



Figure 1. Kit for the collection of CSF.

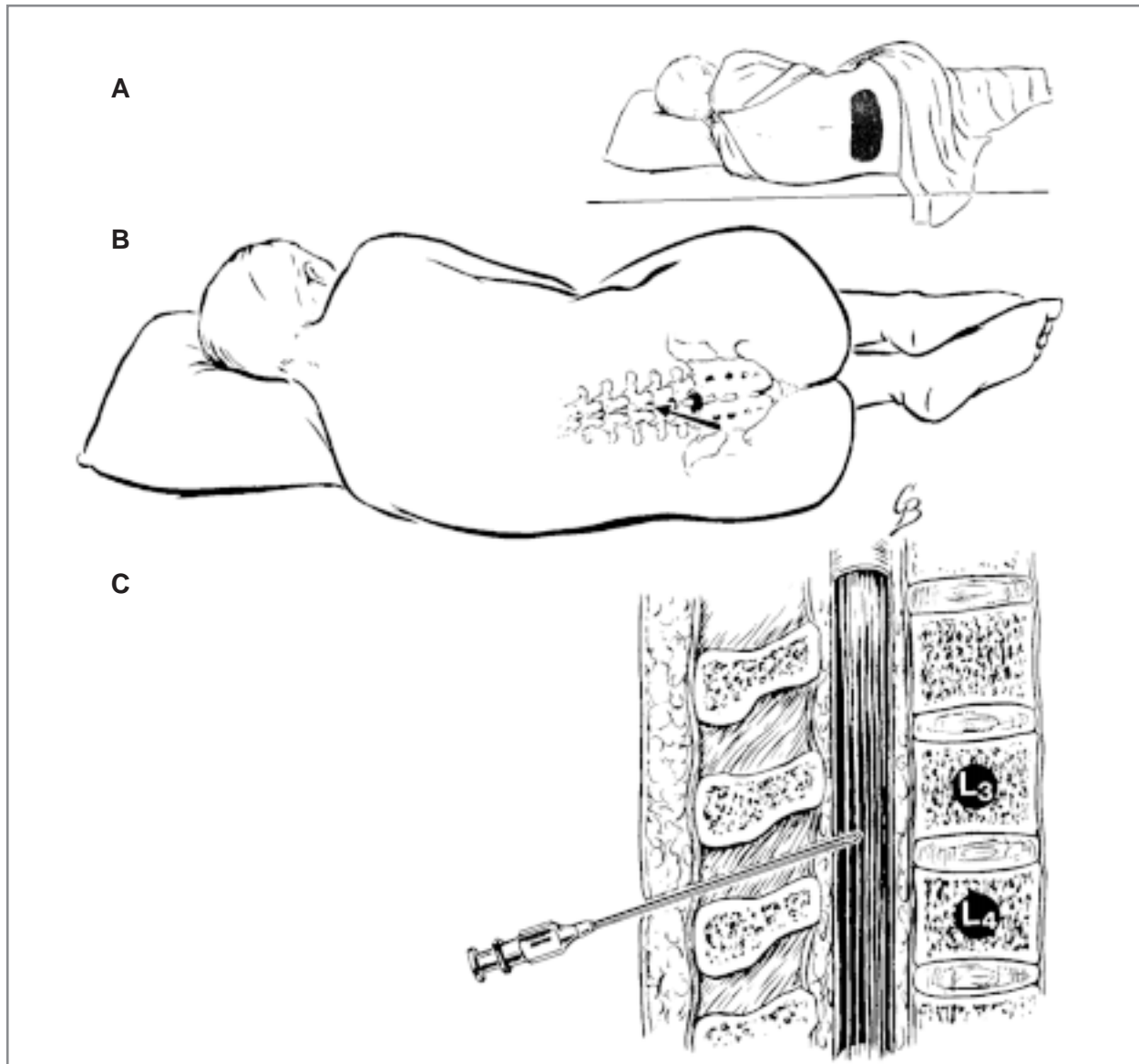


Figure 2. Collection of CSF by lumbar puncture.

- A. The patient lies on his side with knees flexed and back arched to separate the lumbar vertebrae. The patient is surgically draped, and an area overlying the lumbar spine is disinfected.
- B. The space between lumbar vertebrae L3 and L4 is palpated with the sterilely gloved forefinger.
- C. The spinal needle is carefully directed between the spinous processes, through the interspinous processes, through the interspinous ligaments into the spinal canal.

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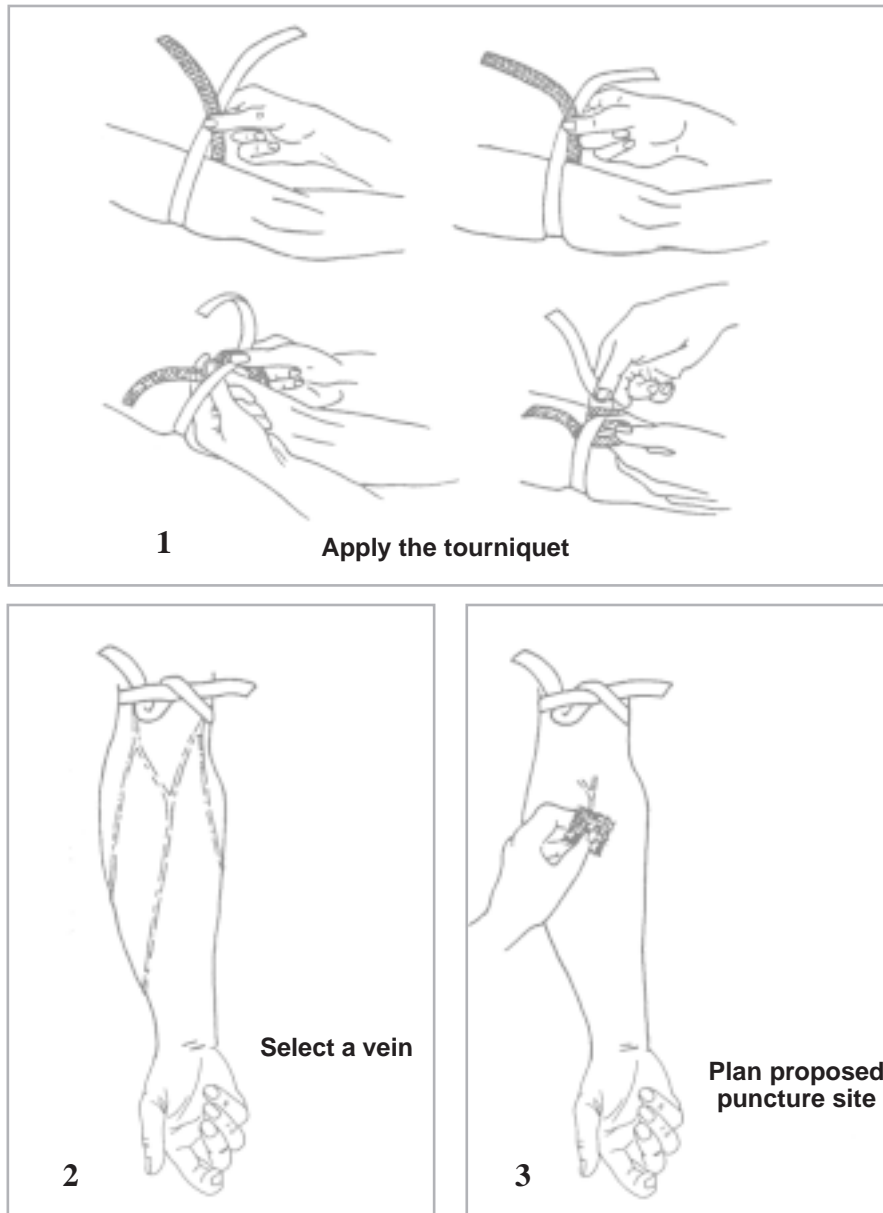


Figure 3. Collection of blood from an arm.

1. Apply the tourniquet
2. Select a vein
3. Plan proposed puncture site

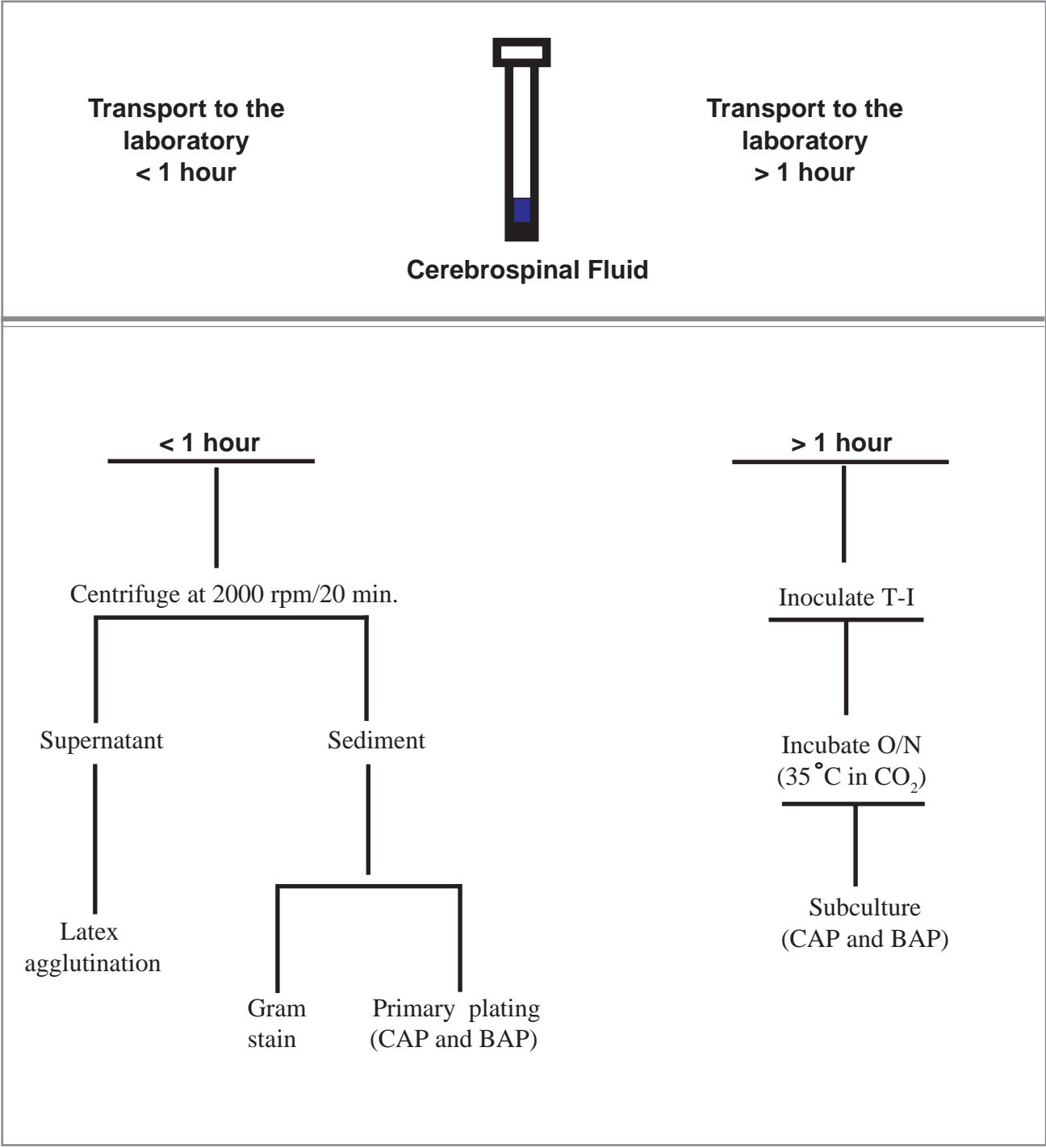


Figure 4. Processing of CSF.



Figure 5. Trans-Isolate medium.

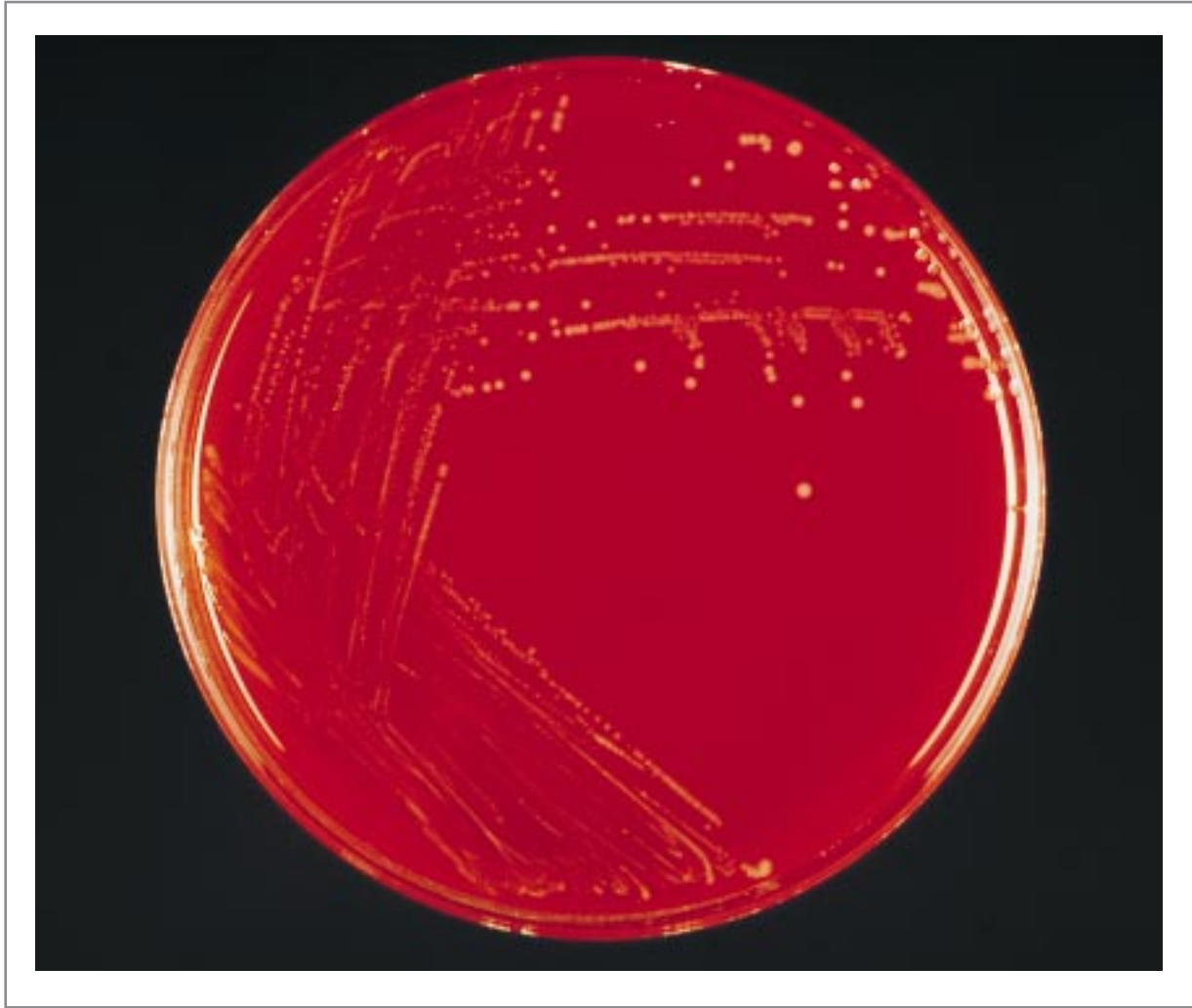


Figure 6. *N. meningitidis* — proper streaking and growth on blood agar plate.



Figure 7. *S. pneumoniae* — proper streaking and growth on blood agar plate.

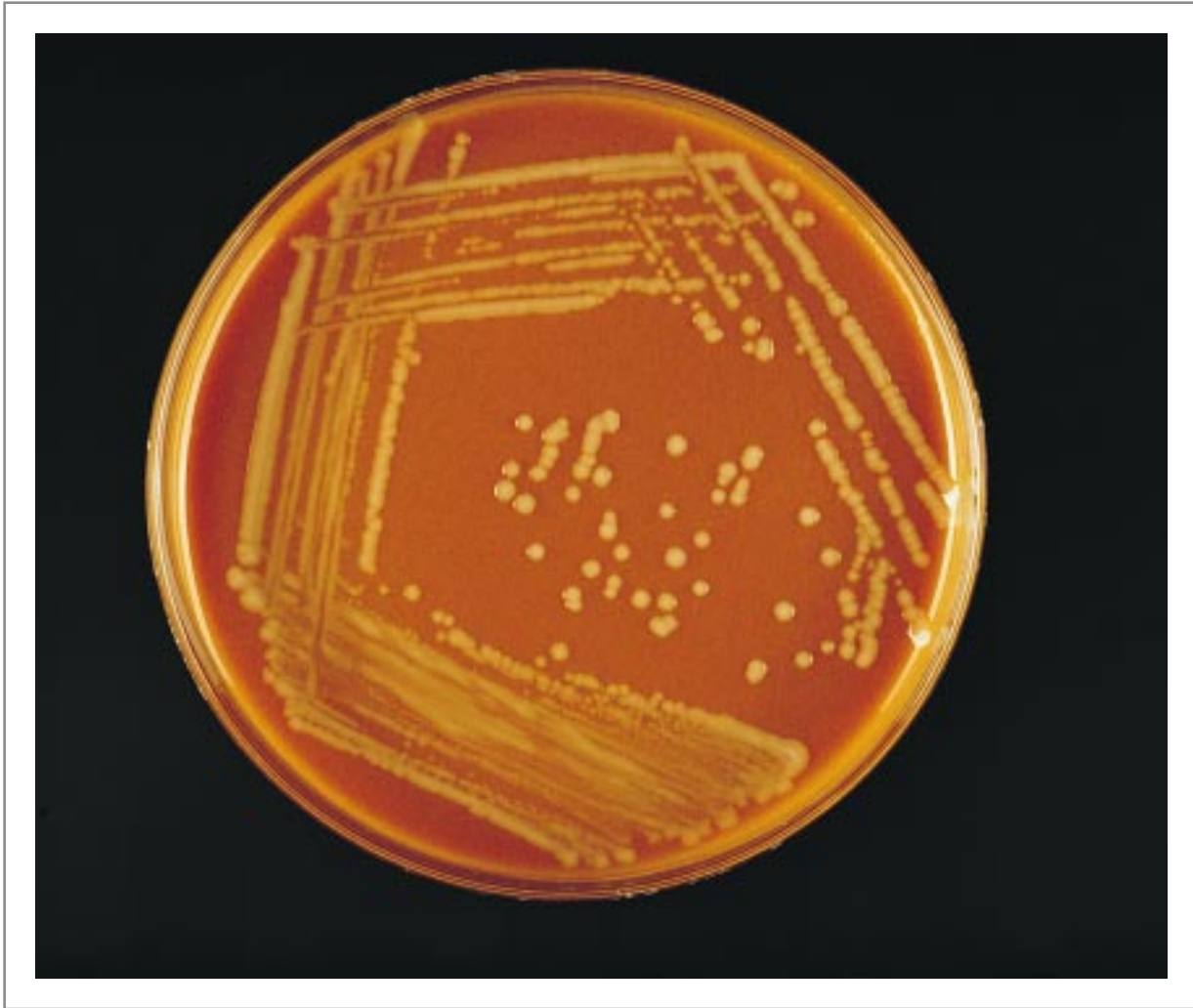


Figure 8. *H. influenzae* — proper streaking and growth on chocolate agar plate.

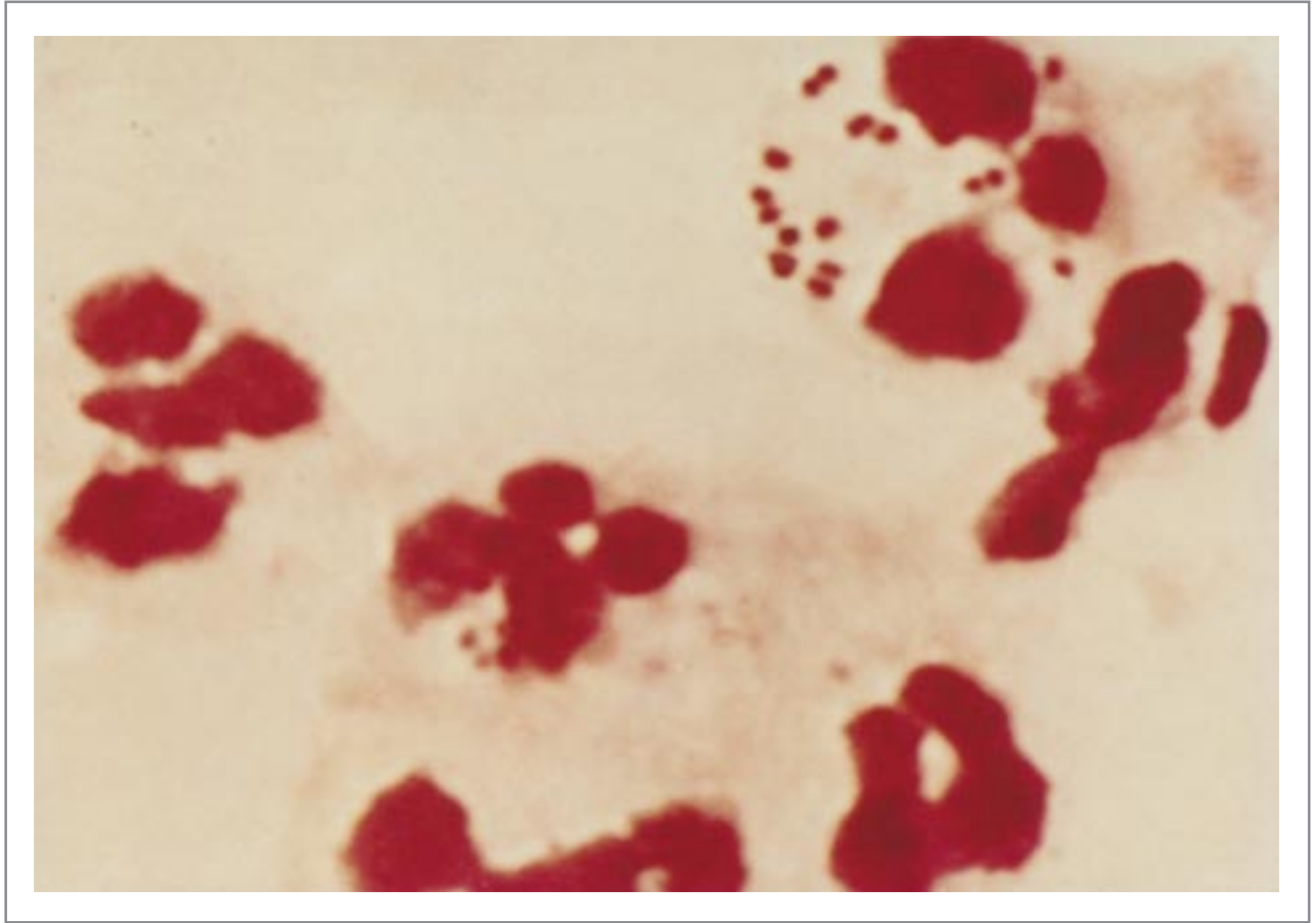


Figure 9a. Gram stain of CSF — *N. meningitidis*: intra-cellular, Gram-negative diplococci.

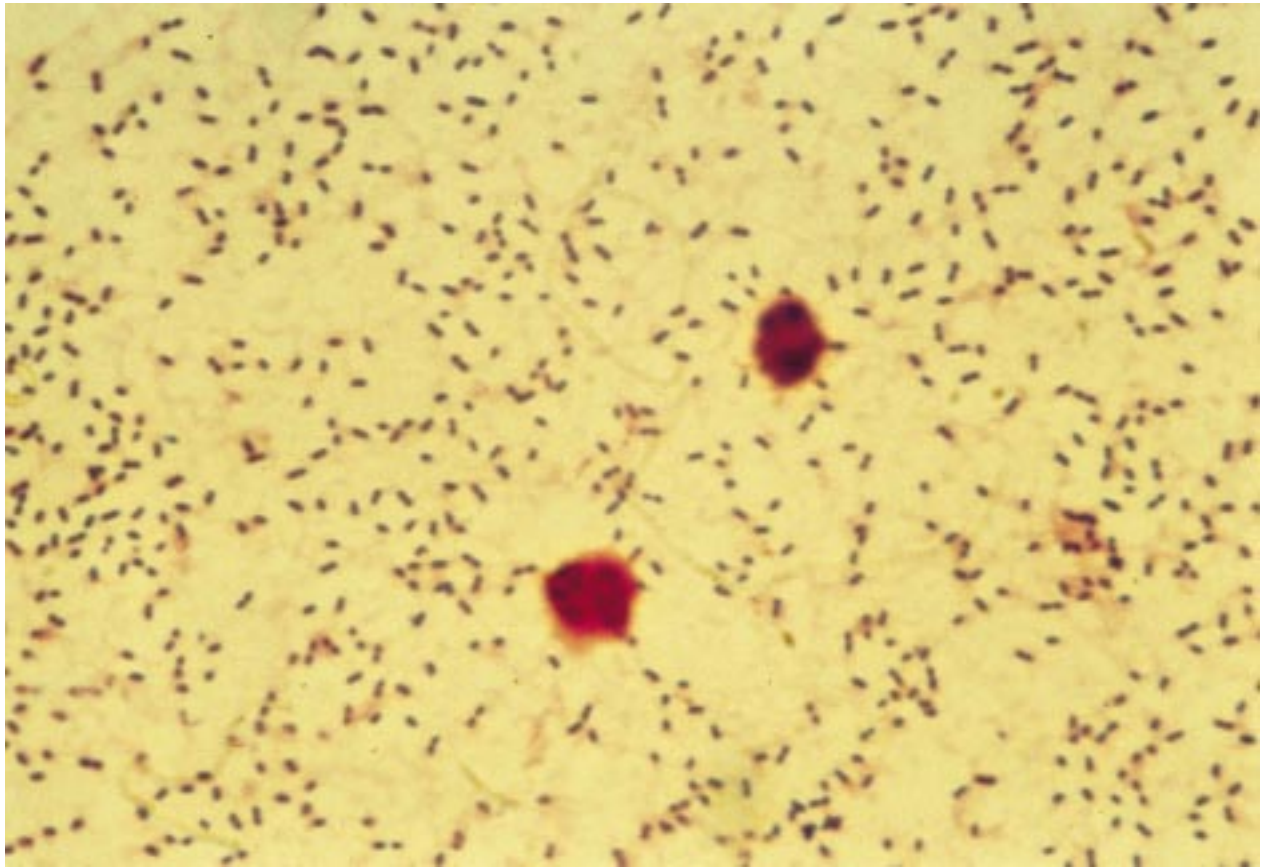


Figure 9b. Gram stain of CSF — *S. pneumoniae*: Gram-positive diplococci. Note that this slide represents a case where an exceptionally large number of organisms are present.

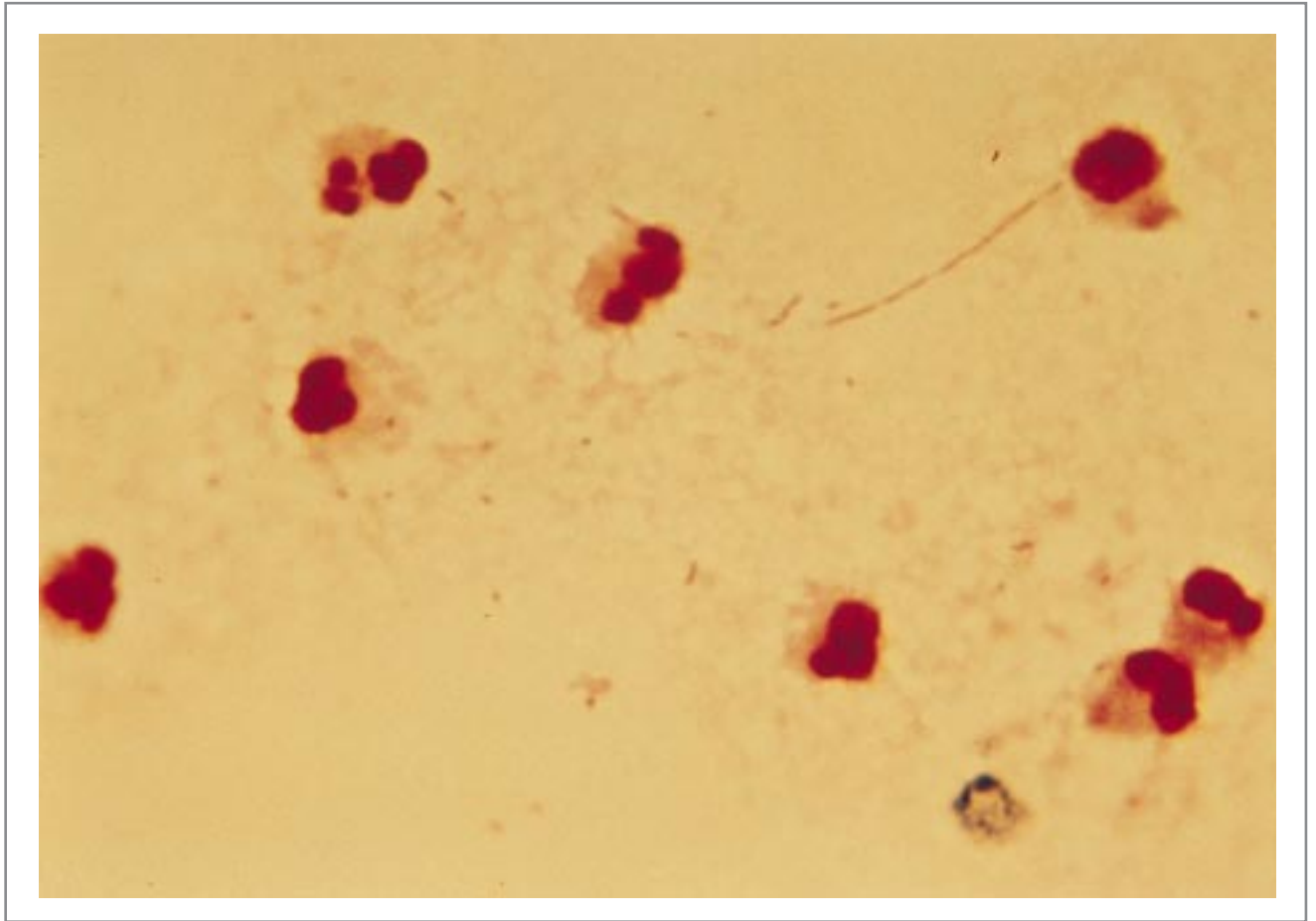


Figure 9c. Gram stain of CSF — *H. influenzae*: Gram-negative pleomorphic coccobacilli.

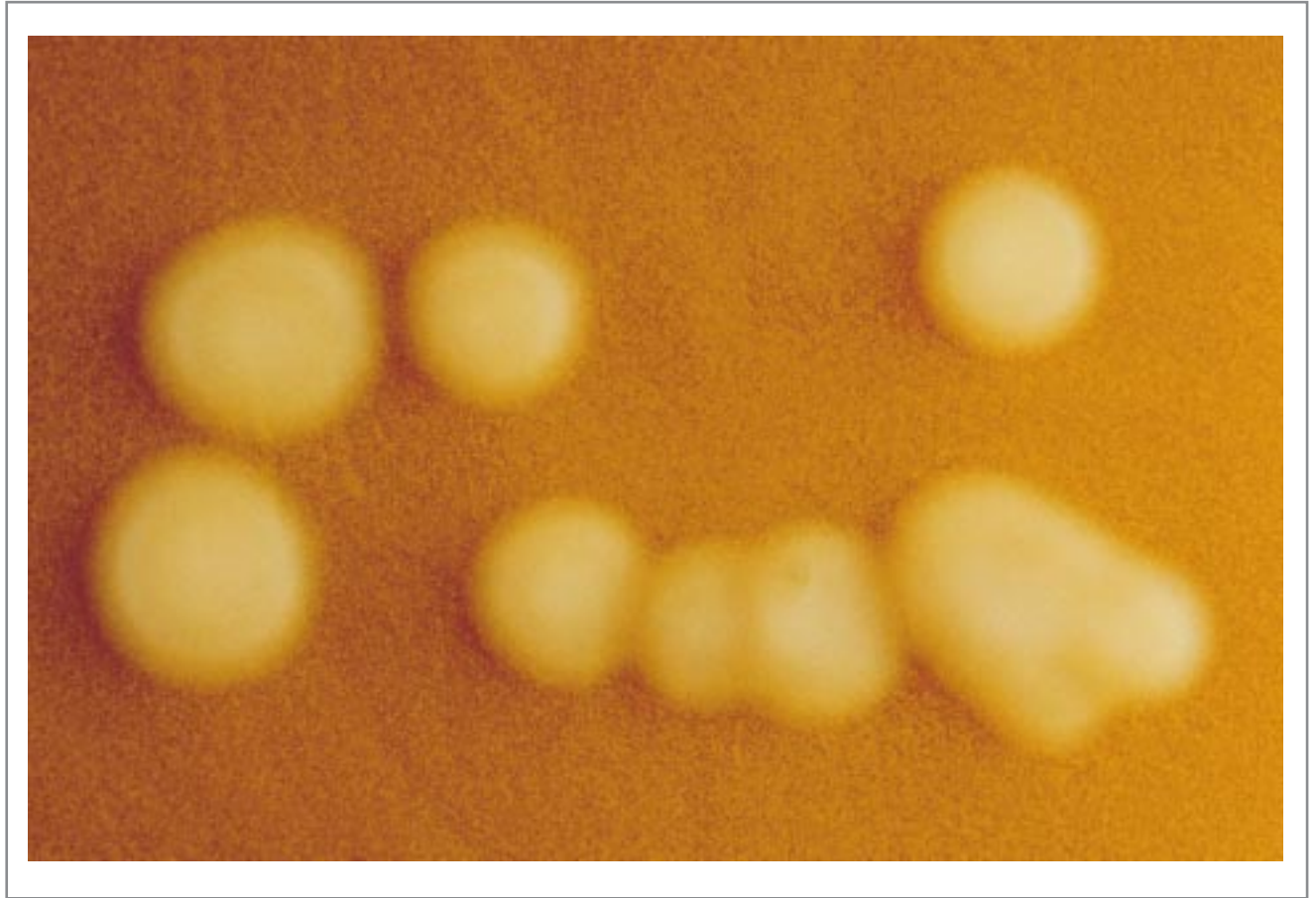


Figure 10. On chocolate agar plate, *H. influenzae* appear as large colourless to grey opaque colonies with no discolouration of the surrounding medium.

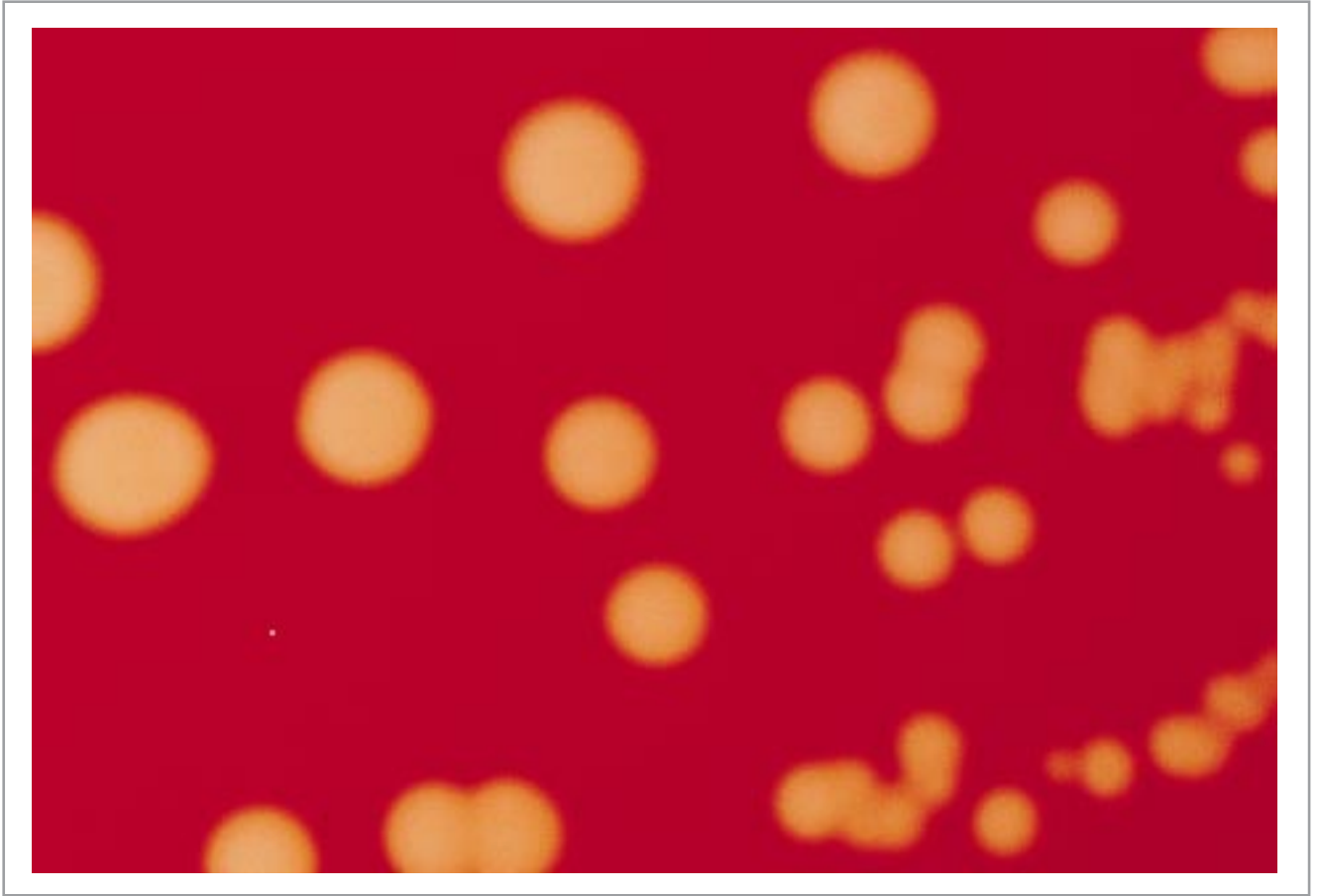


Figure 11. Overnight growth of *N. meningitidis* on blood agar plate appears as round, moist, glistening and convex colonies.

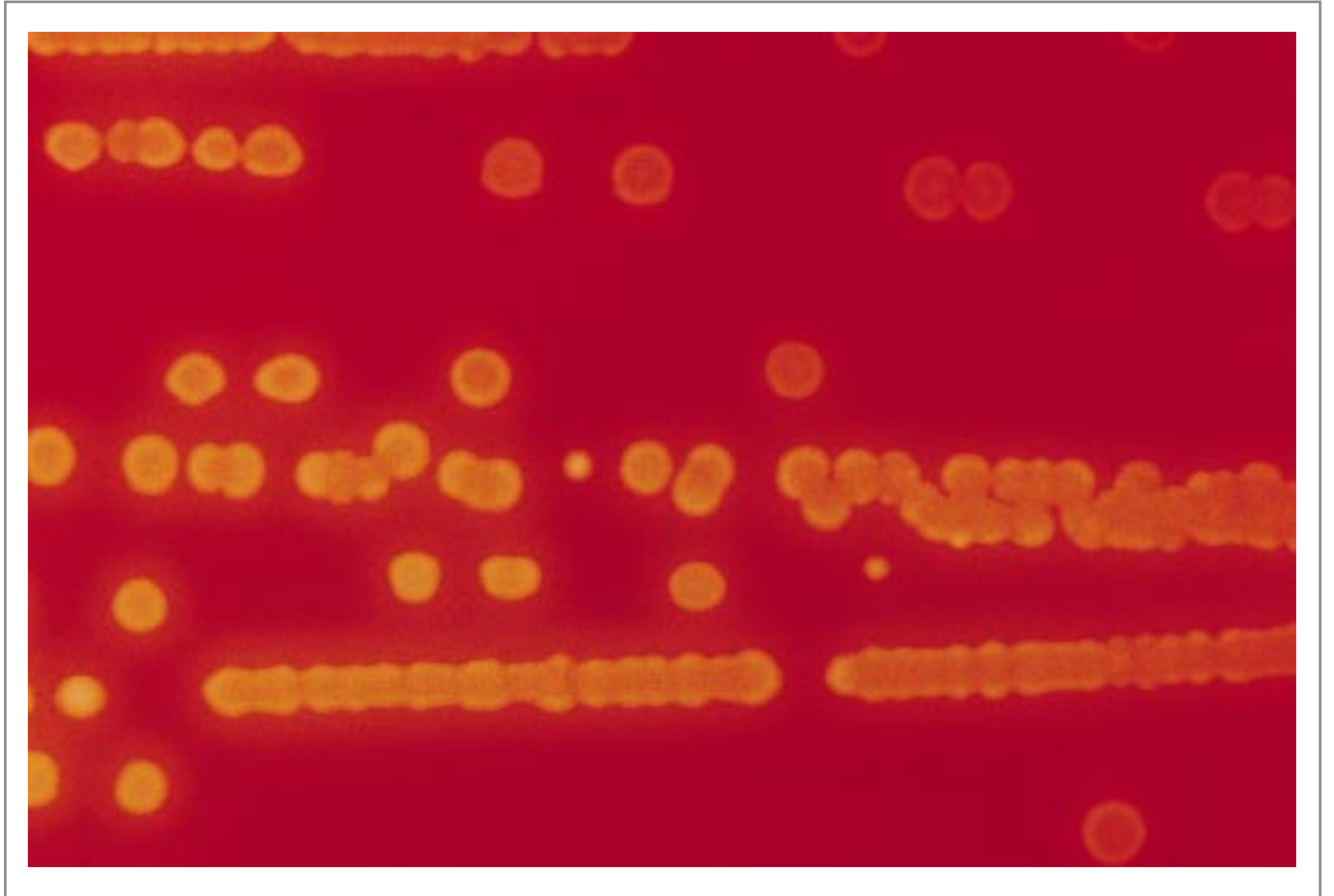


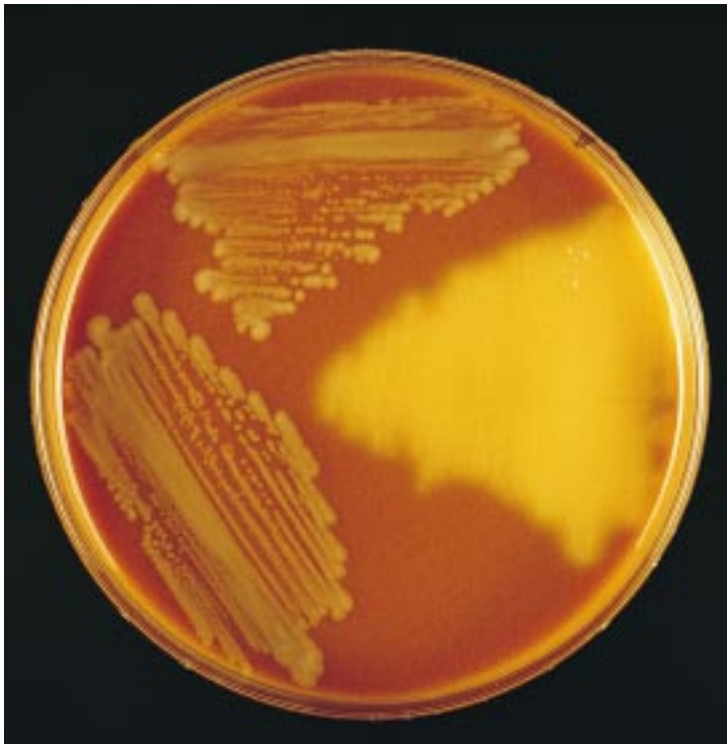
Figure 12. *S. pneumoniae* appear as small greyish mucoid (watery) colonies with a greenish zone of alpha-haemolysis surrounding them on the blood agar plate.

Growth on		Gram stain	Presumptive identification
CAP	BAP (Sheep)		
+	+	Gram-negative diplococci	<i>N. meningitidis</i>
+	+	Gram-positive diplococci	<i>S. pneumoniae</i>
+	—	Gram-negative pleomorphic coccobacilli	<i>H. influenzae</i>

Figure 13. Presumptive identification of *N. meningitidis*, *S. pneumoniae*, and *H. influenzae*.



(a) Growth on blood agar plate.



(b) Growth on chocolate agar plate.

Figure 14. *N. meningitidis* (left), *S. pneumoniae* (right), and *H. influenzae* (top):
(a) growth on blood agar plate and (b) growth on chocolate agar plate.

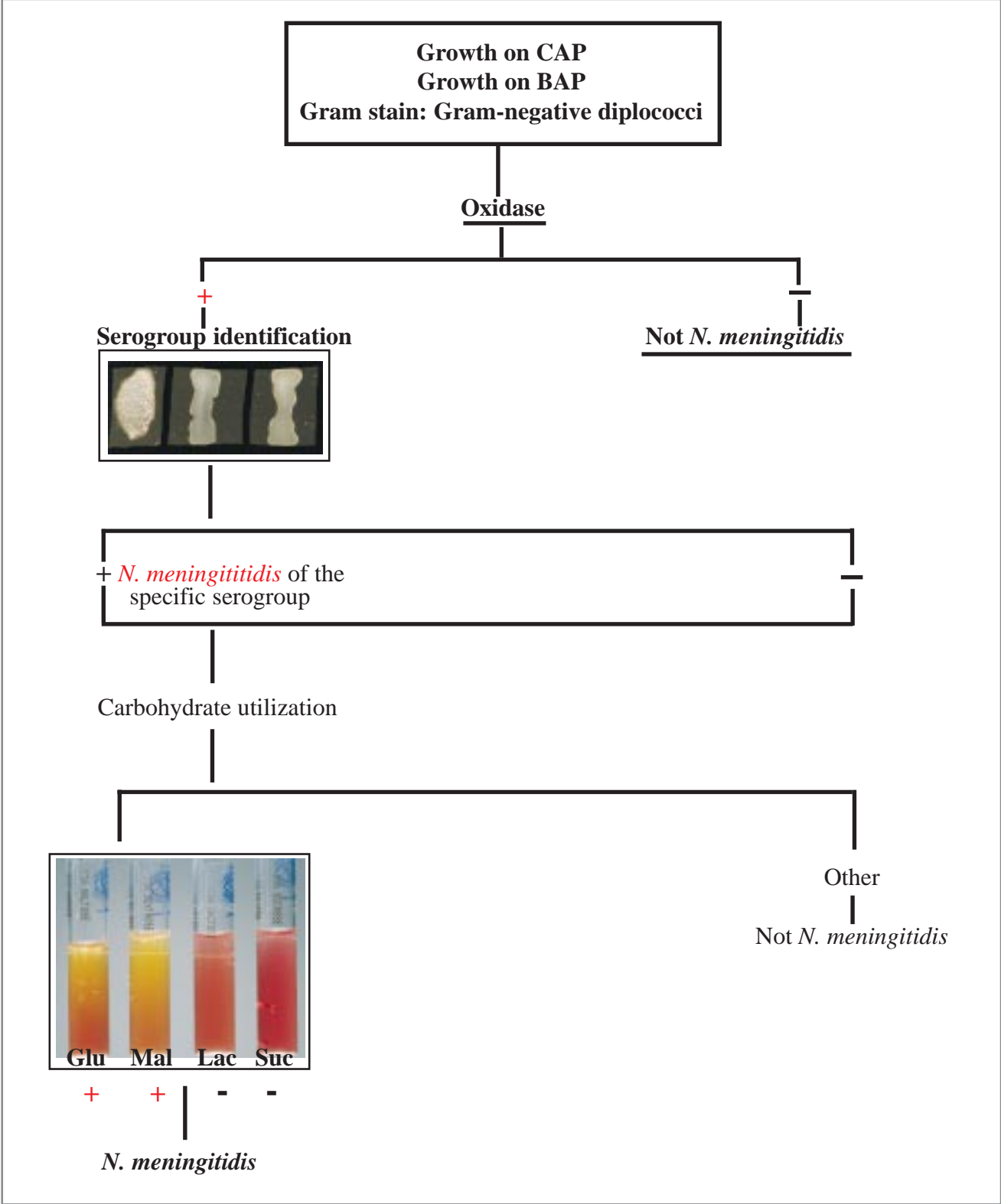


Figure 15. Identification of *N. meningitidis*.

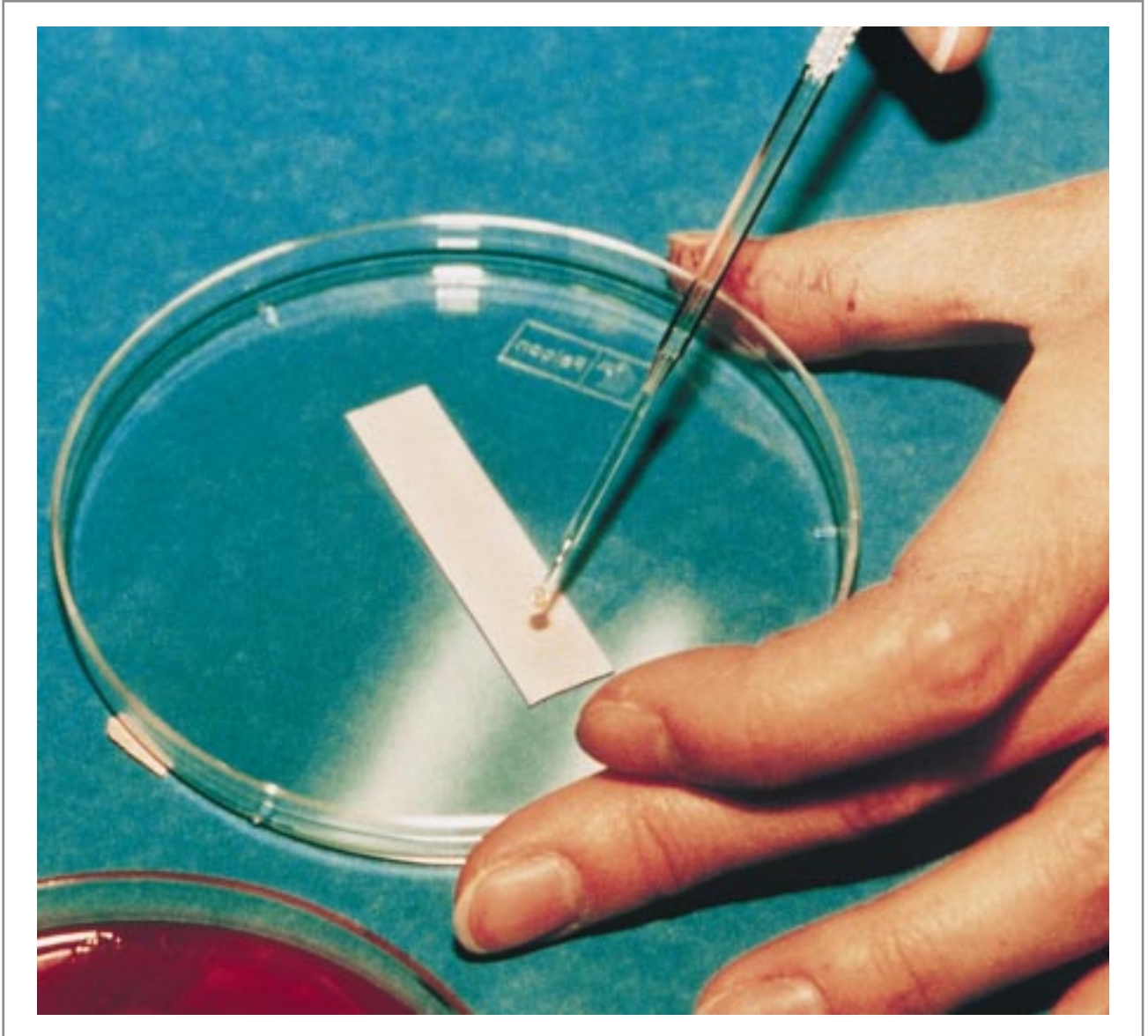


Figure 16. Kovac's oxidase test — positive reaction.

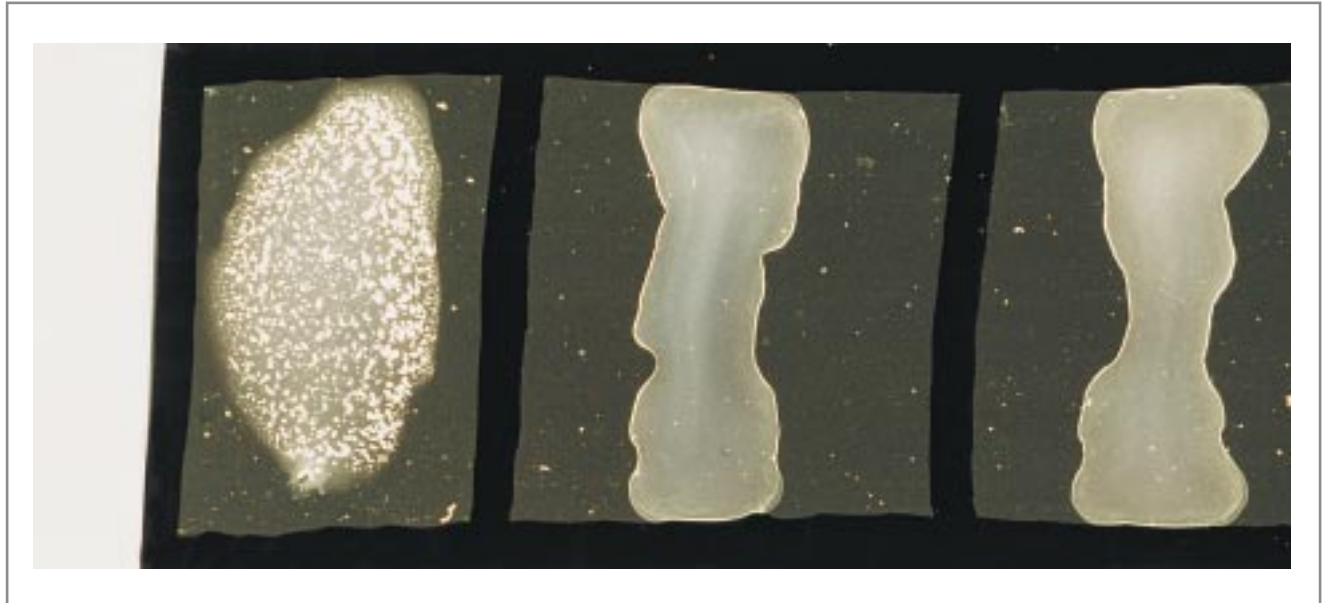


Figure 17. Agglutination, with clearing of the liquid, occurs when a suspension of the isolate is mixed with its homologous antiserum (left). A negative reaction, as in the case of heterologous antiserum (centre) and of saline (right), remains smooth and turbid.

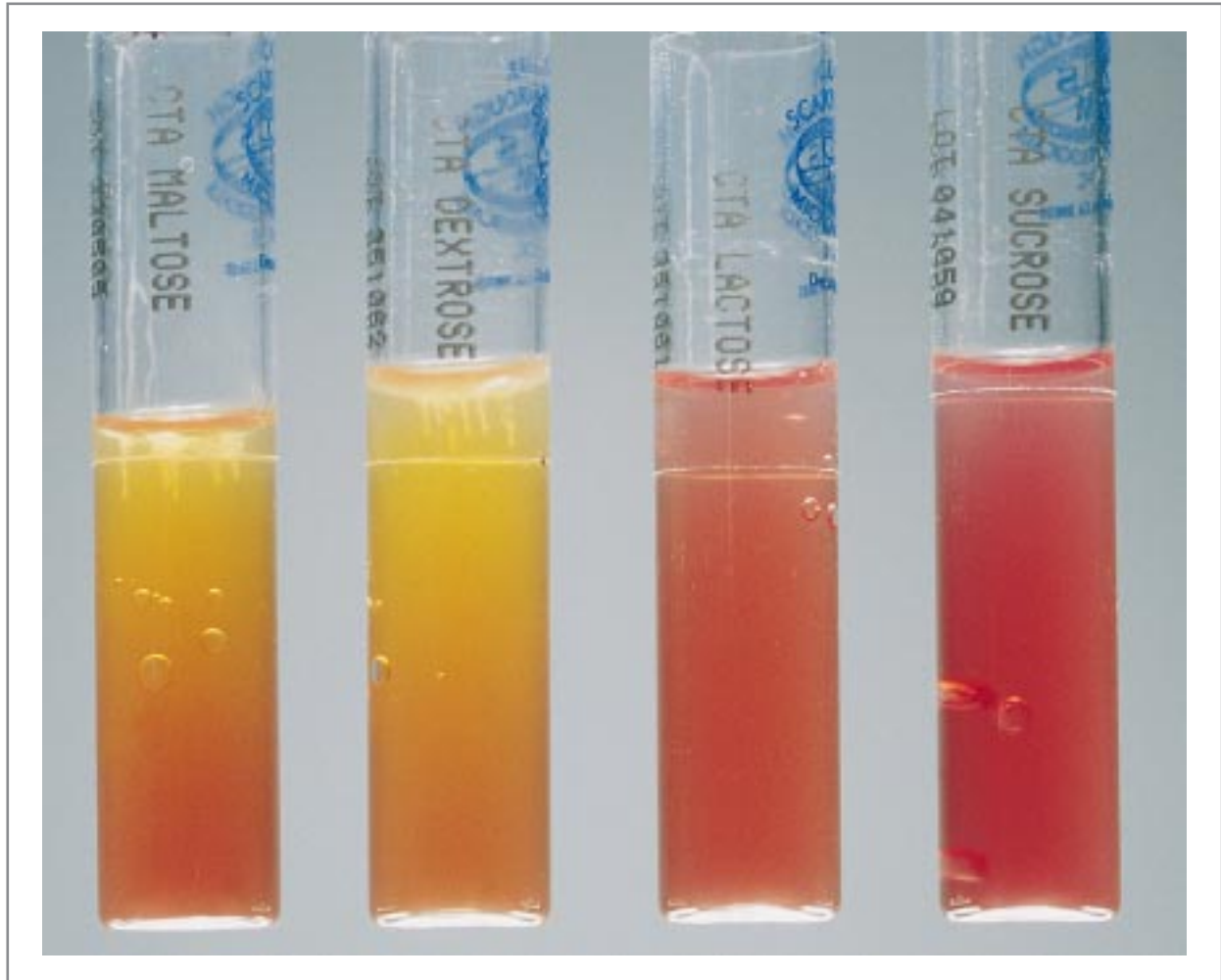
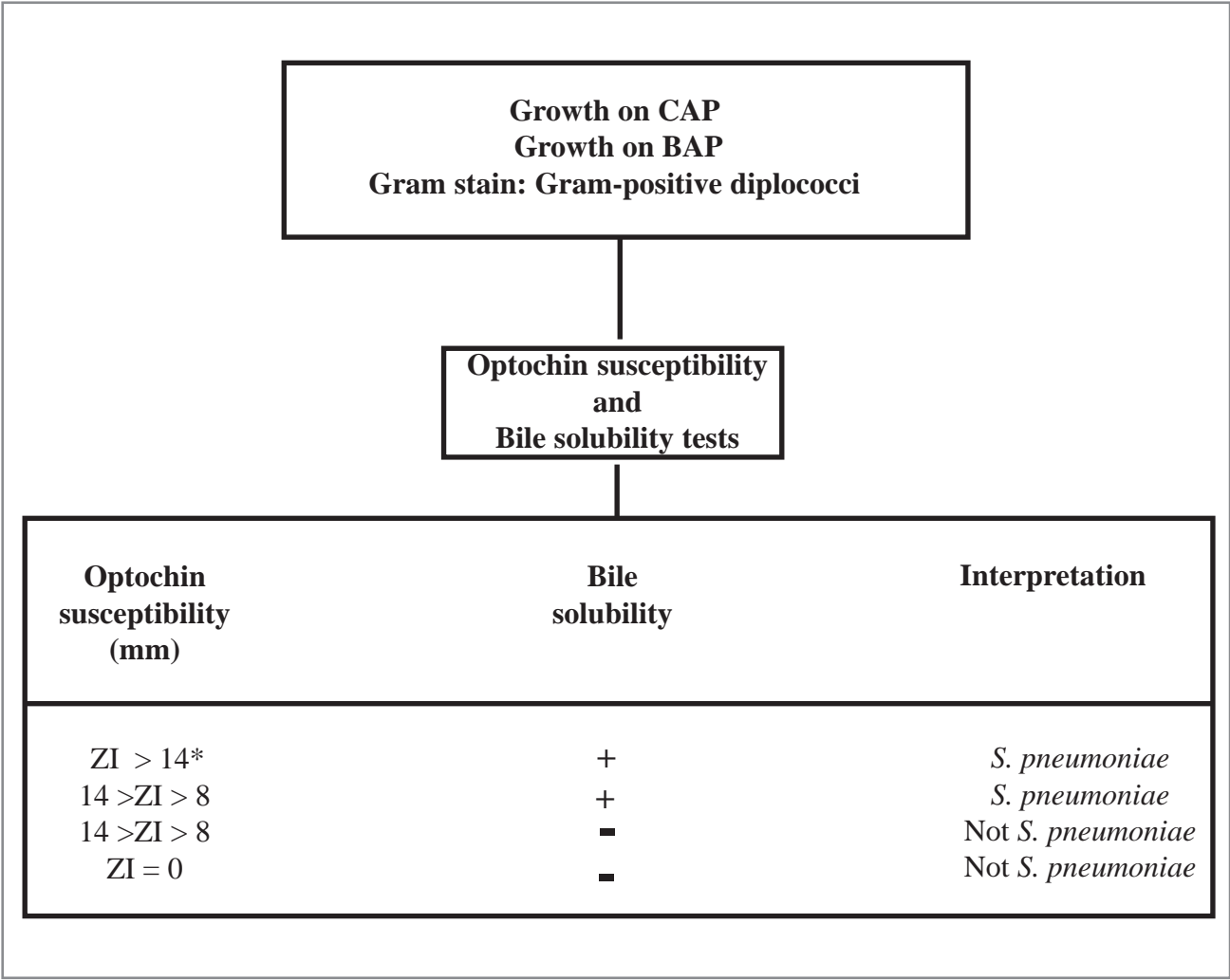


Figure 18. Cystine trypticase agar-sugar reactions differentiating *N. meningitidis* from other *Neisseria* species. Acid production (yellow colour) shows oxidative utilization of dextrose and maltulose with no utilization of lactulose and sucrose.



Note: ZI = Zone of inhibition for BBL (BBL Microbiology Systems) optochin disks. When using any other brand of optochin disks, follow the instructions for the interpretation of zones of inhibition, as specified by that manufacturer.

*95% of clinical *S. pneumoniae* isolates react in this manner

Figure 19. Identification of *S. pneumoniae*.

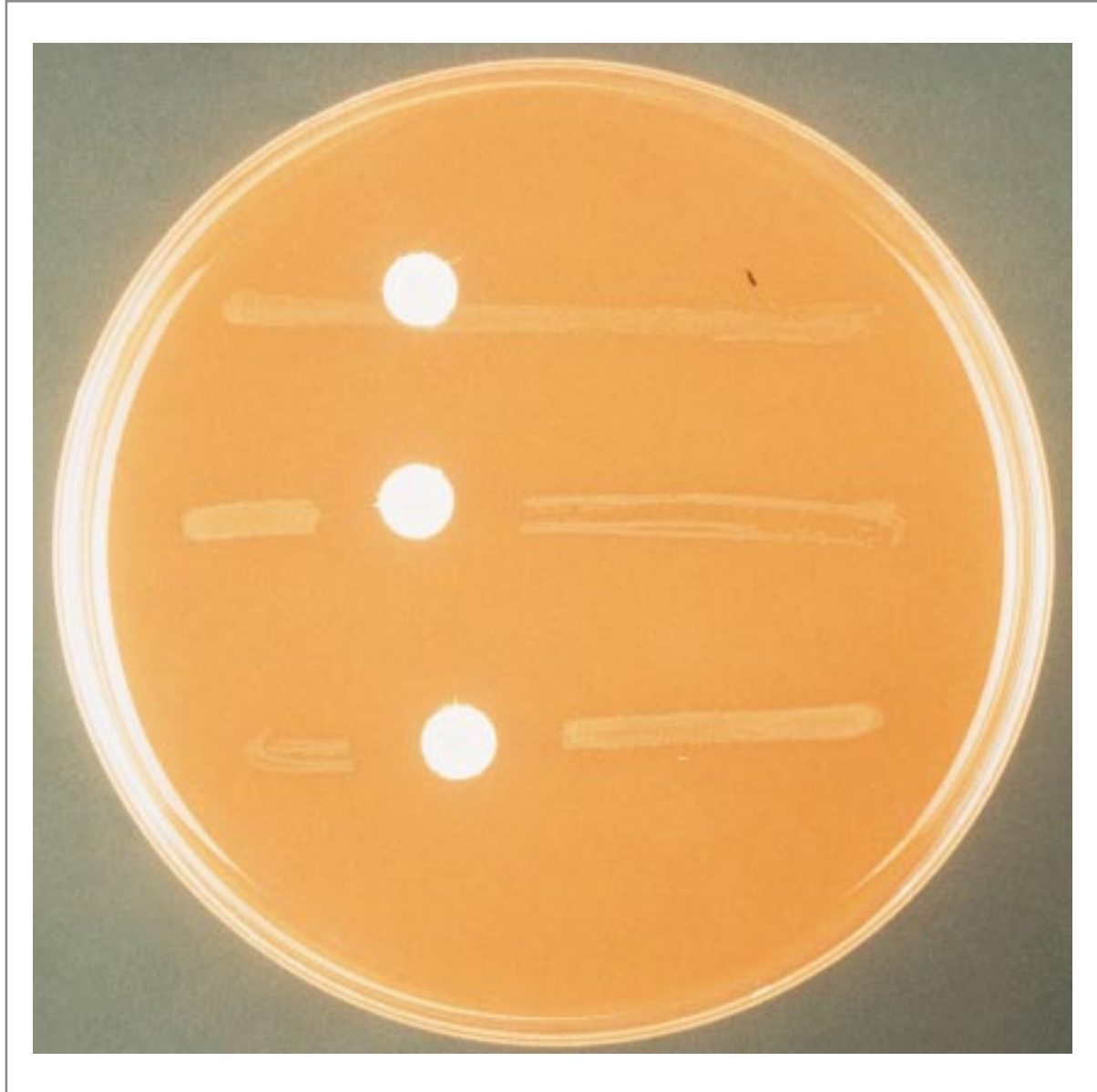


Figure 20. Optochin susceptibility test for identification of *S. pneumoniae*. The strain in the top streak is resistant to optochin and, therefore, is not a pneumococcus. The strains in the centre and lower streaks are susceptible to optochin and appear to be pneumococci.

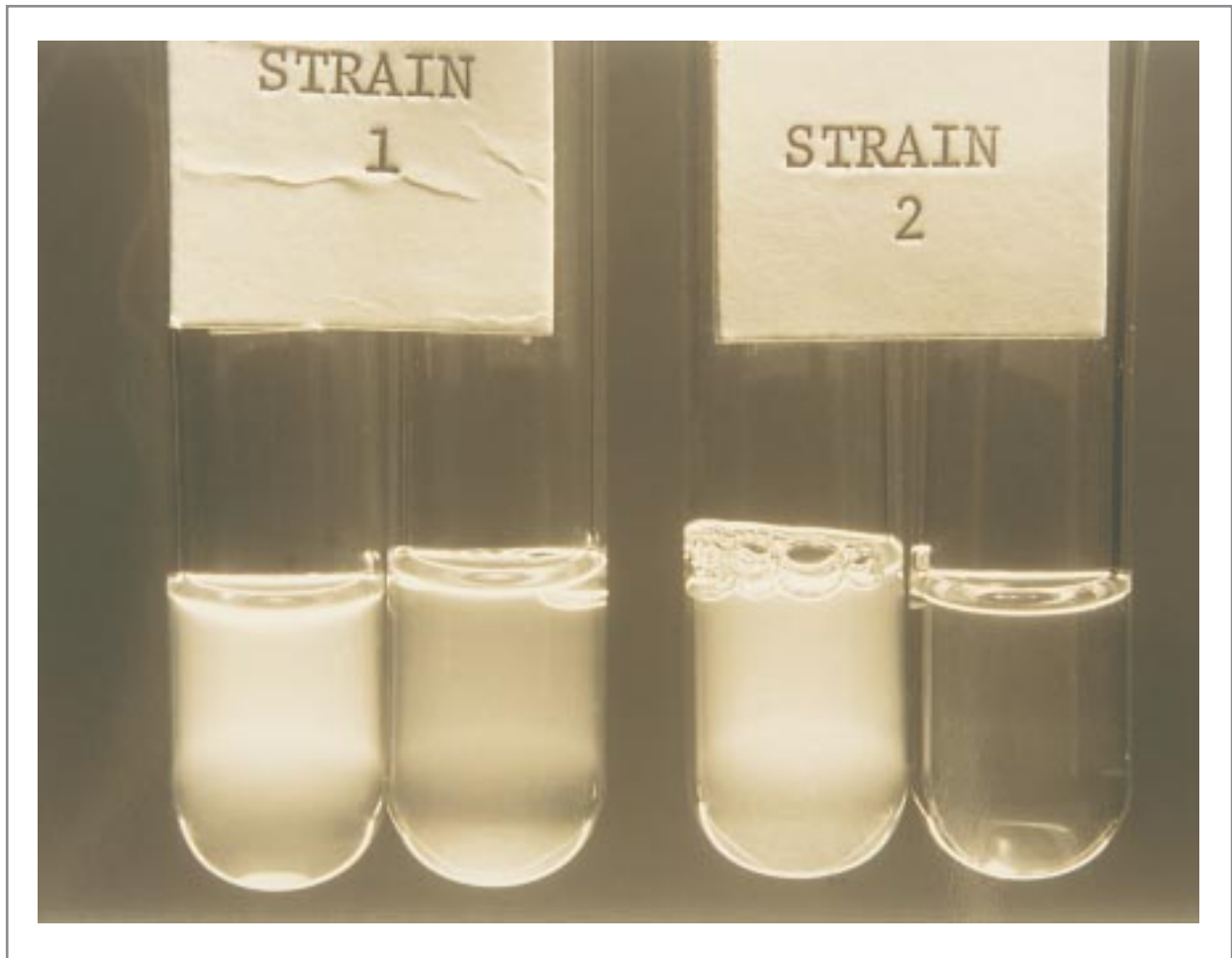


Figure 21. The bile solubility test for two different strains. Strain 1 is not *S. pneumoniae* as both tubes are turbid. There is a slight decrease in turbidity in the tube containing bile salts for strain 1 (2nd tube from the left) but the tube is almost as turbid as the control tube (1st tube on the left). Strain 2 is *S. pneumoniae*. Note that the tube on the far right is clear, all the turbidity due to the cells has disappeared and the cells have lysed; by contrast the control tube (3rd tube from the left) is still very turbid.

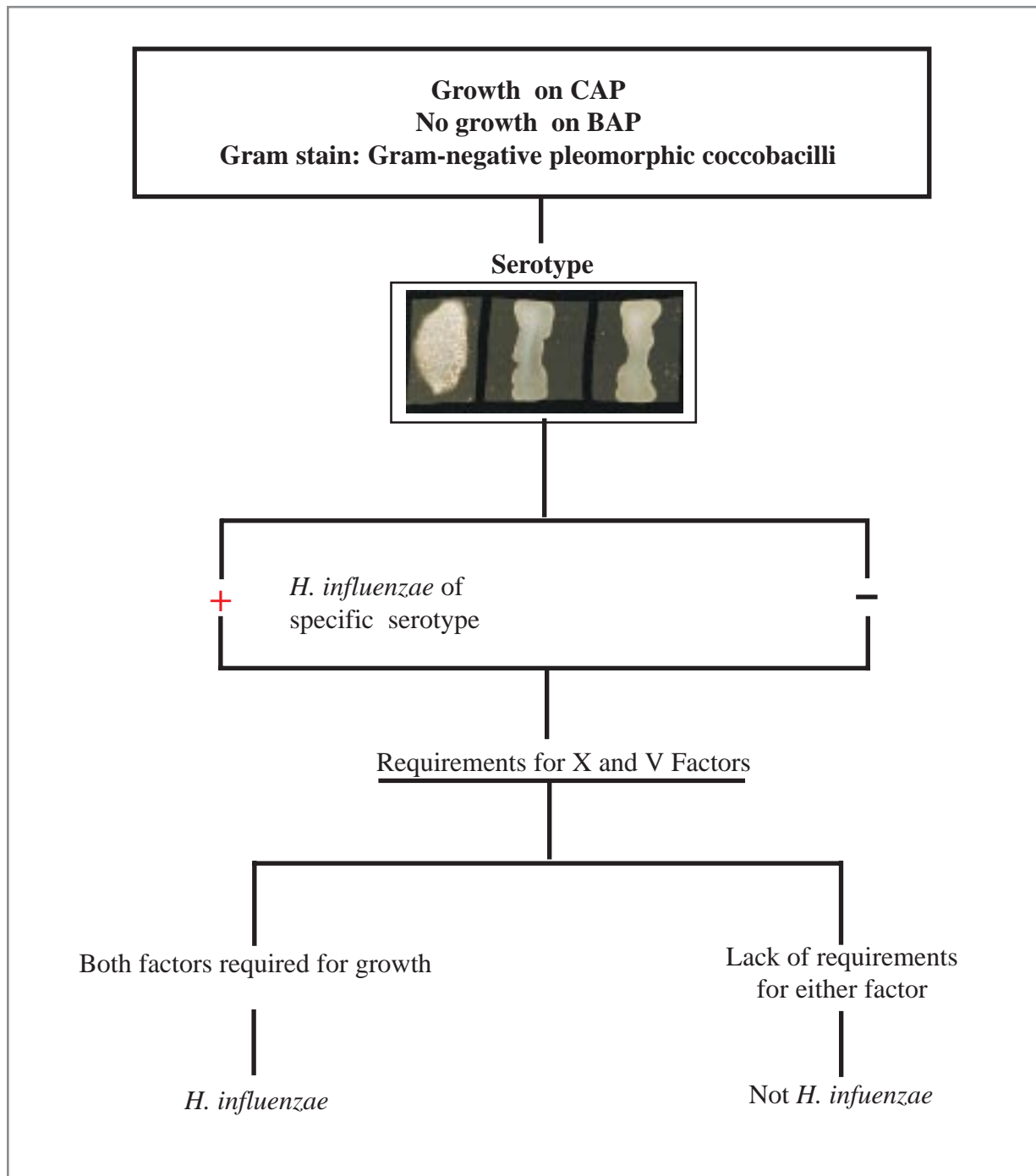


Figure 22. Identification of *H. influenzae*.

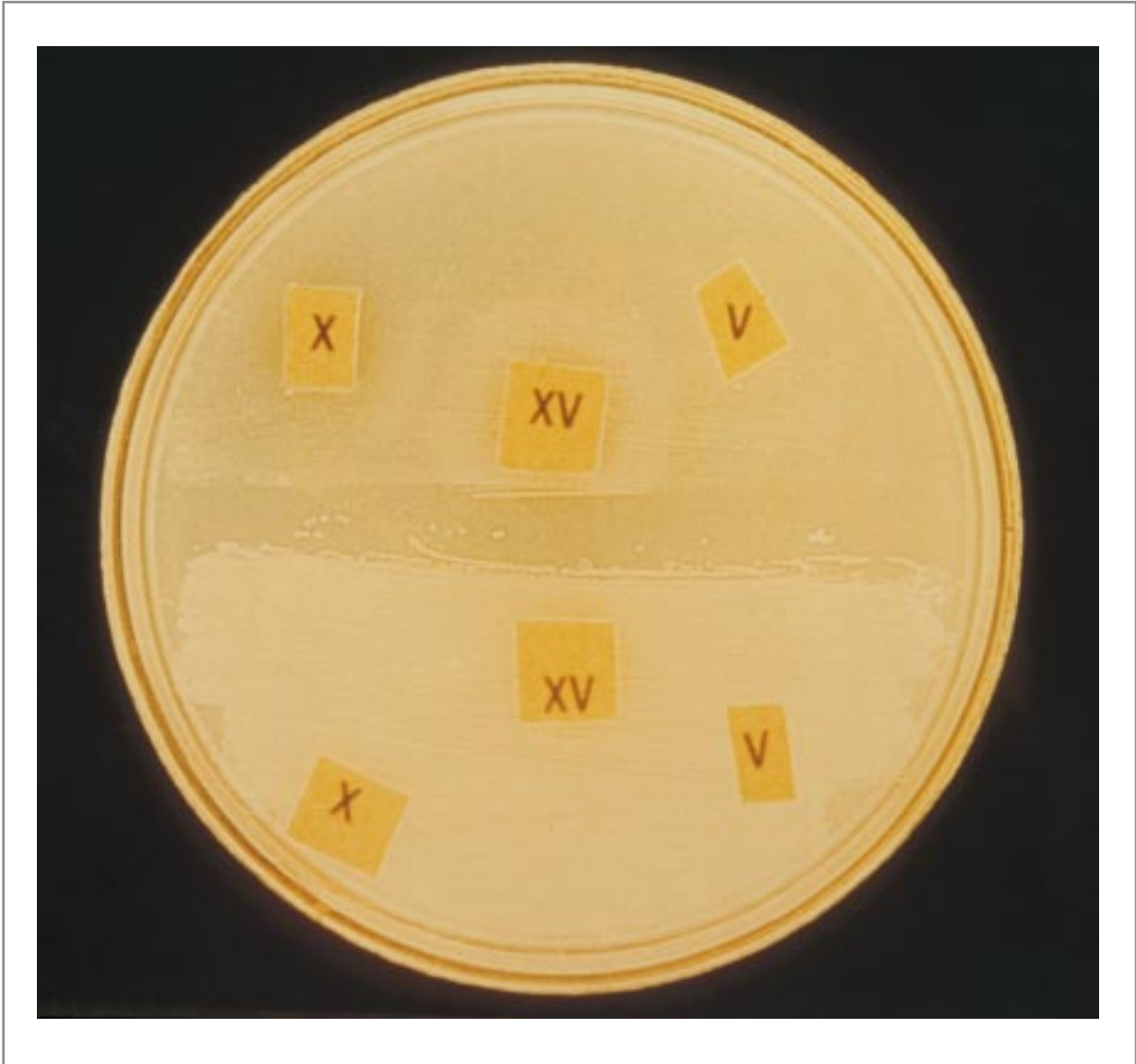


Figure 23. Growth factor requirements. *H. influenzae* will grow only around the disk containing both X and V factors.

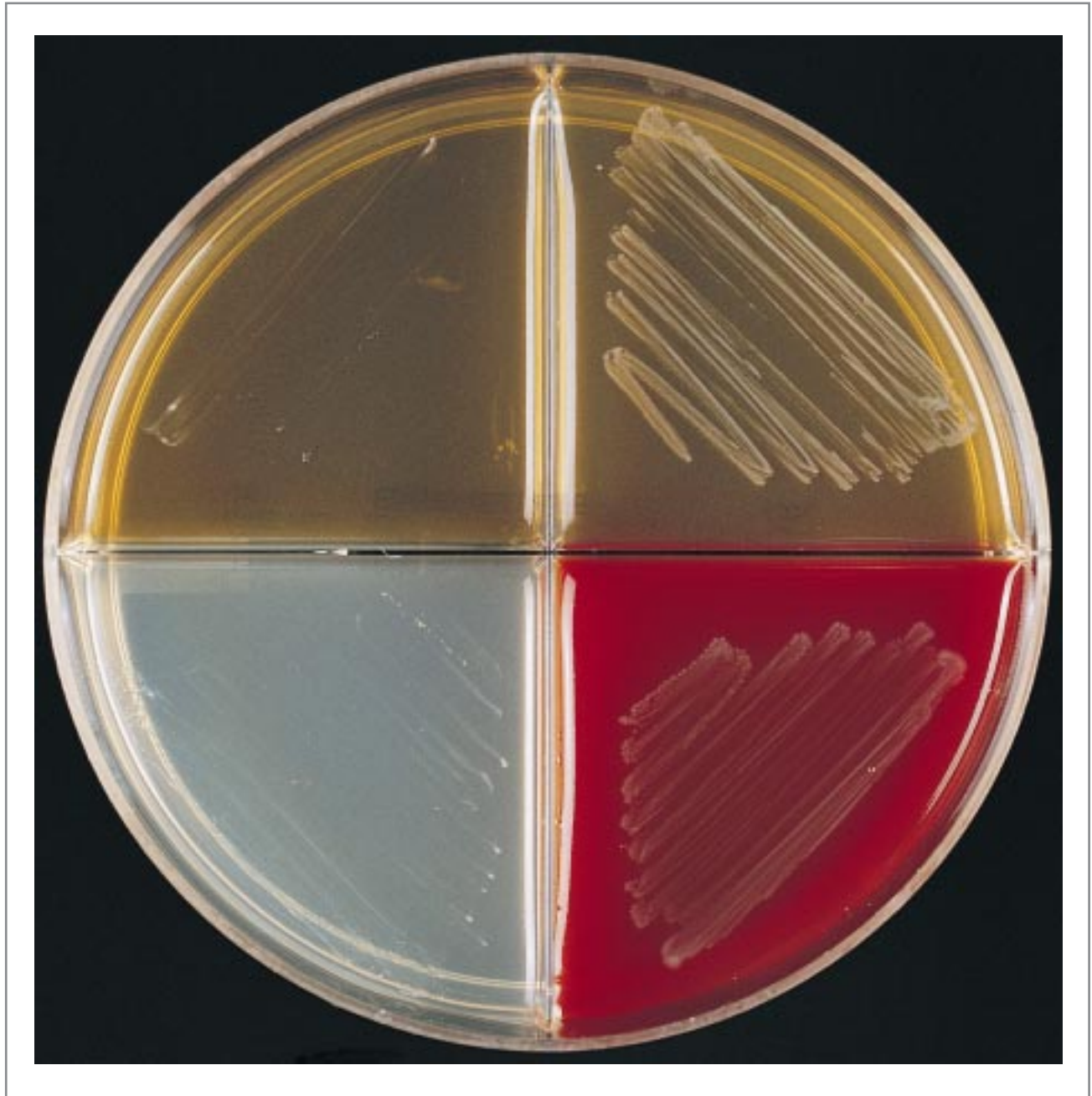


Figure 24. Growth factor requirements for *H. influenzae*: *Haemophilus* quad ID plate. This agar plate is divided into four compartments. One quadrant includes medium containing haemin (X factor) (upper left). One quadrant includes medium containing nicotinamide dinucleotide (V factor) (lower left). Another quadrant contains medium that includes both X and V factors (upper right). The fourth quadrant contains heart infusion agar with 5% horse blood (lower right).

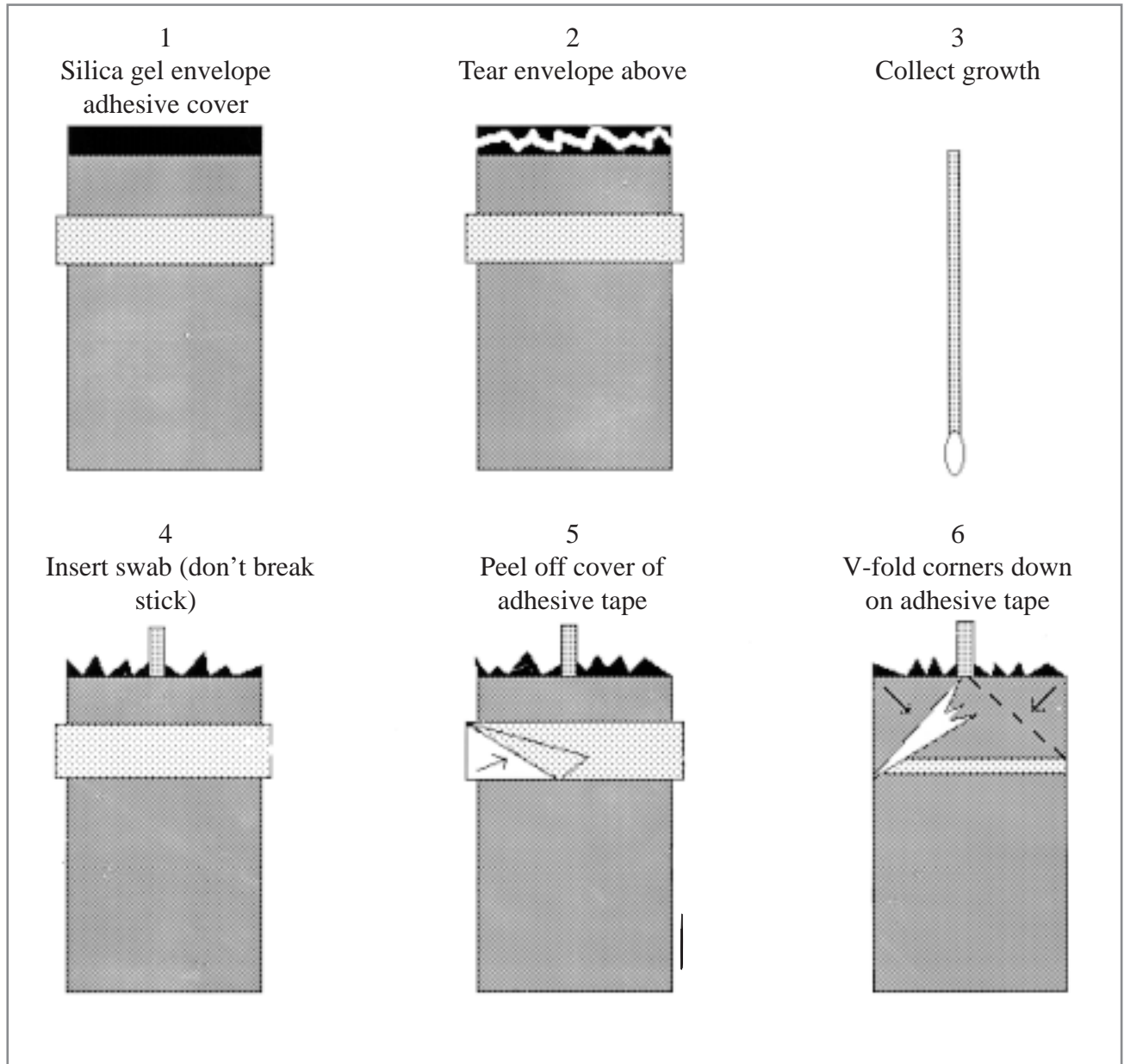


Figure 25. Instructions for using silica gel envelopes for transport of isolates.

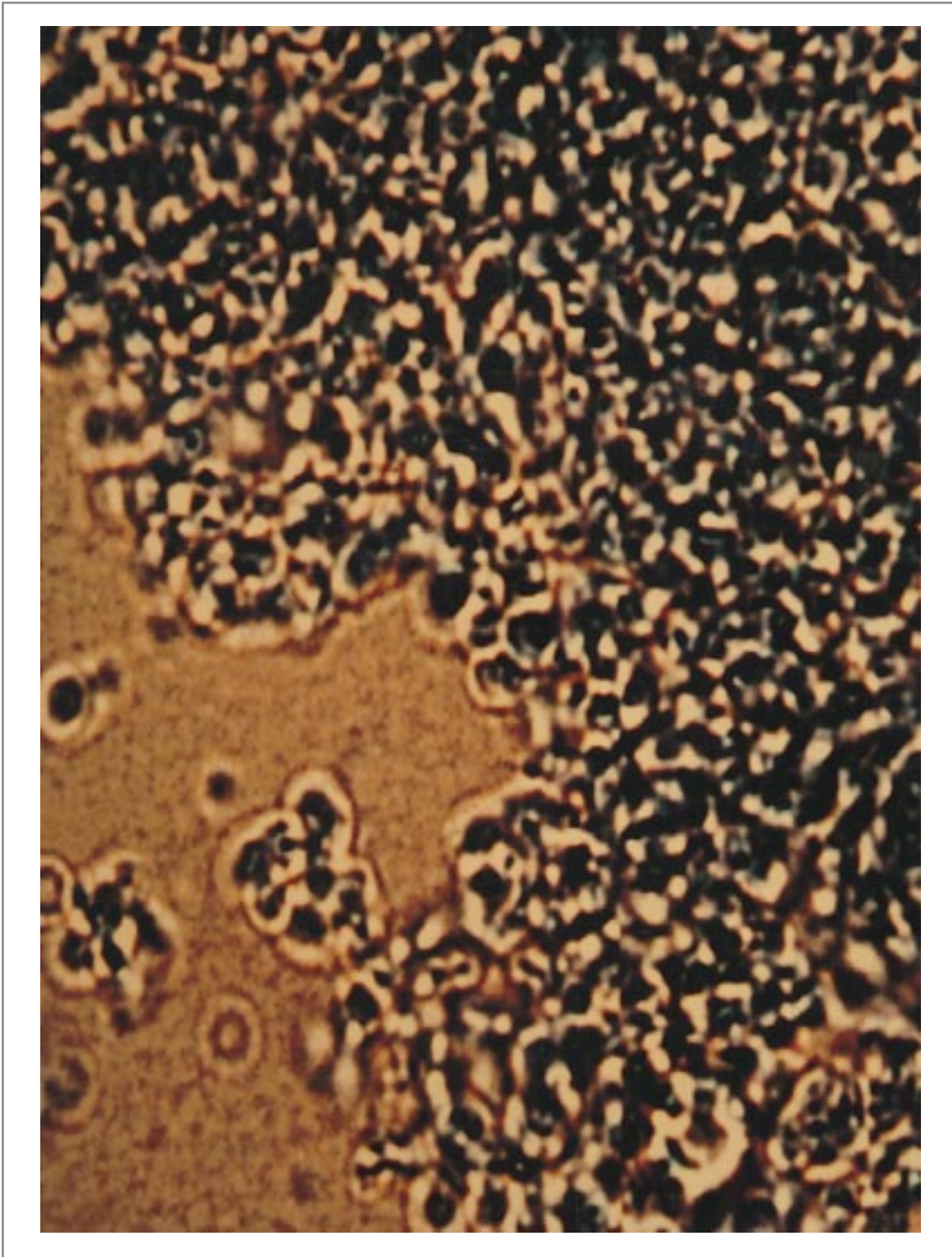


Figure 26. Quellung reaction for identification of *S. pneumoniae*.



World Health Organization