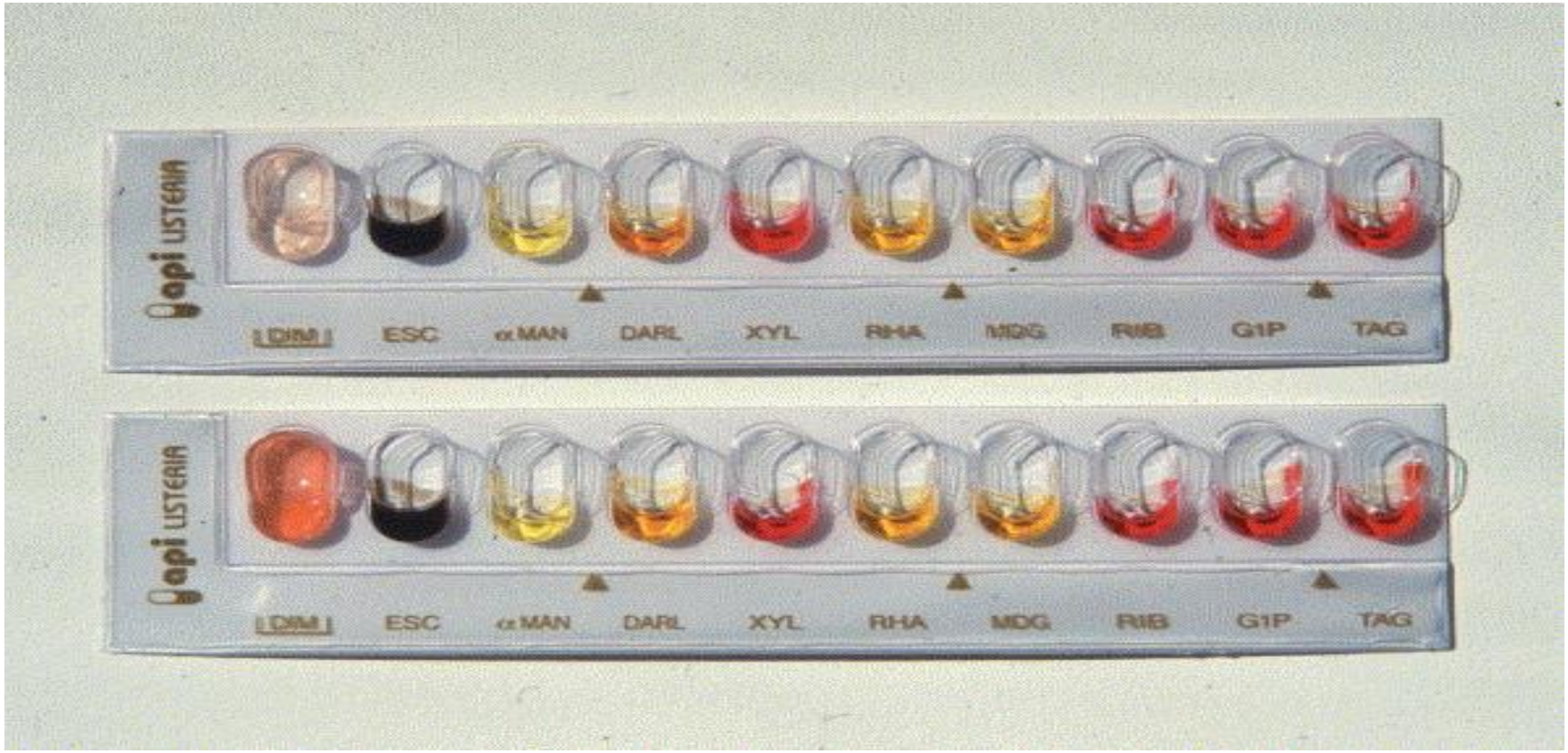
Identification

- **3.Biochemical test and other tests**
- All Listeria spp. hydrolyze aesculin (aesculin broth)
- L.monocytogenes ,particularly ,shows the charateristics (tumbling motility) when incubated in broth culture at 25C◦ for 2-4 hrs. and examined by hanging drop method.
- When growing in semisolid motility media the listeria spp. give an unusual umbrella shaped growth in the subsurface



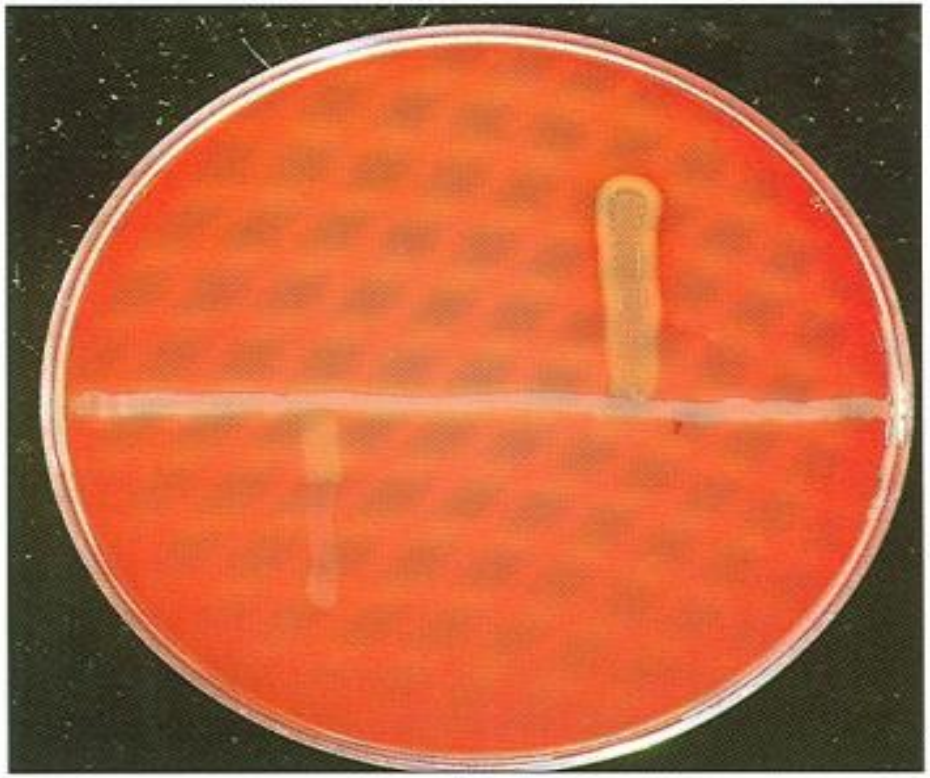
3.Biochemical test and other tests

API 20s The API 20s also is a good test for identification of *L.monocytogenes*



3.Biochemical test and other tests

Modified CAMP test



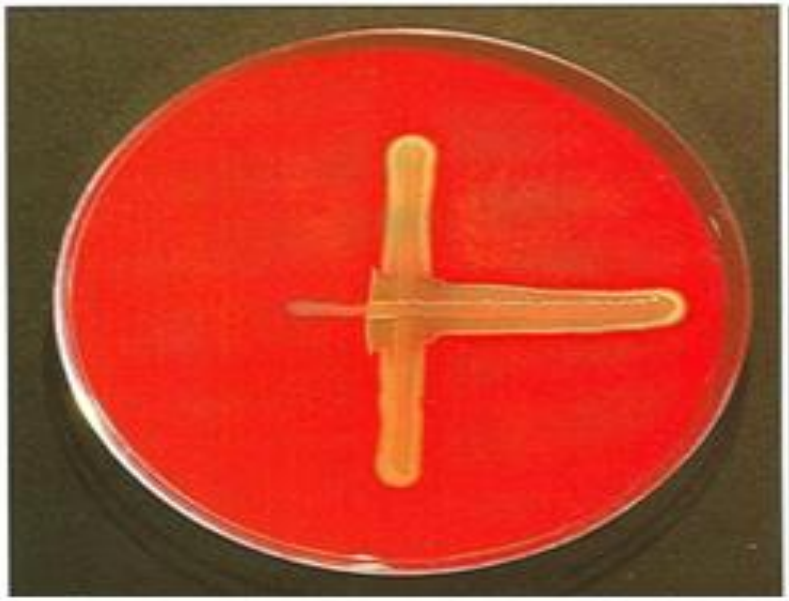
205 CAMP test with *Staphylococcus aureus* (horizontal) showing enhancement of the effect of the staphylococcal beta-haemolysin by *L. monocytogenes* (left) but not by *L. ivanovii* (right).

- Modified CAMP test with staphylococcus aureus and with Rhodococcus equi are useful to differentiate the two pathogenic species

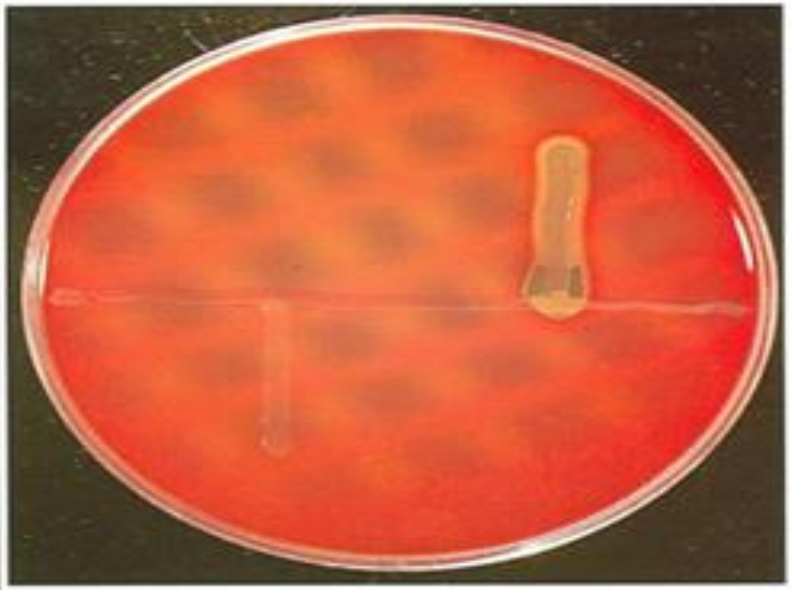
Modified CAMP test

- CAMP test : a culture of Staphylococcus aureus ,with a wide zone of partial hemolysis (beta-haemolysis) is streaked across the center of a sheep or ox blood agar plate.
- A streak of the suspect *L.monocytogenes* is made at right angles to, and taken to within 1 to 1.5 mm of the staphylococcal streak.
- The plate is incubated at 37 for 18-24hour. A positive CAMP test is indicated by an arrow-head of complete haemolysis .
- The L.monocytogenes produce a diffusible metabolite that completes the lysis of the red cells, only partially haemolysed by the beta- haemolysin of the *staphylococcus*

Modified CAMP test

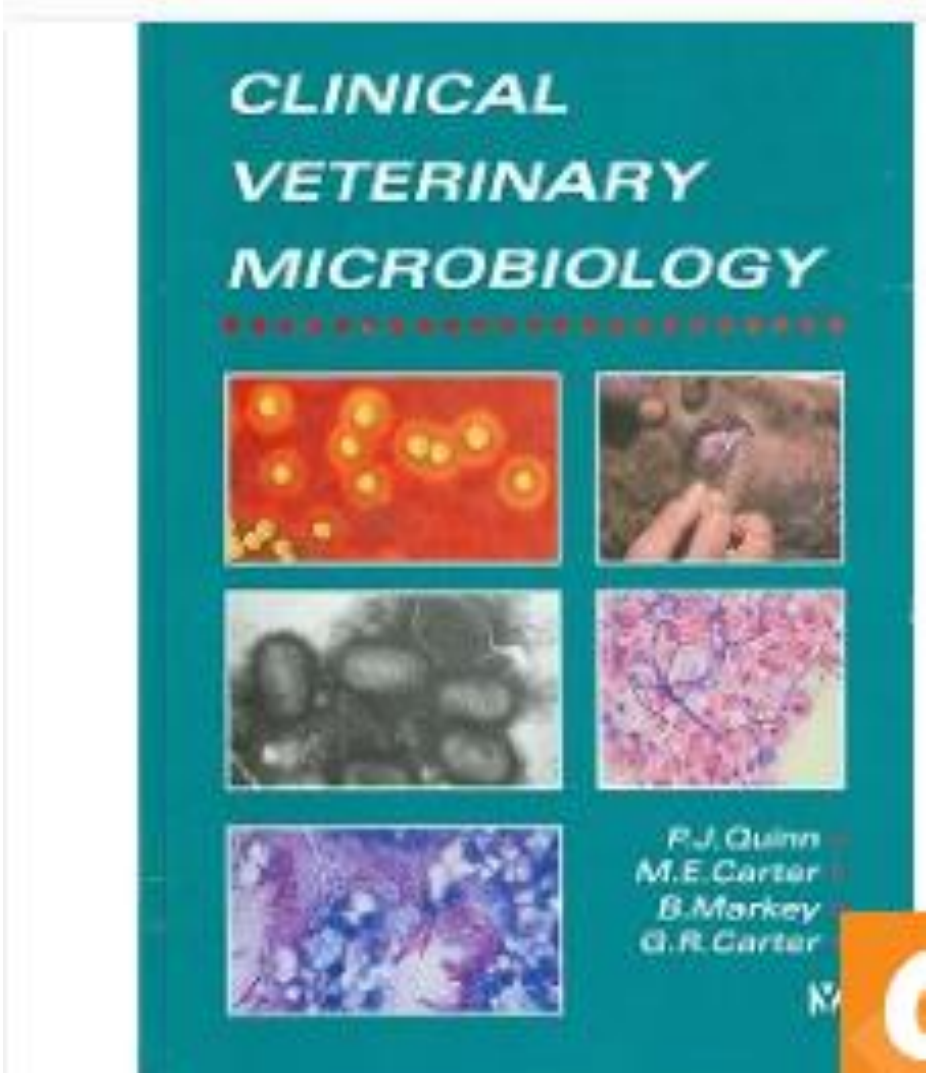


207 *Rhodococcus equi* streaked across (left to right) a vertical streak of *L. ivanovii* giving an enhanced haemolytic effect.



206 CAMP test with *Rhodococcus equi* (horizontal): no reaction by *L. monocytogenes* (left) and enhancement of haemolysis by *L. ivanovii* (right).

References


CLINICAL
VETERINARY
MICROBIOLOGY
P.J. Quinn
M.E. Carter
B. Markey
G.R. Carter

Any Question