

# NEISSERIA

Of the eleven species of *Neisseria* that colonize humans, only two are pathogens. <sup>1</sup>*N. gonorrhoeae* (the gonococcus) is the causative agent of gonorrhoea and is transmitted via sexual contact. Symptoms of infection with *N. gonorrhoeae* differ depending on the site of infection. Infection of the genitals can result in a purulent (or pus-like) discharge from the genitals which may be foul smelling, inflammation, redness, swelling, dysuria and a burning sensation during urination. *N. gonorrhoeae* can also cause conjunctivitis, pharyngitis, proctitis or urethritis, prostatitis and orchitis. Conjunctivitis is common in neonates and silver nitrate or antibiotics are often applied to their eyes as a preventive measure against gonorrhoea. Neonatal gonorrheal conjunctivitis is contracted when the infant is exposed to *N. gonorrhoeae* in the birth canal, and can result in corneal scarring or perforation. Disseminated *N. gonorrhoeae* infections can occur, resulting in endocarditis, meningitis or gonococcal dermatitis-arthritis syndrome.

*Neisseria meningitidis* (the meningococcus) causes significant morbidity and mortality in children and young adults worldwide through epidemic or sporadic meningitis and/or septicemia. *N. meningitidis* is exclusive human pathogen. The epidemiological profile of *N. meningitidis* is variable in different populations and over time and virulence of the meningococcus is based on a transformable/plastic genome and expression of certain capsular polysaccharides (serogroups A, B, C, W-135, Y and X) and non-capsular antigens. *N. meningitidis* colonizes mucosal surfaces using a multifactorial process involving pili, twitching motility, and surface proteins. Certain clonal groups have an increased capacity to gain access to the blood, evade innate immune responses, multiply, and cause systemic disease. Although new vaccines hold great promise, meningococcal infection continues to be reported in both developed and developing countries, where universal vaccine coverage is absent and antibiotic resistance increasingly more common.

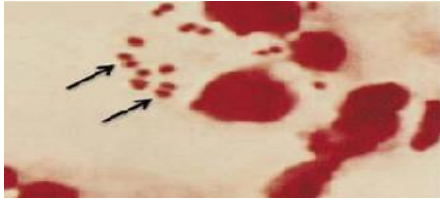
## NEISSERIA - GRAM STAIN

*Neisseria meningitidis* is a Gram-negative, either an encapsulated or unencapsulated, aerobic diplococcus with a “kidney” or “coffee-bean” shape. *N. meningitidis* may occur intracellularly or extracellularly in <sup>2</sup>PMN leukocytes. *N. meningitidis* is a fastidious bacterium, dying within hours on inanimate surfaces.

---

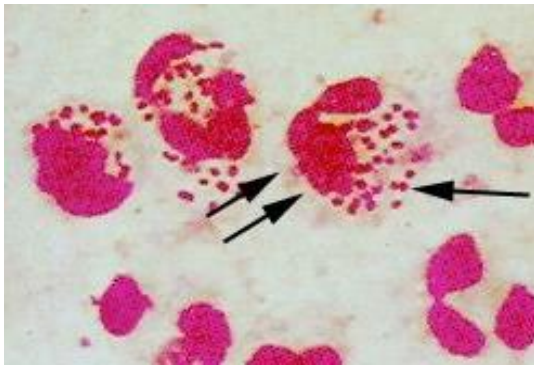
<sup>1</sup> N. – *Neisseria*

<sup>2</sup> PMN - Polymorphonuclear



**Fig. 1 Gram stain of *N. meningitidis* in cerebro-spinal fluid with associated PMNs.**

*Neisseria gonorrhoeae*, also known as gonococcus, is a species of Gram-negative coffee bean-shaped diplococci that typically appear in pairs with the opposing sides flattened. Bacteria are responsible for the sexually transmitted disease gonorrhea.



**Fig. 2 *Neisseria gonorrhoeae* - Gram-negative diplococci.**

## **NEISSERIA - BLOOD AGAR CULTURE**

For cultivation of pathogenic *Neisseria* <sup>3</sup>spp. are used special media for cultivation and isolation of nutritionally fastidious microorganisms.

*Neisseria meningitidis* grows on them without hemolysis. Colonies of *Neisseria meningitidis* are unpigmented and appear round, smooth, glistening, and convex, with a clearly defined edge. Some strains may produce larger, grey, opaque colonies. Cultivation 24 hours in an aerobic atmosphere enriched with 5% carbon dioxide, 37°C.

The organism grows on different media such as blood agar, trypticase soy agar, supplemented chocolate agar, and **Mueller**-Hinton agar.



**Fig. 3 *Neisseria meningitidis* - blood agar culture.**

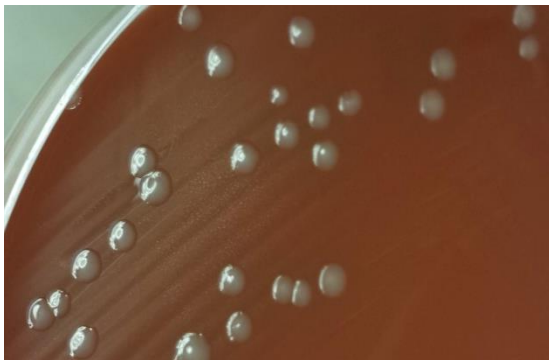
---

<sup>3</sup> spp. - species

*Neisseria gonorrhoeae* are the most fastidious of the *Neisseria* species, require complex growth media and are highly susceptible to toxic substances (e.g., fatty acids). Gonococci are not able to grow on common blood agar. Colonies are positive by the oxidase test and the result is confirmed with carbohydrate reactions (meningococci oxidize glucose and usually maltose, but not sucrose and lactose).

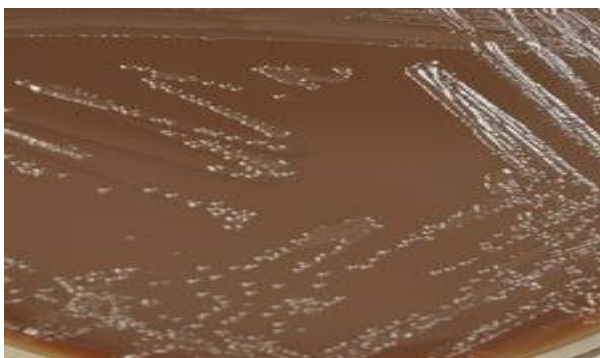
## NEISSERIA - CHOCOLATE AGAR CULTURE

**Chocolate agar** (CHOC) or **chocolate blood agar** (CBA) - is a non-selective, enriched growth medium used for isolation of pathogenic bacteria. It is a variant of the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80°C. Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenzae* and *Neisseria meningitidis*. Chocolate agar with the addition of bacitracin becomes selective, most critically, for the genus *Haemophilus*.



**Fig. 4** *Neisseria meningitidis* on Chocolate Agar.

Media for *N. gonorrhoeae* contain antimicrobials that inhibit the growth of organisms other than *N. gonorrhoeae*; typically vancomycin (inhibits Gram-positive bacteria), colistin (inhibits gram-negative bacteria including the commensal *Neisseria* spp.), trimethoprim (inhibits swarming of *Proteus* spp.) and nystatin or amphotericin B (antifungal agents). Often are used media resembling chocolate agar in appearance (e.g., modified Thayer-Martin agar (MTM) or Martin-Lewis agar (ML)).



**Fig. 5** *Neisseria gonorrhoeae* on Chocolate Agar.

Plates are incubated in a CO<sub>2</sub>-enriched, humid atmosphere (some gonococci require CO<sub>2</sub> for growth, the growth of all species is enhanced by carbon dioxide). Colonies of *N. gonorrhoeae* vary in diameter from 1 to 4,0 mm after 48 hours. The colonies are smooth and nonpigmented (Fig. 27). Some strains may produce atypical small colonies.

## NEISSERIA - BIOCHEMICAL PROPERTIES TESTING

Further testing to differentiate the species includes testing for oxidase (all *Neisseria* show a positive reaction) and the carbohydrates maltose, sucrose, and glucose test in which *N. gonorrhoeae* will only oxidize (that is, utilize) the glucose.

### **Kovac's Oxidase Test**

---

Kovac's oxidase test (cytochromoxidase test) determines the presence of cytochrome oxidase. Kovac's oxidase reagent, tetramethyl-p-phenylenediamine dihydrochloride, is turned into a purple compound by organisms containing cytochrome c as part of their respiratory chain. This test aids in the recognition of *N. meningitidis*, but other members of the genus *Neisseria*, as well as unrelated bacterial species, may also give a positive reaction. Positive and negative quality control strains should be tested along with the unknown isolates to ensure that the oxidase reagent is working properly. Positive reactions will develop within 10 seconds in the form of a purple color. Negative reactions will not produce a color change.

### **Oxidase Test - Filter paper method**

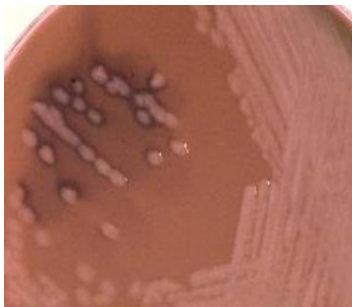
1. Grow the isolate to be tested for 18-24 hours on a blood agar plate (BAP) at 35-37°C with 5% CO<sub>2</sub> (or in a candle-jar).
2. On a nonporous surface (Petri dish or glass plate), wet a strip of filter paper with a few drops of Kovac's oxidase reagent.
3. Let the filter paper strip air dry before use.
4. Use a disposable plastic loop, a platinum inoculating loop, or a wooden applicator stick to pick a portion of a colony from overnight growth on the BAP and rub it onto the treated filter paper.
5. Observe the filter paper for color change to purple (Fig. 28).



**Fig. 6 Oxidase Test - Filter paper method.**

### Oxidase Test - Plate method

1. Grow the isolate to be tested for 18-24 hours on a blood agar plate (BAP) at 35-37°C with 5% CO<sub>2</sub> (or in a candle-jar).
2. Dispense a few drops of Kovac's oxidase reagent directly on top of a few suspicious colonies growing on the 18-24 hour BAP (Fig. 29).
3. Tilt the plate and observe colonies for a color change to purple (Fig. 29).



**Fig. 7 Oxidase Test - Plate method.**

### **Identification of *Neisseria* with a commercial kit (NEISSERIAtest, Erba Lachema).**

---

The NEISSERIAtest is a miniaturized version of conventional procedures for the identification of *Neisseria* species. It is a ready-to-use microwell plate system designed for performance of 7 biochemical tests:

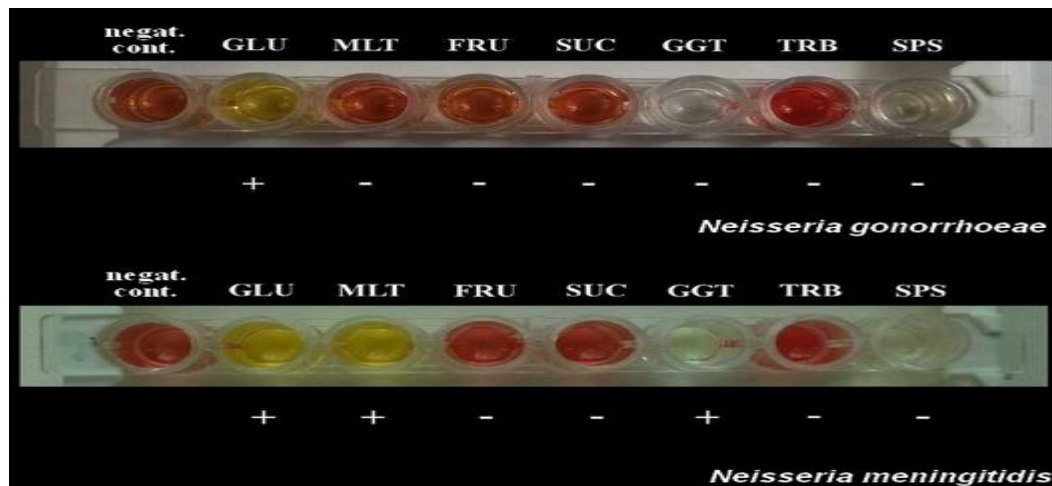
- acid production from **glucose, maltose, fructose** and **sucrose**;
- detection of **γ-glutamyl transferase**, hydrolysis of **tributyrin** and **synthesis of polysaccharide**

(To evaluate the colour reactions of the tests follow the table “Interpretation of reactions” and/or the colour reaction of the control strains).

Any change of the colour reactions of these sugars in comparison to negative control means positive reaction (Fig. 30, Table 1).

*Neisseria* spp. produce acid from carbohydrates by oxidation, not fermentation.

***N. meningitidis* oxidizes glucose and maltose, but not lactose or sucrose.**



**Fig. 8 NEISSERIA test.**

<i>N. meningitidis:</i>	<i>N. gonorrhoeae:</i>
Negative control	Negative control
<u>GLU - Glucose <sup>4</sup>POS</u>	<u>GLU - Glucose POS</u>
<u>MLT - Maltose POS</u>	MLT – Maltose NEG
FRU - Fructose <sup>5</sup> NEG	FRU - Fructose NEG
SUC - Sucrose NEG	SUC - Sucrose NEG
<u>GGT - <math>\gamma</math>-glutamylaminopeptidase POS</u>	GGT - $\gamma$ -glutamylaminopeptidase NEG
TRB - Tributyrin hydrolysis NEG	TRB - Tributyrin hydrolysis NEG
SPS - Polysaccharide synthesis NEG	SPS - Polysaccharide synthesis NEG

**Table 1. NEISSERIA test - results.**

<sup>4</sup> POS - positive  
<sup>5</sup> NEG - negative

---

## SOURCES:

Murray, P. R., E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.). 2007. *Manual of Clinical Microbiology*, 9th ed, vol. ASM Press, Washington

D. C. Segen. "Chocolate agar: Definition". The Free Dictionary. Retrieved 28 September 2012. "Chocolate Agar (CHOC)". Anaerobe free systems. Retrieved 28 September 2012.

Anderson, Cindy (2013). *Great Adventures in the Microbiology Laboratory* (7th ed.). Pearson. p. 175. ISBN 978-1-269-39068-2.

Gunn, B.A. "Chocolate agar: A differential medium for gram positive cocci". PubMed. Retrieved 28 September 2012.

Rouphael, N. G., & Stephens, D. S. (2012). *Neisseria meningitidis: Biology, Microbiology, and Epidemiology. Methods in Molecular Biology* (Clifton, N.J.), 799, 1–20. [http://doi.org/10.1007/978-1-61779-346-2\\_1](http://doi.org/10.1007/978-1-61779-346-2_1)

<http://www.medical-labs.net/wp-content/uploads/2014/11/Neisseria-gonorrhoeae-smear.jpg>

<http://www.medical-labs.net/wp-content/uploads/2014/11/Detail-of-structure-of-Neisseria-Gonorrhoeae.jpg>

<http://www.microbiologyinpictures.com/neisseria%20gonorrhoeae.html>

Murray, P. R., E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.). 2007. *Manual of Clinical Microbiology*, 9th ed, vol. ASM Press, Washington

D. C. Segen. "Chocolate agar: Definition". The Free Dictionary. Retrieved 28 September 2012. "Chocolate Agar (CHOC)". Anaerobe free systems. Retrieved 28 September 2012.

Anderson, Cindy (2013). *Great Adventures in the Microbiology Laboratory* (7th ed.). Pearson. p. 175. ISBN 978-1-269-39068-2.

Gunn, B.A. "Chocolate agar: A differential medium for gram positive cocci". PubMed. Retrieved 28 September 2012.

Rouphael, N. G., & Stephens, D. S. (2012). *Neisseria meningitidis: Biology, Microbiology, and Epidemiology. Methods in Molecular Biology* (Clifton, N.J.), 799, 1–20. [http://doi.org/10.1007/978-1-61779-346-2\\_1](http://doi.org/10.1007/978-1-61779-346-2_1)

<http://www.medical-labs.net/wp-content/uploads/2014/11/Neisseria-gonorrhoeae-smear.jpg>

<http://www.medical-labs.net/wp-content/uploads/2014/11/Detail-of-structure-of-Neisseria-Gonorrhoeae.jpg>

<http://www.microbiologyinpictures.com/neisseria%20gonorrhoeae.html>

<http://www.microbiologyinpictures.com/neisseria%20gonorrhoeae.html>

<http://www.microbiologyinpictures.com/neisseria%20gonorrhoeae.html>

<https://www.studyblue.com/notes/note/n/med-micro-7/deck/6169816>

Arhin, F. F., F. Moreau, J. W. Coulton, and E. L. Mills. Outer membrane proteins and serosubtyping with outer membrane vesicles from clinical isolates of *Neisseria meningitidis*. *Current Microbiology*. 1997;34:18-22.

*Clinical Microbiology Procedures Handbook*, 3rd edition. ASM. Washington, D.C. 2010;2,540 pages.

<http://www.cdc.gov/meningitis/lab-manual/chpt06-culture-id.html>

<http://www.microbiologyinpictures.com/neisseria%20meningitidis.html>

<http://www.cdc.gov/meningitis/lab-manual/chpt07-id-characterization-nm.html>

<http://www.microbiologyinpictures.com/bacteria-photos/neisseria-gonorrhoeae-photos/neisseria-gonorrhoeae-biochemical-tests-for-identification.html>

<http://www.microbiologyinpictures.com/neisseria%20gonorrhoeae.html>

<http://www.microbiologyinpictures.com/neisseria%20gonorrhoeae.html>

<https://www.studyblue.com/notes/note/n/med-micro-7/deck/6169816>

Arhin, F. F., F. Moreau, J. W. Coulton, and E. L. Mills. Outer membrane proteins and serosubtyping with outer membrane vesicles from clinical isolates of *Neisseria meningitidis*. *Current Microbiology*. 1997;34:18-22.

*Clinical Microbiology Procedures Handbook*, 3rd edition. ASM. Washington, D.C. 2010;2,540 pages.

<http://www.cdc.gov/meningitis/lab-manual/chpt06-culture-id.html>

<http://www.microbiologyinpictures.com/neisseria%20meningitidis.html>

<http://www.cdc.gov/meningitis/lab-manual/chpt07-id-characterization-nm.html>

<http://www.microbiologyinpictures.com/bacteria-photos/neisseria-gonorrhoeae-photos/neisseria-gonorrhoeae-biochemical-tests-for-identification.html>

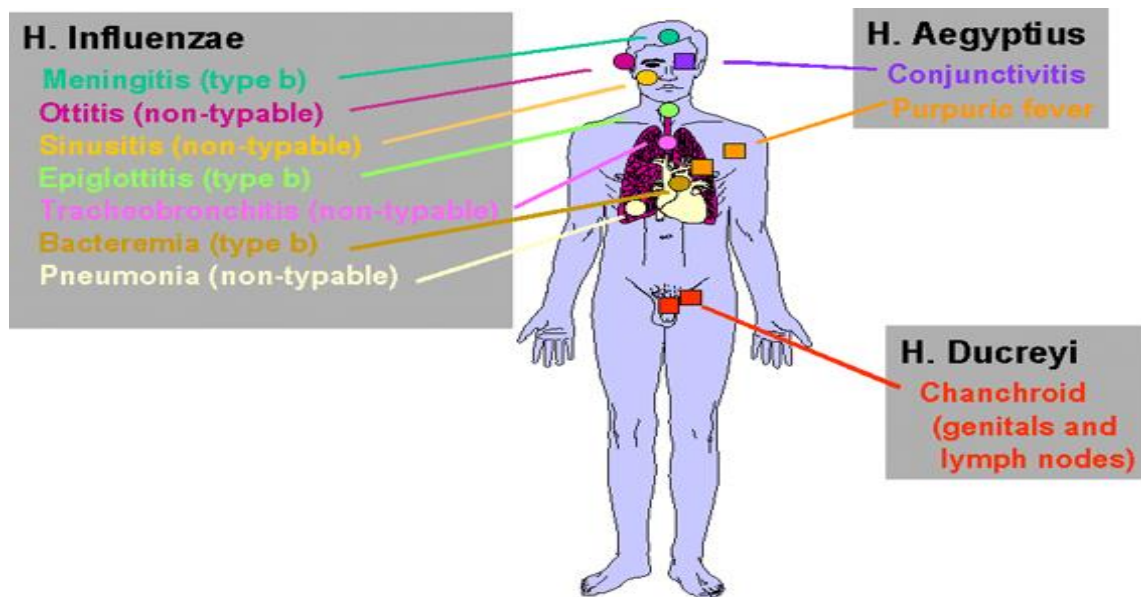
# HAEMOPHILUS

*Haemophilus* is a genus of Gram-negative, pleomorphic, coccobacilli bacteria belonging to the Pasteurellaceae family. The genus includes commensal organisms along with some significant pathogenic species such as <sup>6</sup>*H. influenzae* - a cause of sepsis and bacterial meningitis in young children, and *H. ducreyi*, the causative agent of chancroid (Fig. 31). Other *Haemophilus* species cause disease less frequently. *Haemophilus parainfluenzae* sometimes causes pneumonia or bacterial endocarditis. *Haemophilus aphrophilus* is a member of the normal flora of the mouth and occasionally causes bacterial endocarditis. *Haemophilus aegyptius*, which causes conjunctivitis and Brazilian purpuric fever, and *Haemophilus haemolyticus* used to be separated on the basis of their ability to agglutinate or lyse red blood cells, but both are now included among the nontypable *H influenzae* strains.

---

<sup>6</sup> H - *Haemophilus*





**Fig. 9 Diseases caused by *Haemophilus* species.**

There are two major groups *H. influenzae*:

1. The encapsulated group. and this group consists of six types, they are (a,b,c,d and f).The capsule has a role in virulence as it gives protection from phagocytosis, so the non-capsulated strains of *H. influenzae* are usually less invasive
2. Non-capsulated group. The non-encapsulated strain of *H. influenzae* is present in the nasopharynx of approximately 75 percent of healthy children and adults so *H. influenzae* cultured from the nasopharyngeal cavity or sputum would not indicate *H. influenzae* disease, because these sites are colonized in disease-free individuals. However, *H. influenzae* isolated from cerebrospinal fluid or blood would indicate *H. influenzae* infection.

*H. influenzae* type b (Hib) is the most common bacterium that cause disease such as bacteremia, pneumonia, epiglottitis and acute bacterial meningitis. The bacterial transmission spread person-to-person by direct contact or through respiratory droplets like coughing and sneezing.

## **HAEMOPHILUS - GRAM STAIN**

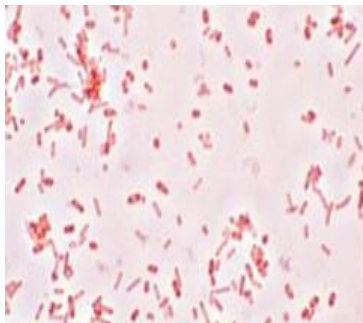
Gram stain and microscopic observation of a specimen of *H. influenzae* will show Gram-negative, rod shaped, with no specific arrangement. The rounded ends of short (0.5-1.5  $\mu\text{m}$ ) bacilli make many appear round, hence the term coccobacilli.



**Fig. 10 *H. influenzae* - Gram stain.**

Non-encapsulated organisms from sputum are pleomorphic and often exhibit long threads and filaments (Fig. 31). The organism may appear gram-positive unless the Gram stain procedure is very carefully carried out.

*H. parainfluenzae* are Gram-negative pleomorphic rods (Fig. 32).



**Fig. 11 *H. parainfluenzae* - Gram stain.**

## **HAEMOPHILUS – CULTIVATION ON BLOOD AGAR**

*H. influenzae* can't grow on blood agar as it lacks the growth factors <sup>7</sup>X and <sup>8</sup>V but in special case the growth is only achieved as a satellite phenomenon around other bacteria.

### **Satellite phenomenon**

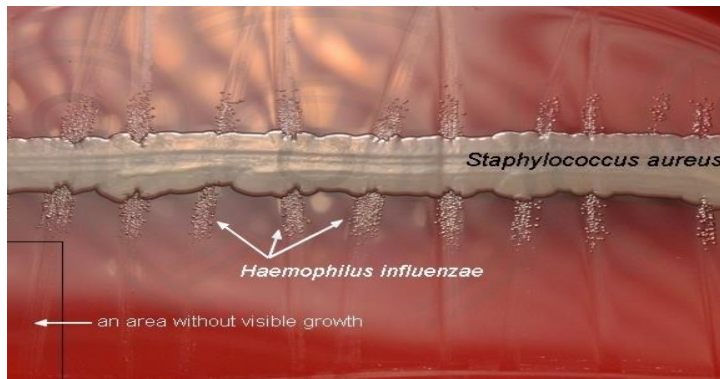
---

*H. influenzae* will grow in the hemolytic zone of *Staphylococcus aureus* on blood agar plates, the hemolysis of cells by *S.aureus* releases factor V which is needed for its growth. *H. influenzae* will not grow outside the hemolytic zone of *S. aureus* due to the lack of nutrients such as factor V in these areas.

---

<sup>7</sup> Factor X - hemin

<sup>8</sup> Factor V - nicotinamide adenine dinucleotide (**NAD**)



**Fig. 12 *Haemophilus influenzae* - Satellite phenomenon.**

*Haemophilus influenzae* will grow in the hemolytic zone of *Staphylococcus aureus* on blood agar plates (Fig. 34). The hemolysis of erythrocytes by *S. aureus* releases nutrients vital to the growth of *H. influenzae* (NAD, factor V). The NAD diffuses into the surrounding medium and stimulates the growth of *Haemophilus influenzae* in the vicinity of the staphylococcus. This is known as satelliting. For *Haemophilus* spp., the satellite test substitutes for the V factor test.

Colonies of *H. influenzae* appear as convex, smooth, pale, grey or transparent colonies. Encapsulated strains may produce larger colonies with a glistening mucoid quality, mouse nest odor is typical. *H. parainfluenzae* colonies morphology: medium to large, smooth, and translucent, nonhemolytic on rabbit or horse blood agar, appear as "schools of fish"

## **HAEMOPHILUS - CULTIVATION ON CHOCOLATE AGAR**

*Haemophilus influenzae* requires X(hemin) and V(NAD) factors for growth so *H. influenzae* culture is performed on chocolate agar, which contain X (hemin) and V (NAD) factors and the plate is placed at 37°C in a CO<sub>2</sub>-enriched incubator.

**Chocolate agar** (CHOC) or **chocolate blood agar** (CBA) - is a non-selective, enriched growth medium used for isolation of pathogenic bacteria. It is a variant of the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80 °C. Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenzae* and *Neisseria meningitidis*. Chocolate agar with the addition of bacitracin becomes selective, most critically, for the genus *Haemophilus*.



Fig. 13 *Haemophilus influenzae* - colonies on Chocolate agar.

## X AND V FACTOR DISKS FOR DIFFERENTIATION OF **HAEMOPHILUS** SPECIES

*Haemophilus influenzae* requires two accessory growth factors: **factor X** (haemin or other porphyrins) and **V factor** (NAD). The X and V factor requirement is usually demonstrated by the absence of growth on porphyrin and NAD deficient but otherwise nutritionally adequate media except near paper disc impregnated with X and V factors.

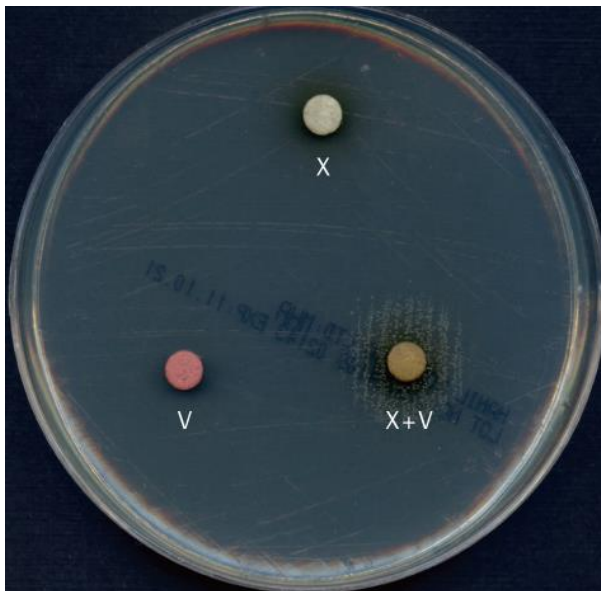
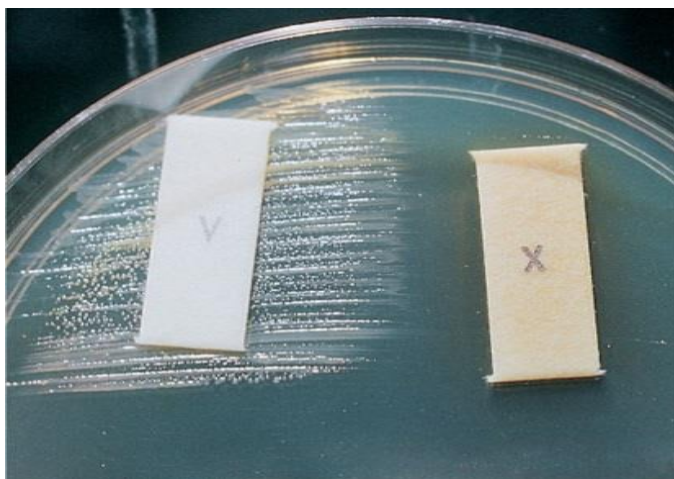


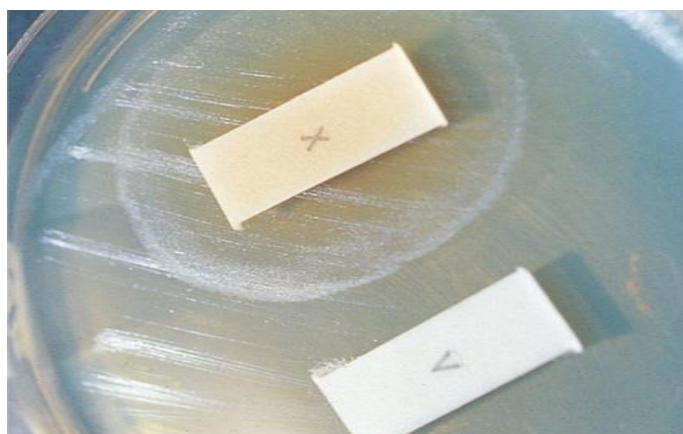
Fig. 14 *H. influenzae* - X and V factor disc test.

**X and V Factor Disks** are paper disks impregnated with X (hemin) and V (nicotinamide adenine dinucleotide) growth factors. They are used for the differentiation of *Haemophilus* species (Fig. 36, Table 2). We usually use **Mueller** Hinton Agar for the disk test. *Haemophilus influenzae* require both X and V factor to grow.



**Fig. 15 *H. parainfluenzae* - X and V factor disc test.**

*Haemophilus parainfluenzae* requires V factor only for growth.



**Fig. 16 *H. ducreyi* - X and V factor disc test.**

*Haemophilus ducreyi* requires only X factor without need of V factor.

<i>Haemophilus</i> species	Growth Around Disk		
	X	V	XV
<i>Haemophilus aegyptius</i>	-	-	+
<i>Haemophilus aphrophilus</i>	v/-	-	+
<i>Haemophilus ducreyi</i>	+	-	+
<i>Haemophilus haemolyticus</i>	-	-	+
<i>Haemophilus influenzae</i>	-	-	+
<i>Haemophilus parahaemolyticus</i>	-	+	+
<i>Haemophilus parainfluenzae</i>	-	+	+

**Table 2 *Haemophilus* species - growth around X and V discs.**



# QUELLUNG TEST

Quellung Test is an increase in the opacity and visibility of the capsule of encapsulated organisms resulting from exposure to specific, agglutinating, anticapsular antibodies. This test is also called Neufeld reaction or quellung reaction. The Quellung reaction is a biochemical reaction in which antibodies bind to the bacterial capsule of *Haemophilus influenzae* (or *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Neisseria meningitidis*) and thus allow them to be visualized under a microscope. If the reaction is positive, the capsule becomes opaque and appears to enlarge.



Fig. 17 Quellung reaction - swelling of bacterial capsules.

---

## SOURCES:

Musher DM. *Haemophilus* Species. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 30. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK8458/>

Holt JG (editor) (1994). *Bergey's Manual of Determinative Bacteriology* (9th ed.). Williams & Wilkins. ISBN 0-683-00603-7.

Kuhnert P; Christensen H (editors). (2008). *Pasteurellaceae: Biology, Genomics and Molecular Aspects*. Caister Academic Press.

Mary Catherine McEllistrem: Genetic Diversity of the Pneumococcal Capsule: Implications for Molecular-based Serotyping, *Future Microbiol.* 2009;4(7):857-865.

<http://www.microbiologybook.org/ghaffar/h-influ.jpg>

<http://www.medical-labs.net/wp-content/uploads/2014/04/Haemophilus-influenzae-gram-stain-pink-pleomorphic-rods.png>

<http://www.microbiologyinpictures.com/bacteria-photos/haemophilus-influenzae-images/satellite-test.html>

<http://www.medical-labs.net/x-and-v-factor-disks-for-the-differentiation-of-haemophilus-species-2649/>

[http://www.medical-labs.net/x-and-v-factor-disks-for-the-differentiation-of-haemophilus-species-2649/ISBN 978-1-904455-34-9.](http://www.medical-labs.net/x-and-v-factor-disks-for-the-differentiation-of-haemophilus-species-2649/ISBN%20978-1-904455-34-9)

[http://www.snipview.com/q/Quellung\\_reaction](http://www.snipview.com/q/Quellung_reaction)

