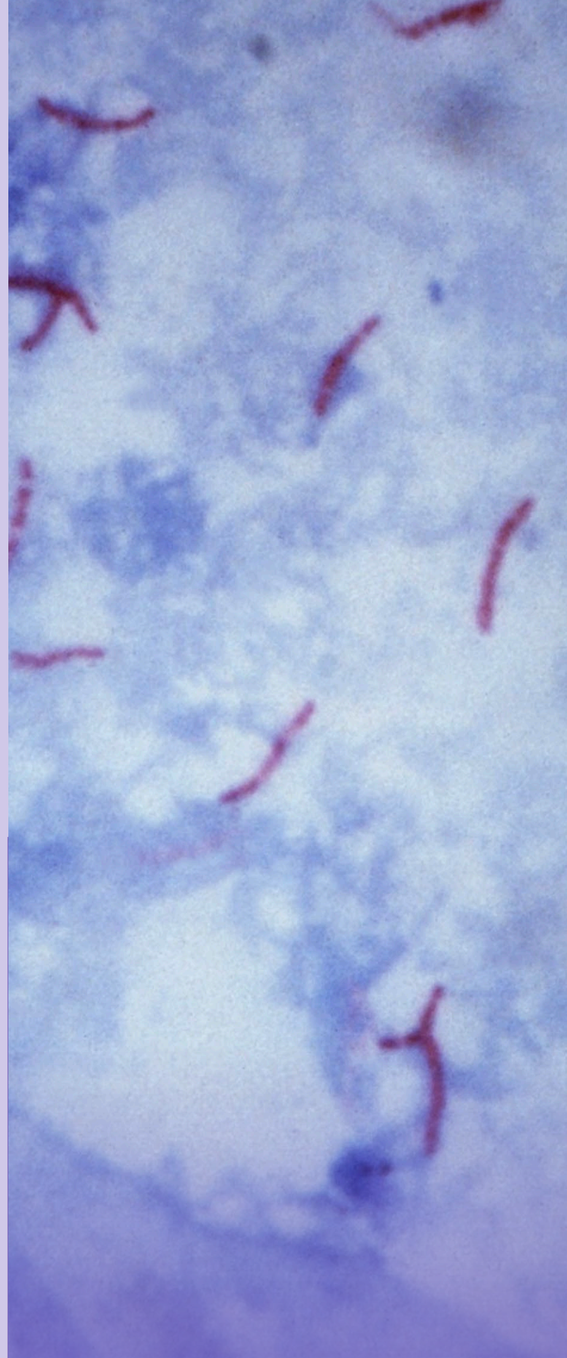
Blood culture: a history of its uses in microbiology

Many microbial diseases are characterised by the presence of the causative agent in circulating blood, and its investigation plays a significant role in the diagnosis and monitoring of infection and infestations.

Growth of microorganisms in a blood culture bottle



Lysis centrifugation for the isolation of *Mycobacterium tuberculosis* was described in 1914

Manual methodology

- Up to the end of the 19th century blood was either directly inoculated onto agar plates or injected into animals.
- In 1907 Muir and Richie added 1 mL of blood to 50 mL of bouillon in a cotton wool stoppered flask and subculturing after incubation. Some blood was also to be smeared on to agar or added to molten nutrient agar and then poured into a Petri dish.
- In a later paper they recommend broth with ox bile or a 0.5% bile salts in 1% solution of sodium citrate for enteric bacteria and the addition of trypsin to prevent clotting and to destroy blood's antibacterial properties.
- Early workers also use 'clot culture'. The clot was placed in broth or in a syringe and expressed through the nozzle, to disrupt it, into broth.
- Lysis centrifugation for the isolation of *Mycobacterium tuberculosis* in cases of miliary tuberculosis was described by Clough in 1914.
- In 1934 Mackie and McCartney describe a method for making blood culture bottles using 6oz (170 mL) with a perforated cap and rubber washer. The type of broth in these bottles was identified by a coloured glass bead and cap.
  - This type of bottle became commercially available and was the standard approach until the last quarter of the 20th century when commercial automated systems were introduced.
- Over the century many modifications to the culture media were suggested:
  - Glucose to enhance the growth of streptococci; calcium carbonate to neutralise acid production; sodium citrate, saponin and trypsin to prevent clotting
  - Optimum blood to broth ratio of 5–10% blood.
  - During the 1930s 'Liquoid' (sodium polyanethol sulfonate) was found to have anticoagulant, anticomplement and antiphagocytic properties and was added to the media but concentrations over 0.05% inhibited anaerobes.
  - For anaerobes, Robertson's Cooked Meat, thioglycolate broth and semi-solid agar were used.
  - To neutralise the inhibitory effect of circulating sulphonamides p-amino benzoic acid was added and then penicillinase when penicillin was introduced.
  - In the late 1980s mixtures of resins and charcoal were added to absorb antibiotics and other growth inhibiting substances.
  - Bottles were vented and incubated in atmospheres of 10% CO_2 or anaerobically.
- In 1947 Castaneda described a bi-phasic bottle for the isolation of *Brucella* spp. containing an agar slope and broth. The bottle was tipped to allow the broth to wash over the agar and then re-incubated.
  - In the 1980s Septi-Chek was introduced in which a tube containing an agar-coated paddle was fitted to the bottle.

The Bactec 460 radiometric machine launched in 1976 held 60 bottles

Castaneda's bi-phasic bottle for the isolation of *Brucella* spp., containing an agar slope and broth.

Automated techniques

- In the manual methods growth in the broth was detected visually, by Gram stain and by subculture.
- In the 1970s and '80s attention turned to detecting the products of bacterial growth; initially in the head space gas.
- In 1968 Dr William Johnson developed the Bactec 110 which detected ^{14}C released from radiolabelled culture media.
- The first commercially available blood culture instrument, the Bactec 225, was introduced in 1971. It held 25 bottles, and was read manually. Followed by the cheaper 301 version.
- In 1976 the final radiometric machine was the 460 which held 60 bottles, read offline. Automated testing took one hour.
- In 1983 Becton Dickinson introduced the fully automated NR-660, which used infrared spectroscopy to measure CO_2.
- The 1992 the Bactec 9000 series was introduced, with non-invasive fluorescence technology incorporated in the bottle to detect the CO_2 (measurements taken every 10 minutes).
- Oxoid introduced the Signal System in 1987. This detects increase in pressure by displacement of media into an attached reservoir.
- The Difco ESP was introduced in 1992, and the VersaTREK in 2004 – continuous systems that also detected changes in head space pressure, both positive and negative.
- Automation in a 'Black Box', the BacT/Alert was introduced by Organon Teknica in 1989 using continuous monitoring with LED to detect CO_2 production with a pH-sensitive indicator sealed in the base of the bottle behind a permeable membrane.

Oxoid Signal blood culture bottles

Direct non-cultural detection

- All of the adjacent methods depend on the organism growing in broth, which slows diagnosis, does not detect those that do not grow, viruses or parasites. The 21st century has seen the development of molecular methods for direct detection of microorganisms in blood.

Blood Lines:
A Resource Not To Be Taken In Vein
Produced by the IBMS History Committee for Congress 2023