Actualización sobre las meningitis bacterianas: diagnóstico, vigilancia, y tratamiento

Modulo 3. Diferenciación y caracterización de patógenos causantes de meningitis Manejo clínico y estrategias de prevención y control de Nm

El laboratorio en el diagnóstico de los patógenos de meningitis: Bioseguridad en el laboratorio

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The First World War years of Sydney Domville Rowland: an early case of possible laboratory-acquired meningococcal disease

Peter C Wever,[1,2] A J Hodges[3]

ABSTRACT

Sydney Domville Rowland was a bacteriologist and staff member at the Lister Institute of Preventive Medicine when the First World War broke out in 1914. Following a request to the Director of the Lister Institute to staff and equip a mobile field laboratory as quickly as possible, Rowland was appointed to take charge of No. 1 Mobile Laboratory and took up a temporary commission at the rank of Lieutenant in the Royal Army Medical Corps. On 9 October 1914, Rowland set out for the European mainland and was subsequently attached to General Headquarters in Saint-Omer, France (October 1914-June 1915), No. 10 Casualty Clearing Station in Lijssenthoek, Belgium (June 1915-February 1916, during which period he was promoted Major), and No. 26 General Hospital in Étaples, France (February 1916-March 1917). His research focused on gas gangrene, typhoid fever, trench fever, wound infection and cerebrospinal fever. In February of 1917, while engaged in identifying meningococcal carriers, Rowland contracted cerebrospinal meningitis to which he succumbed at age 44 on 6 March 1917. His untimely death might have been caused by laboratory-acquired meningococcal disease, especially since Rowland's work with Neisseria meningitidis isolates had extended beyond routine laboratory techniques and included risk procedures like immunisation of rabbits with pathogenic strains isolated from cerebrospinal fluid. Currently, microbiology laboratory workers who are routinely exposed to N. meningitidis isolates are recognised as a population at increased risk for meningococcal disease, for which reason recommended preventive measures include vaccination and handling of isolates within a class II biosafety cabinet.

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THE PRE-WAR YEARS

Sydney Domville Rowland was born on 29 March 1872 in Mylor, Cornwall, as the eldest child of William John Rowland, the Curate of St Peter's Church in nearby Flushing.[1 2] In 1889, he proceeded to Downing College in Cambridge, where he took the first and second parts of the Natural Science Tripos and the first and second MB. He finished his medical studies at St Bartholomew's Hospital in London, qualifying in 1897, but as the examiners for the Cambridge MB would not accept knowledge of physiology and general science in lieu of that in midwifery, he never graduated in medicine.[2 3] Notably, in May 1896, 5 months after Röntgen's discovery of X-rays, the world's first radiological journal the *Archives of Clinical Skiagraphy* was published in London under the editorship of 24-year-old medical student Rowland (Figure 1).[4 5]

With his unbusinesslike habits, clinical medicine had no real attraction to Rowland and his attention was largely given to natural science and physics. After qualification, he held a minor post on the editorial staff of *The British Medical Journal* (*BMJ*), of which his uncle Ernest Hart was editor, and also practised as an X-ray specialist. Neither position was very successful. At the end of 1889, he received an appointment as assistant bacteriologist at the Lister Institute of Preventive Medicine, to which he remained attached for the rest of his life. There, he found ample scope for his inventive and independent character and was able to devote practically all of his time to research with little interruption from routine duties.[2 3]

An important part of his pre-war years at the Lister Institute was devoted to bubonic plague. Rowland was seconded by the Lister Institute to the Advisory Committee for Plague Investigation in India and went to Bombay in 1905 to work for 2 years on the relationship between fleas and plague bacilli. A year after his return, he was seconded again to the service of the Committee this time to improve methods of vaccination and preparation of curative sera, for which he was transferred to the Serum Department of the Lister Institute at Elstree. From 1909 to 1914, he published a number of papers on the subject in the *Journal of Hygiene*. Rowland had just about completed these studies when the First World War broke out.[2 3]

NO. 1 MOBILE LABORATORY

On 4 August 1914, Great Britain declared war on Germany. It soon became evident to the Headquarter Medical Staff in the field that a mobile field laboratory was a necessity. The war was not much more than a month old when a telegram was

Figure 2 Lieutenant Sydney Domville Rowland (left) and his servant Bray in front of No. 1 Mobile Laboratory at Elstree. The picture is presumably taken in the period between 28 September and 9 October 1914 (courtesy of the Wellcome Library, London).

Figure 1 One of the earliest X-ray photographs taken in England by Sydney Domville Rowland in 1896 showing the hand of a healthy adult (courtesy of the Wellcome Library, London).

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documents cover are now the property of this organization. These, together with certain instruments of measurement developed by experts of the American Child Health Association and additional books and pamphlets, the copyrights of which are still owned by the original recorder, are now available through the Book Service.

By keeping alive and current these valuable aspects of the work of the now dissolved American Child Health Association, we shall hope to continue the service so competently rendered to the public health profession by that agency in the past.

ANNA M. PABST

RESEARCH has claimed another victim. The Public Health Service has recorded on its special honor roll Anna M. Pabst, who died of meningitis on Christmas night in Washington, D. C. Miss Pabst was working on meningitis serum. While injecting an animal on December 17 at the National Institute of Health, it moved and some of the culture was squirted into her eye. In spite of prompt cleansing she was stricken on December 21 and died on December 25. She was very highly regarded at the National Institute of Health.

Those who use biological products, which have done so much to take away the dread of many diseases, do not realize the risks that laboratory workers run in their study. Science has an entirely too long roll of honor brought about by accidental infection in studying disease. This *Journal* and the Association which it represents unite with others everywhere to honor this latest martyr to science.

MMWR™
Morbidity and Mortality Weekly Report
Weekly February 22, 2002 / Vol. 51 / No. 7

Laboratory-Acquired Meningococcal Disease — United States, 2000

Neisseria meningitidis is a leading cause of bacterial meningitis and sepsis among older children and young adults in the United States. *N. meningitidis* usually is transmitted through close contact with aerosols or secretions from the human nasopharynx. Although *N. meningitidis* is regularly isolated in clinical laboratories, it has infrequently been reported as a cause of laboratory-acquired infection. This report describes two probable cases of fatal laboratory-acquired meningococcal disease and the results of an inquiry to identify previously unreported cases. The findings indicate that *N. meningitidis* isolates pose a risk for microbiologists and should be handled in a manner that minimizes risk for exposure to aerosols or droplets.

Case Reports

Case 1. On July 15, 2000, an Alabama microbiologist aged 35 years presented to the emergency department of hospital A with acute onset of generalized malaise, fever, and diffuse myalgias. The patient was given a prescription for oral antibiotics and released. On July 16, the patient returned to hospital A, became tachycardic and hypotensive, and died 3 hours later. Blood cultures were positive for *N. meningitidis* serogroup C. Three days before the onset of symptoms, the patient had prepared a Gram's stain from the blood culture of a patient who was subsequently shown to have meningococcal disease; the microbiologist also had handled and subcultured agar plates containing cerebrospinal fluid (CSF) cultures of *N. meningitidis* serogroup C from the same patient. Co-workers reported that in the laboratory, aspiration of materials from blood culture bottles was performed at the open laboratory bench; biosafety cabinets, eye protection, or masks were not used routinely for this procedure. Results of pulsed-field gel electrophoresis (PFGE) and multilocus enzyme electrophoresis (MEE) testing at CDC indicated that the two isolates were indistinguishable. The laboratory at hospital A infrequently processed isolates of *N. meningitidis* and had not processed another meningococcal isolate during the previous 4 years.

Case 2. On December 24, 2000, a Michigan microbiologist aged 52 years had acute onset of sore throat, vomiting, headache, and fever; by December 25, the patient had developed a petechial rash on both legs, which quickly evolved to widespread purpura. The patient presented to the emergency department of hospital B and died later that day of overwhelming sepsis. Blood cultures were positive for *N. meningitidis* serogroup C. The patient was a microbiologist in the state public health laboratory and had worked on several *N. meningitidis* serogroup C isolates during the 2 weeks before becoming ill. That laboratory had handled a median of four meningococcal isolates per month (range: 0–11) during the previous 4 years. Co-workers reported that the patient had performed slide agglutination testing and recorded colonial morphology using typical biosafety level 2 (BSL 2) precautions; this did not entail the use of a biosafety cabinet. PFGE was performed at the state public health laboratory and at CDC on all four specimens handled by the microbiologist; results of this testing indicated that the isolates from the patient and from one of the recently handled laboratory samples were indistinguishable.

INSIDE

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SHORT REPORTS

Risk of laboratory-acquired meningococcal disease

R. Boutet*, J. M. Stuart*, E. B. Kaczmarski†, S. J. Gray†, D. M. Jones† and N. Andrews‡

**PHLS Communicable Disease Surveillance Centre (South West), Public Health Laboratory, Gloucestershire Royal Hospital, Gloucester, † PHLS Meningococcal Reference Unit, Public Health Laboratory, Manchester and ‡ PHLS Statistics Unit, London, UK*

Summary: Five probable secondary cases of meningococcal disease were identified in microbiology laboratory workers in England and Wales during a 15-year period. All cases had prepared suspensions of *Neisseria meningitidis* outside a safety cabinet upto seven days before onset of illness. Relative risk in laboratory workers compared with the background adult population was 184 (95% CI 60–431). In view of the potentially serious outcome from this infection, a safety cabinet should always be used when preparing or working with suspensions of meningococci. Vaccination policy for microbiology laboratory workers should be reviewed.

Keywords: *Neisseria meningitidis*; laboratories; risk.

J Occup Health 2007; 49: 399–401

Case Study

Laboratory-Acquired Serogroup A Meningococcal Meningitis

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Key words: Meningitis, Meningococcus, Serogroup A, Laboratory-acquired

Neisseria meningitidis causes fulminant meningitis and sepsis. Worldwide, *N. meningitidis* serogroup A is responsible for large epidemic outbreaks (e.g., sub-Saharan Africa) and serogroups B, C, Y and W-135 cause epidemic and endemic disease[1–3]. Meningococci are usually transmitted from person to person through close contact with contaminated aerosols and secretions from the human nasopharynx. Laboratory-acquired infection has been reported infrequently but laboratory technicians are at increased risk[4–6]. Most of the reported cases of laboratory-acquired infections occur in clinical microbiology laboratories and have been due to serogroups B and C. For reasons that are unclear, the reported mortality of laboratory-acquired *N. meningitidis* sepsis or meningitis is ~50%[4, 5], which is higher than mortality from endemic infections.

We present the first reported case of laboratory-acquired serogroup A *N. meningitidis* meningitis in a 21 yr-old research laboratory assistant.

(CSF) showed 14,000 white blood cells (95% polymorphonuclear leukocytes) and 367 red blood cells/mm^3. Cerebrospinal fluid (CSF) protein was 265 mg/d*l* and the glucose level was 4 mg/d*l*. Gram stain, culture and bacterial antigen testing of CSF were negative as were blood cultures, all taken reportedly before initiation of antibiotics.

The patient was started on antibiotic coverage for bacterial meningitis with ceftriaxone and vancomycin and required external ventricular drain placement for intracranial pressure reduction. On hospital day two, meningococcal polymerase chain reaction (PCR) performed on the initial CSF by the Centers for Disease Control and Prevention (CDC) was reactive for *N. meningitidis* serogroup A. During the hospitalization, the patient reported plating *N. meningitidis* serogroup A in the research laboratory and admitted that this was not conducted under a biosafety cabinet. He also then denied previous vaccination with meningococcal vaccine. He completed a 10-d course of antibiotics with intravenous penicillin, and rifampin was given to eradicate nasopharyngeal colonization; he had a full recovery. Significant contacts were traced and given chemoprophylaxis, and there were no secondary cases.

Discussion

Laboratory-acquired meningococcal disease is infrequent but the risk appears to be underappreciated[6]. A case of laboratory-acquired meningococcal infection is defined as meningococcal disease in a laboratory worker who had laboratory exposure to a *N. meningitidis* isolate within 14 d before the onset of illness and who has illness with the serogroup that matches the source

Fatal Meningococcal Disease in a Laboratory Worker — California, 2012

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Occupationally acquired meningococcal disease is rare (*1*). Adherence to recommendations for safe handling of *Neisseria meningitidis* in the laboratory greatly reduces the risk for transmission to laboratory workers (*2*). A California microbiologist developed fatal serogroup B meningococcal disease after working with *N. meningitidis* patient isolates in a research laboratory (laboratory A). The California Department of Public Health (CDPH), the local health department, the California Division of Occupational Safety and Health (CalOSHA), and the federal Occupational Safety and Health Administration (OSHA) collaborated on an investigation of laboratory A, which revealed several breaches in recommended laboratory practice for safe handling of *N. meningitidis*, including manipulating cultures on the bench top. Additionally, laboratory workers had not been offered meningococcal vaccine in accordance with Advisory Committee on Immunization Practices (ACIP) recommendations and CalOSHA Aerosol Transmissible Diseases Standard requirements (*3*,*4*). In accordance with OSHA and CalOSHA regulations, laboratory staff members must receive laboratory biosafety training and use appropriate personal protective equipment, and those who routinely work with *N. meningitidis* isolates should receive meningococcal vaccine.

Case Report

On the evening of Friday, April 27, 2012, a microbiologist aged 25 years had onset of headache, fever, neck pain, and stiffness. The following morning, April 28, he was transported by automobile to the emergency department at hospital A, where he was employed in laboratory A as a researcher. While on the way to the hospital he lost consciousness. Upon arrival, the patient was noted to have a petechial rash, was suspected of having meningococcal disease, and was treated with ceftriaxone. He later had a respiratory arrest. Attempted resuscitation was unsuccessful, and he was declared dead approximately 3 hours after his arrival.

On the day of the patient's death, hospital A notified the local health department and CDPH of the case of suspected meningococcal disease. On April 29, hospital A notified OSHA, which notified CalOSHA that the deceased had worked in a laboratory conducting *N. meningitidis* vaccine research. Hospital A evaluated potentially exposed emergency department staff members and research laboratory employees; all persons found to have been exposed were immediately assessed for symptoms of meningococcal disease and offered postexposure chemoprophylaxis. Laboratory A voluntarily closed on April 30. No additional cases of meningococcal disease were identified among emergency department or laboratory staff members. The local health department identified other close contacts of the patient and ensured that they received postexposure chemoprophylaxis.

Blood and tissue specimens from the patient were sent to the CDPH Microbial Diseases Laboratory for isolation and serogroup identification. *N. meningitidis* serogroup B was identified in the clinical specimens by polymerase chain reaction. The patient had worked with *N. meningitidis* serogroup B isolates in the weeks and days before his death.

Investigation Findings

CalOSHA, OSHA, and CDPH initiated an investigation. Laboratory A was inspected, and employees were interviewed about their training as well as laboratory practices and protocols and were asked to demonstrate how procedures were performed. Multiple breaches in recommended laboratory safety practices were identified (Tables 1 and 2), including manipulation of *N. meningitidis* isolates on an open laboratory bench (*2*,*5*). The inspection team made recommendations for safe handling of *N. meningitidis* isolates and use of appropriate personal protective equipment. Laboratory A microbiologists working with *N. meningitidis* isolates had not been offered quadrivalent meningococcal vaccine, as recommended by ACIP (*4*). At the conclusion of the investigation, OSHA issued three citations classified as serious for failure to protect laboratory workers.

Discussion

Although occupationally acquired meningococcal disease is rare, it is a known risk among microbiologists who work with *N. meningitidis* isolates (*6*–*8*). Investigations of laboratory-acquired cases of meningococcal disease in the United States have demonstrated a many-fold higher attack rate for microbiologists compared with the U.S. general population aged 30–59 years and a case fatality rate of 50%, more than triple the 12%–15% case fatality rate associated with disease in the general population (*9*). In almost all cases, infected microbiologists had manipulated sterile-site isolates on an open laboratory bench outside of a biosafety cabinet (*2*,*6*). Manipulating *N. meningitidis* isolates outside a biosafety cabinet is known

TABLE 1. Selected breaches in recommended laboratory practices for *Neisseria meningitidis* that were observed by an inspection team after the death of a laboratory worker — California, 2012

Activity	Observed practice	Recommended practice
Flaming of Gram stain slide	Slide not allowed to completely air dry before flaming. This activity was conducted on the open bench.	Allow the slide to air dry before applying fixation. Use alternative methods (e.g., alcohol fixation) in the BSC.
Plate spreading	A disposable plate spreader was used to saturate the plate with the organism. The activity was conducted on the open bench.	A cotton-tipped swab could be used instead of a plastic spreader to reduce the amount of generated aerosol. If plate spreading is necessary, it should be conducted in the BSC.
Plate scraping	A disposable plastic plate scraper was used to harvest the bacteria on the plate. This activity was conducted on the open bench.	Plate scraping is not recommended, but if necessary should be performed in the BSC with appropriate PPE.
Flaming loops	Transfer loops used to inoculate media were flamed on the open bench.	Open flames are no longer universally recommended. Electric furnaces are an alternative. Disposable transfer loops used in the BSC are preferable.
Re-suspension of solution	A solution containing substantial concentrations of viable organism was inoculated with an inactivating enzyme. The solution was vigorously pipetted to create a homogenous solution. This activity occurred 10 minutes into the enzymatic reaction.	This activity should be performed in the BSC. Manufacturer recommends a 20–30 minute treatment time for the enzymatic reaction.
Opening discard bin	The biohazard discard bin lid was foot-pedal operated and opening can rapidly generate an aerosol.	Infectious material should be manipulated in the BSC. Discards should be disposed of in a biohazard bag in the BSC. Biohazard bags should be sealed and wiped down before they are transferred to the biohazard bin outside the BSC.
Discarding plate scraper and spreader	Microbiologists dropped contaminated scrapers and spreaders into an open discard bin located on the floor after working with them on the open bench, potentially generating aerosols.	Spreaders and scrapers should only be used in the BSC. Contaminated spreaders and scrapers should be placed in either a discard pan or biohazard bag. The bag or container should be sealed or covered with a lid and wiped down before removal from the BSC.

Abbreviations: BSC = biological safety cabinet; PPE = personal protective equipment.

Laboratorians working with infectious agents are at risk of laboratory-acquired infections as a result of accidents or unrecognized incidents.

The degree of hazard depends upon the virulence and dose of the biological agent, route of exposure, host resistance, proper biosafety training, and experience with biohazards.

Laboratory-acquired infections occur when microorganisms are inadvertently ingested, inhaled, or introduced into tissues. Multiple instances of laboratory acquired meningococcal infection have been reported with a case fatality rate of 50%.

While laboratory-acquired *H. influenzae* and *S. pneumoniae* infections are not as extensively reported, deadly infections with any of these organisms are possible if appropriate biosafety procedures are not strictly followed in a properly equipped laboratory.

Biosafety Level 2 (BSL2) practices are required for work involving these agents as they present a potential hazard to personnel and the environment.

Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae.
WHO manual – 2nd Edition

Safe Laboratory Handling of *Neisseria meningitidis*

The following requirements have been established for laboratorians working in BSL-2 facilities:

Borrow R, Findlow J, Gray S, Taylor S, Kaczmarski E. Safe laboratory handling of Neisseria meningitidis. J Infect. 2014 Apr;68(4):305-12. doi: 10.1016/j.jinf.2014.01.003.

Detailed work planning
BIO SAFETY CABINET

Plan the work in detail

Prepare the biological safety cabinet

Place materials and equipment

Carry out activities **according to good practices**

Remove materials and equipment

Decontaminate the biological safety cabinet

Laboratory code of pratice when working with *N. meningidis*

Laboratory security and access

Training and competency assessments

Equipment qualification

Personal protective equipment

Spillages (clinical infectious / potentially infectious)

Microbiological infectious / potentially infectious

Post – exposure monitoring

Immunisation

Borrow R, Findlow J, Gray S, Taylor S, Kaczmarski E. Safe laboratory handling of *Neisseria meningitidis*. J Infect. 2014 Apr;68(4):305-12. doi: 10.1016/j.jinf.2014.01.003.

BOX 1. Meningococcal vaccination recommendations — Advisory Committee on Immunization Practices, United States, 2020

ACIP recommends MenACWY vaccination for the following groups:

- Routine vaccination for adolescents aged 11 or 12 years, with a booster dose at age 16 years.
- Routine vaccination of persons aged ≥2 months at increased risk for meningococcal disease (dosing schedule varies by age and indication, and interval for booster dose varies by age at time of previous vaccination):
  - Persons with certain medical conditions including anatomic or functional asplenia, complement component deficiencies (e.g., C3, C5-C9, properdin, factor H, or factor D), complement inhibitor (e.g., eculizumab [Soliris] or ravulizumab [Ultomiris]) use, or human immunodeficiency virus infection.
  - Microbiologists with routine exposure to *Neisseria meningitidis* isolates.
  - Persons at increased risk during an outbreak (e.g., in community or organizational settings, and among men who have sex with men [MSM]).
  - Persons who travel to or live in countries in which meningococcal disease is hyperendemic or epidemic.
  - Unvaccinated or undervaccinated first-year college students living in residence halls.
  - Military recruits.
- Booster doses for previously vaccinated persons who become or remain at increased risk.

ACIP recommends MenB vaccination for the following groups:

- Routine vaccination of persons aged ≥10 years at increased risk for meningococcal disease (dosing schedule varies by vaccine brand; boosters should be administered at 1 year after primary series completion, then every 2–3 years thereafter):
  - Persons with certain medical conditions, such as anatomic or functional asplenia, complement component deficiencies, or complement inhibitor use.
  - Microbiologists with routine exposure to *N. meningitidis* isolates.
  - Persons at increased risk during an outbreak (e.g., in community or organizational settings, and among MSM).
- Vaccination of adolescents and young adults aged 16–23 years with a 2-dose MenB series on the basis of shared clinical decision-making. The preferred age for MenB vaccination is 16–18 years. Booster doses are not recommended unless the person becomes at increased risk for meningococcal disease.
- Booster doses for previously vaccinated persons who become or remain at increased risk.

Abbreviations: ACIP = Advisory Committee on Immunization Practices; MenACWY = quadrivalent (serogroups A, C, W, Y) meningococcal conjugate vaccine; MenB = serogroup B meningococcal vaccine.

Immunisation of healthcare and laboratory staff

Staff handling specific organisms

For some infections, the probability that clinical specimens and environmental samples of UK origin contain the implicated organism, and therefore present any risk to staff, is extremely low. For these infections, routine immunisation of laboratory workers is not indicated. Staff handling or conducting research on specific organisms and those working in higher risk settings, such as reference laboratories or infectious disease hospitals, may have a level of exposure sufficient to justify vaccination. The following vaccines are recommended for those who work with the relevant organism and should be considered for those working with related organisms, as well as those in reference laboratories or specialist centres:

- hepatitis A
- Japanese encephalitis
- cholera
- meningococcal ACW135Y
- smallpox

Public Health England. The Green Book. Guidance: Chapter 12 Immunisation of healthcare and laboratory staff. Available at: https://www.gov.uk/government/publications/immunisation-ofhealthcare-and-laboratory-staff-the-green-book-chapter-12 [last accessed August 2021].

Guidance on regulations for the **Transport of Infectious Substances**

2021-2022

Applicable as from 1 January 2021

World Health Organization

6.2 Packing Instruction P650 (Category B Infectious Substance Requirements)

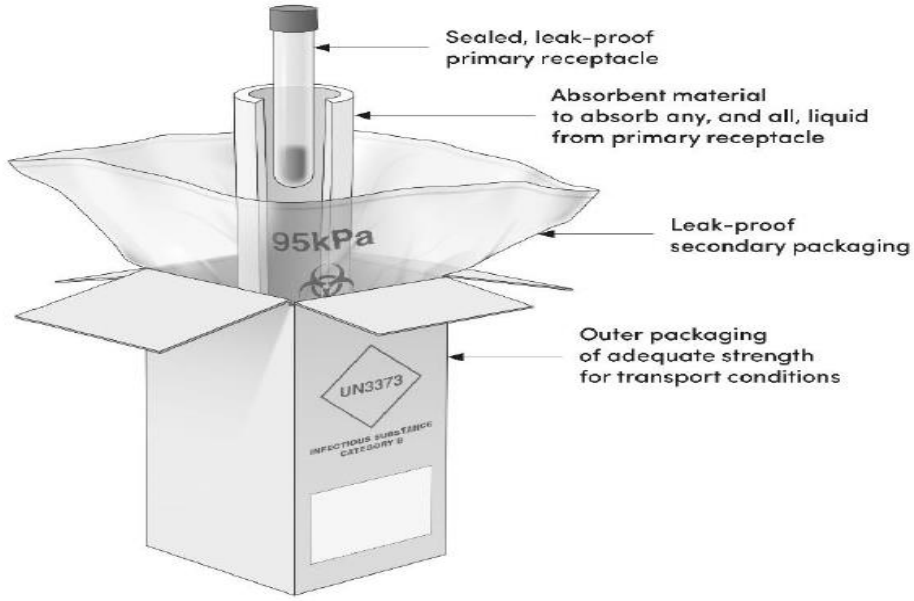
Packing instruction P650 provides a slightly more detailed set of triple packaging requirements than that of the basic triple packaging system. Infectious substances sub-classified as Biological Substance, Category B (UN3373) and packaged in accordance with P650 may be considered safe and compliant for all modes of transportation and are not subject to any other packaging requirement outlined in the UN model regulations, for example the more detailed testing and approval processes which will be required for packaging of Category A infectious substances. For this reason, it is generally feasible to source P650 compliant packaging materials from local manufacturers and/suppliers. In this case, the manufacturers/supplier should provide clear instructions for the user (shipper, sender or consignee) on how to correctly fill and close the package ensuring full compliance with P650.

Quantity Limits (Category B)

For shipments being carried by air (passenger or cargo aircraft), the primary inner receptacle must not contain more than 1L and the outer packaging must not contain more than 4L of material. This excludes any quantity of coolants used, such as dry ice or liquid nitrogen.

For shipments being carried via surface transport (road, rail or maritime), there are no quantity limits per package.

It should be noted that there is no comprehensive list of suppliers of packagings that comply with Packing Instructions P650 or P620. However, an Internet search using a suitable international or national search engine usually provides appropriate information, as well as access to national regulations. Search phrases such as "UN packaging" and "UN infectious substance packaging" produce extensive results. Carriers and forwarding agents (couriers or logistics companies) should also be able to supply details of local suppliers or local companies that can provide such information.



Actualización sobre las meningitis bacterianas: diagnóstico, vigilancia, y tratamiento

Modulo 3. Diferenciación y caracterización de patógenos causantes de meningitis Manejo clínico y estrategias de prevención y control de Nm

El laboratorio en el diagnóstico de los patógenos de meningitis: Caracterización serológica

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Serogroups B, C, W, and Y are commonly associated with invasive **meningococcal disease**, and rapid diagnosis of the serogroup is key in monitoring the epidemiology of the disease and in developing prevention strategies

More than 100 different capsular types (serotypes) of **pneumococci** have been reported, but pneumococcal conjugate vaccines (PCV) include polysaccharide antigens against only 10, 13, 15 or 20 serotypes. It is therefore important to track the emergence of serotypes due to the clonal expansion of non-vaccine serotypes

There are six different **H. influenzae** serotypes (Hia, Hib, Hic, Hid, Hie and Hif), which each express a unique polysaccharide capsule, as well as nontypeable Hi (NTHi) strains, which lack capsule expression. Since the implementation of the Hib vaccine, the burden of Hib disease has decreased dramatically. However, NTHi and other non-b serotypes continue to cause disease, highlighting the continued need to monitor the distribution of Hi serotypes.

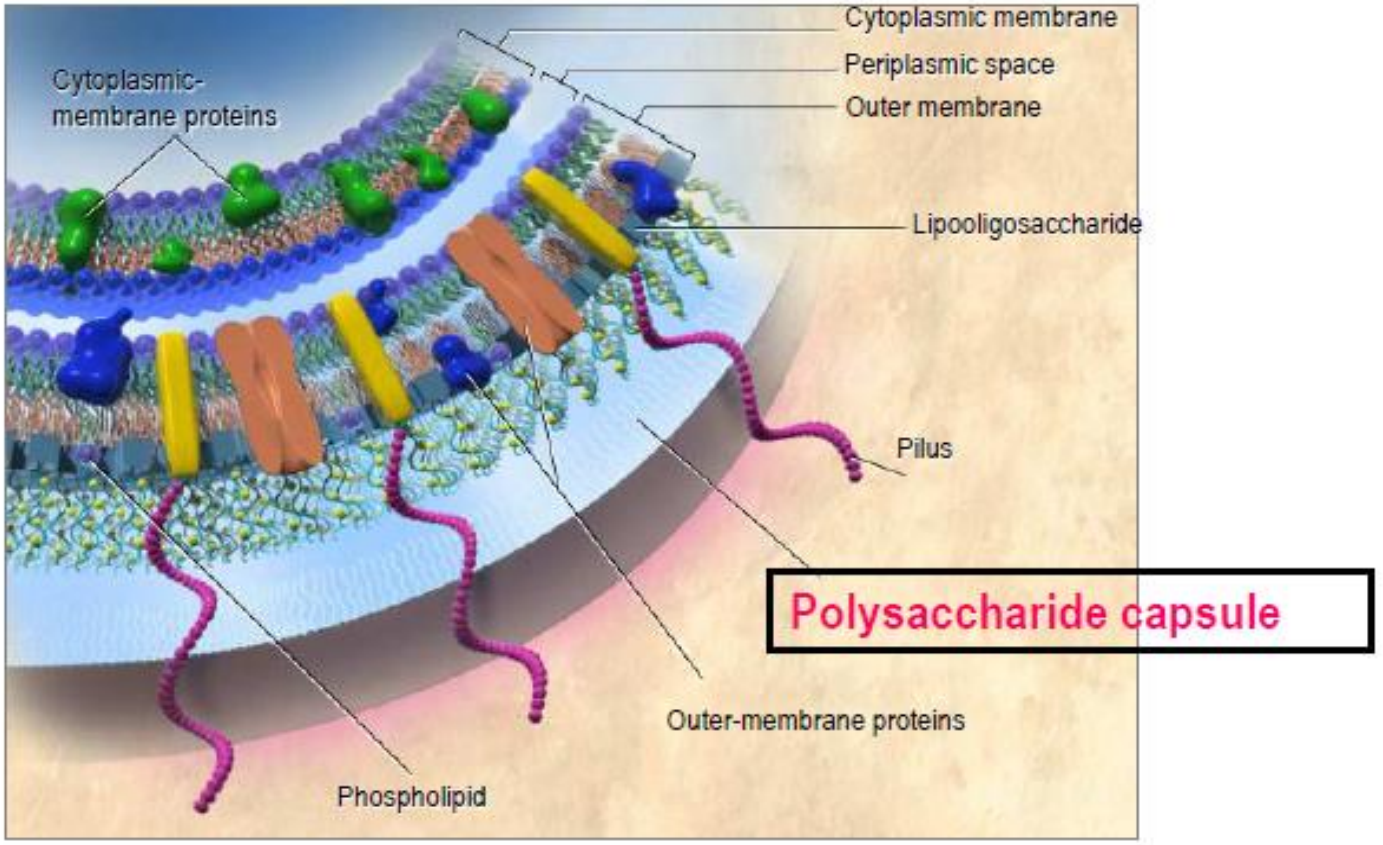
Jauneikaite E. et al. Current methods for capsular typing of Streptococcus pneumoniae. J Microbiol Methods. 2015 Jun;113:41-9. doi: 10.1016/j.mimet.2015.03.006; Harrison OB et al. Description and nomenclature of Neisseria meningitidis capsule locus. Emerg Infect Dis. 2013 Apr;19(4):566-73. doi: 10.3201/eid1904.111799; Jauneikaite E et al. Current methods for capsular typing of Streptococcus pneumoniae. J Microbiol Methods. 2015 Jun;113:41-9. doi: 10.1016/j.mimet.2015.03.006.

Validation of bacterial killing

Heating at 50°C for 1 h was found to be effective for killing meningococci and samples while remaining effective for agglutination, co-agglutination, latex kit testing, serotyping by dot-blot ELISA and nucleic acid extraction. This was increased to 60°C for 60 min with retained effectiveness for the serological detection assays. Other examples of adopting the precautionary principals are the preparation of meningococcal suspensions for PCR or DNA extraction, five minutes at 100°C had been shown to be effective but this could be increased to 10 min with no detriment to performance while adding to safety.

Borrow R, Findlow J, Gray S, Taylor S, Kaczmarski E. Safe laboratory handling of *Neisseria meningitidis*. J Infect. 2014 Apr;68(4):305-12. doi: 10.1016/j.jinf.2014.01.003.

N. meningitidis cell surface components

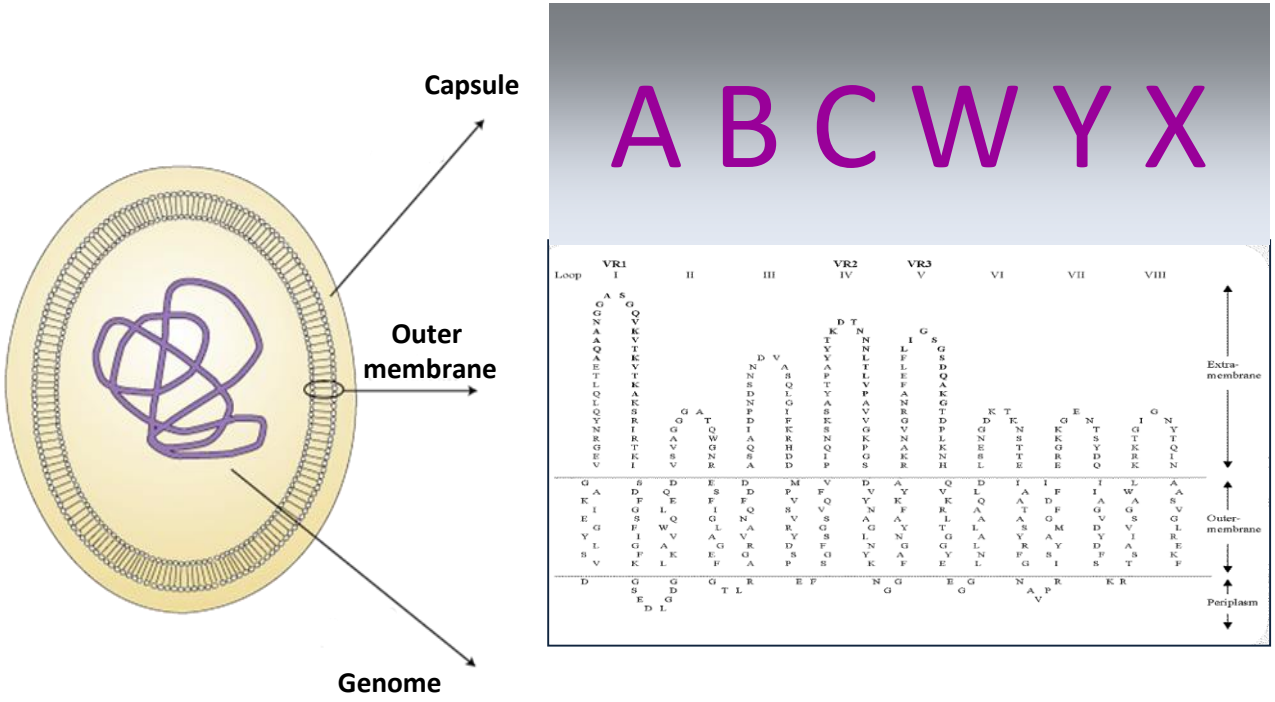


Capsular polysaccharide vaccines work for serogroups (e.g. A,C,W,Y) but don't work for MenB

- MenB capsule is a self antigen

Rosenstein NE et al. N.Engl J Med 2001, 344 :1378-88

Schematic representation of the various facets of meningococcal typing

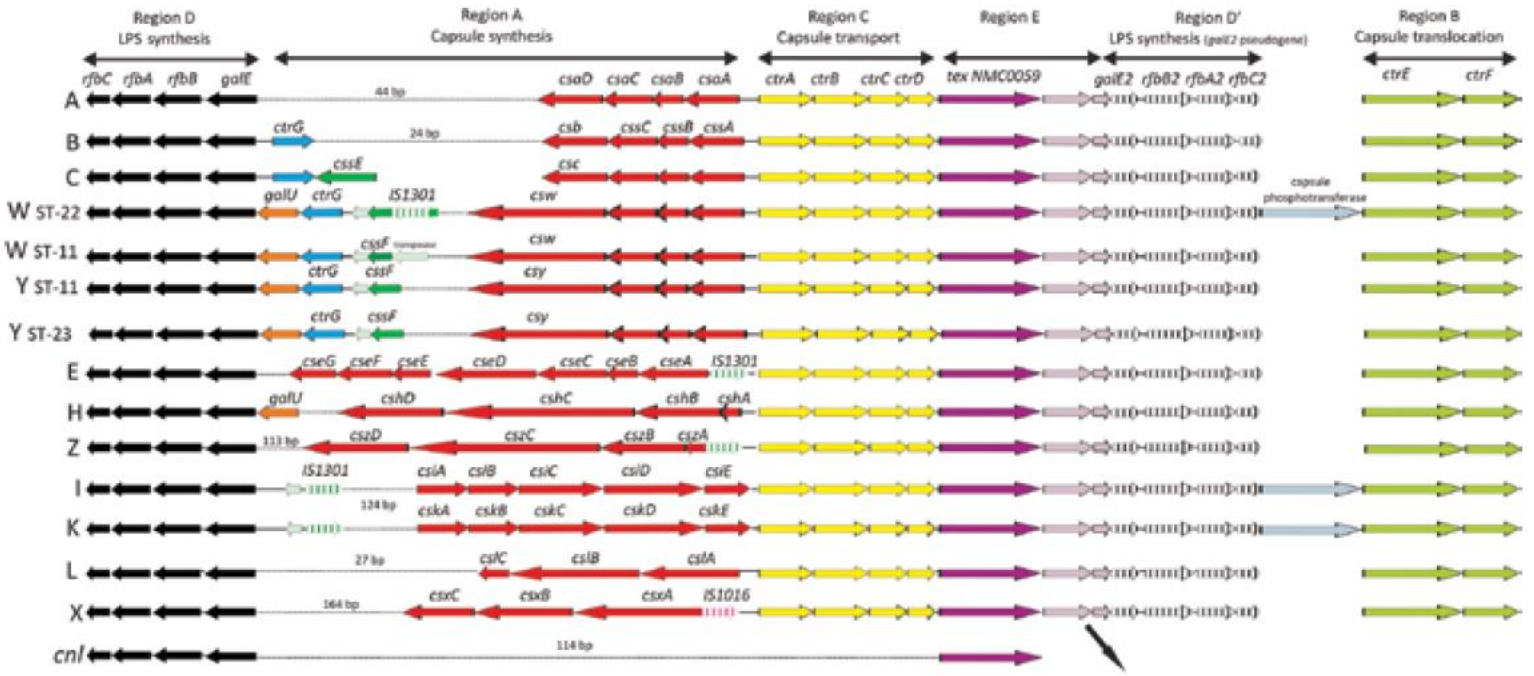


Diggle MA, et al. *Expert Rev Mol Diagn.* 2006;6:79-87; 2. Jolley KA, et al. In: Frosch M, Maiden MCJ, eds. *Handbook of Meningococcal Disease: Infection Biology, Vaccination, Clinical Management*. 2006.

Description and Nomenclature of *Neisseria meningitidis* Capsule Locus

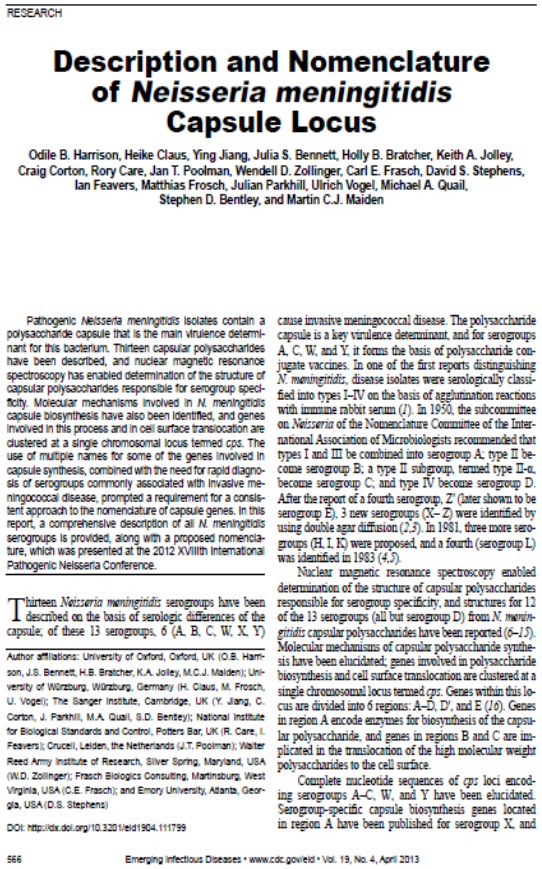
Odile B. Harrison, Heike Claus, Ying Jiang, Julia S. Bennett, Holly B. Bratcher, Keith A. Jolley, Craig Corton, Rory Care, Jan T. Poolman, Wendell D. Zollinger, Carl E. Frasch, David S. Stephens, Ian Feavers, Matthias Frosch, Julian Parkhill, Ulrich Vogel, Michael A. Quail, Stephen D. Bentley, and Martin C.J. Maiden

N. meningitidis Capsule Locus



Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, Corton C, Care R, Poolman JT, Zollinger WD, Frasch CE, Stephens DS, Feavers I, Frosch M, Parkhill J, Vogel U, Quail MA, Bentley SD, Maiden MC. Description and nomenclature of Neisseria meningitidis capsule locus. Emerg Infect Dis. 2013 Apr;19(4):566-73. doi: 10.3201/eid1904.111799. PMID: 23628376

Proposed Nomenclature – *Neisseria menigitidis*



RESEARCH

Description and Nomenclature of *Neisseria meningitidis* Capsule Locus

Odile B. Harrison, Heike Claus, Ying Jiang, Julia S. Bennett, Holly B. Bratcher, Keith A. Jolley, Craig Corton, Rory Care, Jan T. Poolman, Wendell D. Zollinger, Carl E. Frasch, David S. Stephens, Ian Feavers, Matthias Frosch, Julian Parkhill, Ulrich Vogel, Michael A. Quail, Stephen D. Bentley, and Martin C.J. Maiden

Pathogenic *Neisseria meningitidis* isolates contain a polysaccharide capsule that is the main virulence determinant for this bacterium. Thirteen capsular polysaccharides have been described, and nuclear magnetic resonance spectroscopy has enabled determination of the structure of capsular polysaccharides responsible for serogroup specificity. Molecular mechanisms involved in *N. meningitidis* capsule biosynthesis have also been identified, and genes involved in this process and in cell surface translocation are clustered at a single chromosomal locus termed *cps*. The use of multiple names for some of the genes involved in capsule synthesis, combined with the need for rapid diagnosis of serogroups commonly associated with invasive meningococcal disease, prompted a requirement for a consistent approach to the nomenclature of capsule genes. In this report, a comprehensive description of all *N. meningitidis* serogroups is provided, along with a proposed nomenclature, which was presented at the 2012 XVIIIth International Pathogenic Neisseria Conference.

Thirteen *Neisseria meningitidis* serogroups have been described on the basis of serologic differences of the capsule; of these 13 serogroups, 6 (A, B, C, W, X, Y) cause invasive meningococcal disease. The polysaccharide capsule is a key virulence determinant, and for serogroups A, C, W, and Y, it forms the basis of polysaccharide conjugate vaccines. In one of the first reports distinguishing *N. meningitidis*, disease isolates were serologically classified into types I–IV on the basis of agglutination reactions with immune rabbit serum (*1*). In 1950, the subcommittee on *Neisseria* of the Nomenclature Committee of the International Association of Microbiologists recommended that types I and III be combined into serogroup A, type II become serogroup B, a type II subgroup, termed type II-α, become serogroup C, and type IV become serogroup D. After the report of a fourth serogroup, Z′ (later shown to be serogroup E), 3 new serogroups (X–Z) were identified by using double agar diffusion (*2*,*3*). In 1981, three more serogroups (H, I, K) were proposed, and a fourth (serogroup L) was identified in 1983 (*4*,*5*).

Nuclear magnetic resonance spectroscopy enabled determination of the structure of capsular polysaccharides responsible for serogroup specificity, and structures for 12 of the 13 serogroups (all but serogroup D) from *N. meningitidis* capsular polysaccharides have been reported (*6*–*15*). Molecular mechanisms of capsular polysaccharide synthesis have been elucidated; genes involved in polysaccharide biosynthesis and cell surface translocation are clustered at a single chromosomal locus termed *cps*. Genes within this locus are divided into 6 regions: A–D, D′, and E (*16*). Genes in region A encode enzymes for biosynthesis of the capsular polysaccharide, and genes in regions B and C are implicated in the translocation of the high molecular weight polysaccharides to the cell surface.

Complete nucleotide sequences of *cps* loci encoding serogroups A–C, W, and Y have been elucidated. Serogroup-specific capsule biosynthesis genes located in region A have been published for serogroup X, and

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DOI: http://dx.doi.org/10.3201/eid1904.111799

sis genes. Distinct capsule operons corresponding to serogroups A, B, C, E, H, I, K, L, W, X, Y, and Z have been described herein, with the serogroup D capsule described as being an unencapsulated serogroup C variant. Nucleotide sequence data for each capsule locus have been depos-

The W-135 and 29E serogroup designations originated at the Walter Reed Army Institute of Research as a result of a paper published by Evans et al. (35). We propose to rename these W and E because the numbers are historic and supply no useful information.

Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, Corton C, Care R, Poolman JT, Zollinger WD, Frasch CE, Stephens DS, Feavers I, Frosch M, Parkhill J, Vogel U, Quail MA, Bentley SD, Maiden MC. Description and nomenclature of Neisseria meningitidis capsule locus. Emerg Infect Dis. 2013 Apr;19(4):566-73. doi: 10.3201/eid1904.111799. PMID: 23628376

Determining the meningococcal serogroup by agglutination reaction - Serogrouping

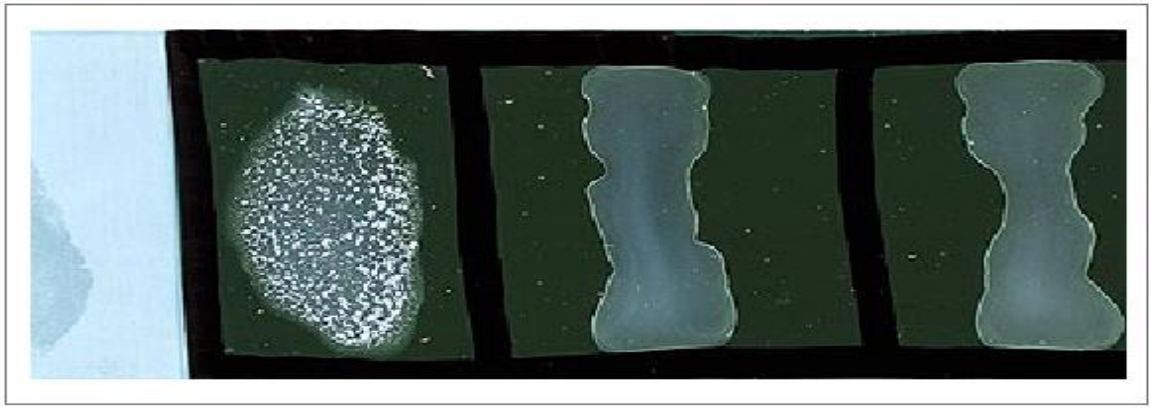

Figure 17. Agglutination, with clearing of the liquid, occurs when a suspension of the isolate is mixed with its homologous antiserum (left). A negative reaction, which should occur with heterologous antiserum (center) and with saline (right), remains smooth and turbid.

Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae.
WHO manual – 2nd Edition

Serotyping and serosubtyping of *Neisseria meningitidis* with monoclonal antibodies (Mabs)

J. Med. Microbiol. — Vol. 31 (1990), 195–201

0022–2615/90/0031–0195/$10.00

Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, dot-blotting and ELISA

E. WEDEGE, E. A. HØIBY*, E. ROSENQVIST and L. O. FRØHOLM

*Departments of Methodology and *Bacteriology, National Institute of Public Health, Geitmyrsveien 75, 0462 Oslo 4, Norway*

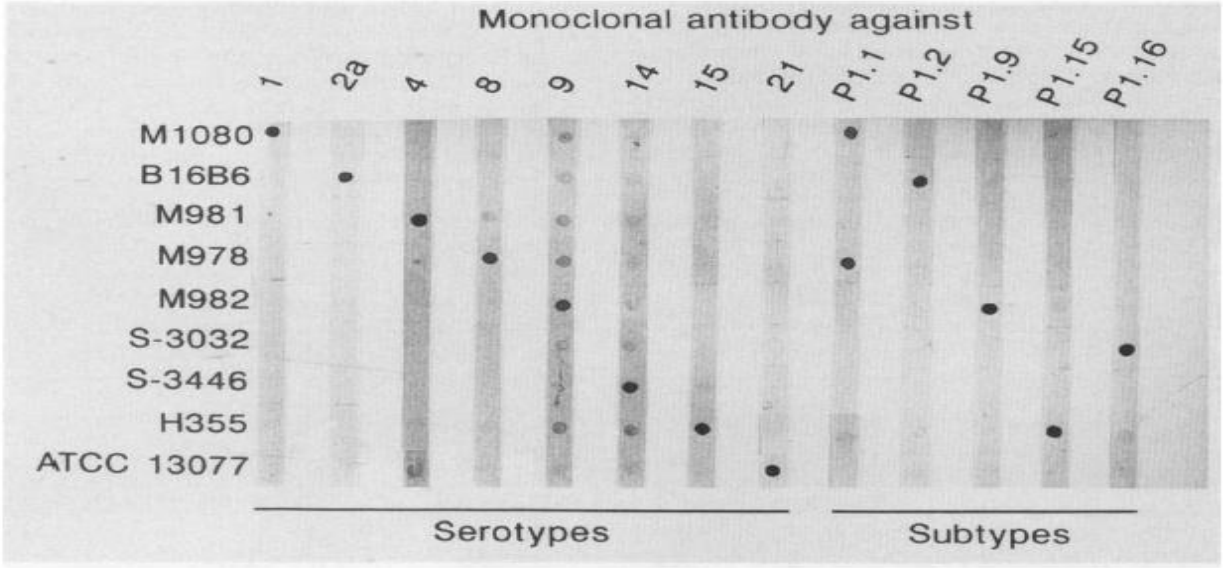

Fig. 1. Dot-blots of whole-cell suspensions of nine meningococcal reference strains incubated with various serotype-specific and subtype-specific MAbs.

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2000, p. 855–857 Vol. 38, No. 2
0095-1137/00/$04.00+0

NOTES

Simultaneous Approach for Nonculture PCR-Based Identification and Serogroup Prediction of *Neisseria meningitidis*

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Received 27 May 1999/Returned for modification 12 August 1999/Accepted 4 November 1999

A nonculture PCR-based method to characterize *Neisseria meningitidis* was used to test 225 clinical specimens. PCR correctly identified and predicted the serogroups of *N. meningitidis* of culture-proven meningococcal diseases and confirmed this diagnosis in 35% of suspected samples. This approach could be useful when culture fails to isolate *N. meningitidis*.

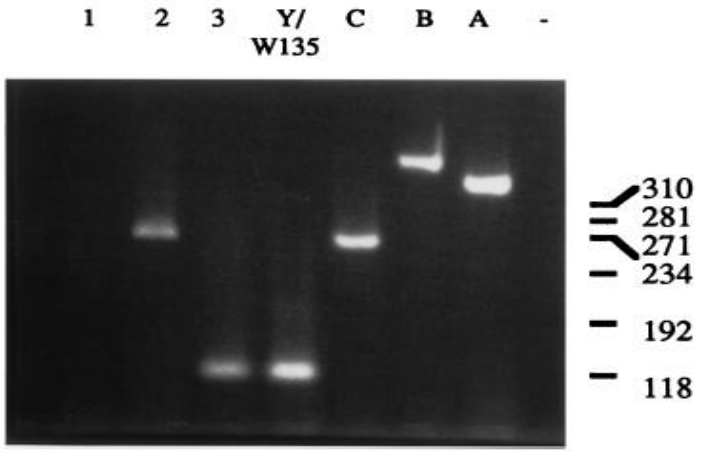


FIG. 1. PCR amplification of the *siaD* (serogroups B, C, and Y and W135) and *orf-2* (serogroup A) genes from strains belonging to serogroups A (strain LNP10824), B (strain LNP10846), C (strain LNP13331), and Y and W135 (strains LNP13145 and LNP13230, respectively) and from three clinical specimens (lanes 1, 2, and 3). The strains used were previously described (6). Lane − is the negative control (no bacteria). Electrophoresis was done on a 2% agarose gel. Size markers (bacteriophage ϕX174 digested by *Hae*III) are indicated in base pairs at the right.

Serogroup type capsule type and gene targets for genogrouping real-time PCR assays

Table 1. Serogroup capsule type and gene targets for genotyping real-time PCR assays

Sero-group	Capsule type	Gene Target Name	Alternate Gene Names	Ref
A	(α1→6)-*N*-acetyl-D-mannosamine-1-phosphate	*sacB*		(31)
B	(α2→8)- N-acetylneuraminic acid	*synD*	*siaD* *siaD* of B $siaD_B$	(5, 9, 23, 48, 61)
C	(α2→9)- N-acetylneuraminic acid	*synE*	*siaD* of C $siaD_C$	(5, 9, 50, 58, 61)
W135	6-D-Gal(α1→4)-N-acetylneuraminic acid(α2→6)	*synG*	*siaD* of W135 $siaD_W$	(4, 9, 14, 35, 49, 61)
X	(α1→4)-*N*-acetyl-D-glucosamine-1-phosphate	*xcbB*		(2, 6)
Y	6-D-Glc(α1→4)-N-acetylneuraminic acid(α2→6)	*synF*	*siaD* of Y $siaD_Y$	(4, 9, 14, 35, 49, 61)

For serogroups B, C, Y and W135, the first three genes of the *syn* operon encode functions for the synthesis of capsule polysaccharide precursors (32, 48, 51). The fourth gene product is a polymerase that catalyzes the formation of polymers with the serogroup-specific linkage. In serogroups B and C, the products of a four-gene operon (*synABC* plus the polysialyltransferase gene *synD* [Nmen B] or *synE* [Nmen C]) are responsible for biosynthesis of the sialic acid (also known as *N*-acetylneuraminic acid, NeuNAc, or NANA) homopolymer. Expression of the poly-*N*-acetylmamosamine-1-phosphate capsule polymer of serogroup A requires the *sacABCD* operon (formerly known as *mynABCD* (49), while expression of the poly-*N*-acetyl-D-glucosamine-1-phosphate capsule of serogroup X requires the *xcbABC* operon.

Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae.
WHO manual – 2nd Edition

Serotyping of *Streptococcus pneumoniae*

More than 100 different capsular types (serotypes)
Staten Serum Institute, Denmark

Pool	P	Q	R	S	T	Non-vaccine Groups/types
A	1	18 (18F, 18A, 18B, 18C)	4	5	2	
B	19 (19F, 19A, 19B, 19C)	6 (6A, 6B, 6C, 6D)	3	8		
C	7 (7F, 7A, 7B, 7C)				20	24 (24F, 24A, 24B) 31, 40
D			9 (9A, 9L, 9N, 9V)		11 (11F, 11A, 11B, 11C, 11D)	16 (16F, 16A) 36, 37
E			12 (12F, 12A, 12B)	10 (10F, 10A, 10B, 10C)	33 (33F, 33A, 33B, 33C, 33D)	21, 39
F				17 (17F, 17A)	22 (22F, 22A)	27 32 (32F, 32A) 41 (41F, 41A)
G						29, 34 35 (35F, 35A, 35B, 35C) 42 47 (47F, 47A)
H	14	23 (23F, 23A, 23B)		15 (15F, 15A, 15B, 15C)		13 28 (28F, 28A)
I						25 (25F, 25A) 38, 43, 44, 45, 46, 48

Quellung reaction

Serotyping of *Streptococcus pneumoniae*

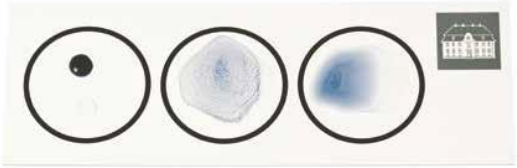
ImmuLex Pneumotest kit, 14 x 1.5 mL

Art. no. 51823

AVAILABILITY: PRODUCED TO ORDER

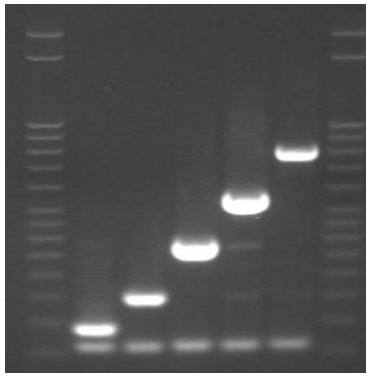
Latex agglutination

\+ -



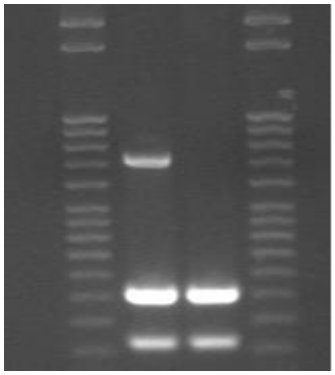
Conventional PCR Deduction of 40 Pneumococcal Serotypes or Serogroups

Reation 1



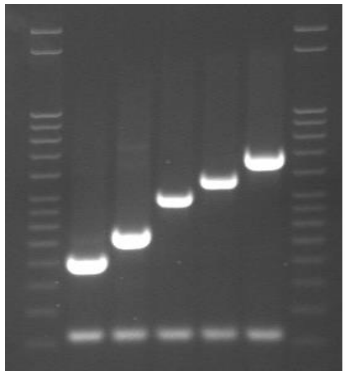
14 6 23F 19A 9VA

Reaction 2



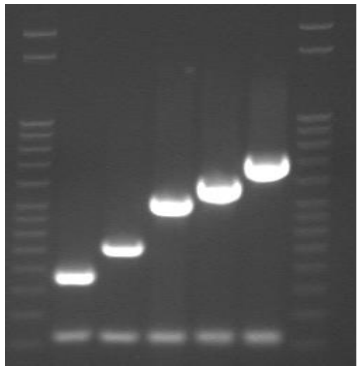
6CD 6AB

Reaction 3



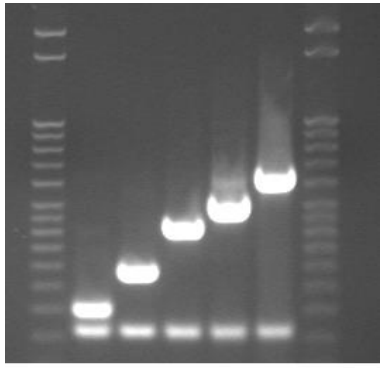
19F 3 15BC 18 17F

Reaction 4



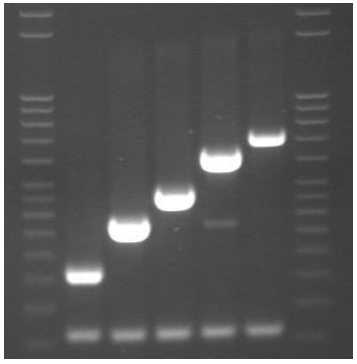
1 5 9NL 7FA 16F

Reaction 4



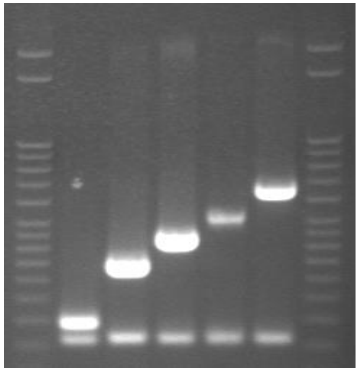
8 2 4 20 22FA

Reaction 5



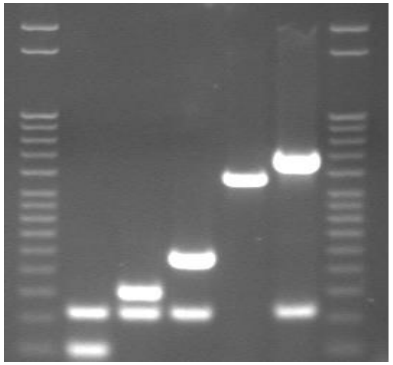
7CB40 12 11AD 10A 23A

Reaction 6



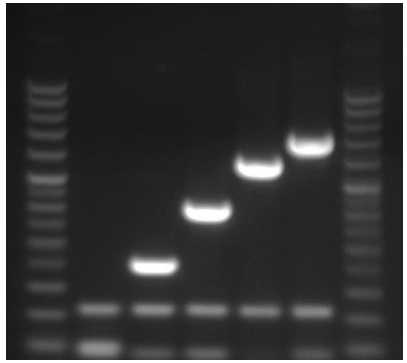
21 33FA37 15AF 35F47 13

Reaction 7



39 23B 35AC42 38 35B

Reaction 8



24 10FC33C 34 19Fv 31

www.cdc.gov/streplab/downloads/pcr-latin-amer-clinical-specimens.pdf

Real-time PCR Deduction of Pneumococcal Serotypes or Serogroups

Quadriplex real-time PCR identification of pneumococcal serotypes/serogroups. Forty-eight real-time PCR assays in 12 quadriplex reactions are available for detection of 64 serotypes as individual serotypes or small serogroups.

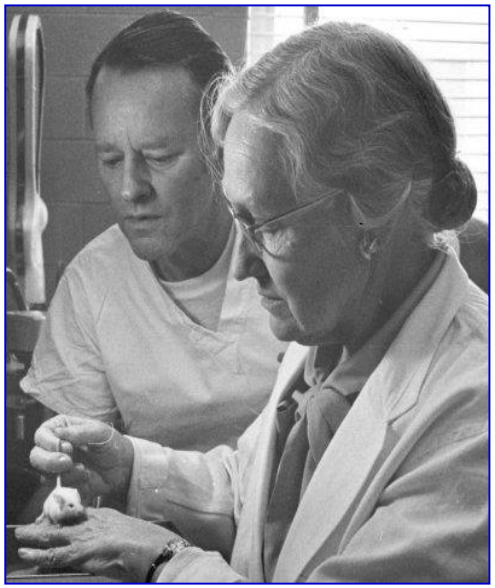
Triplex real time PCR identification of pneumococcal serotypes/serogroups. Twenty-one real-tireal tme PCR assays are currently available for detection of pneumococcal serotypes or serogroups.

Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae.
WHO manual – 2nd Edition

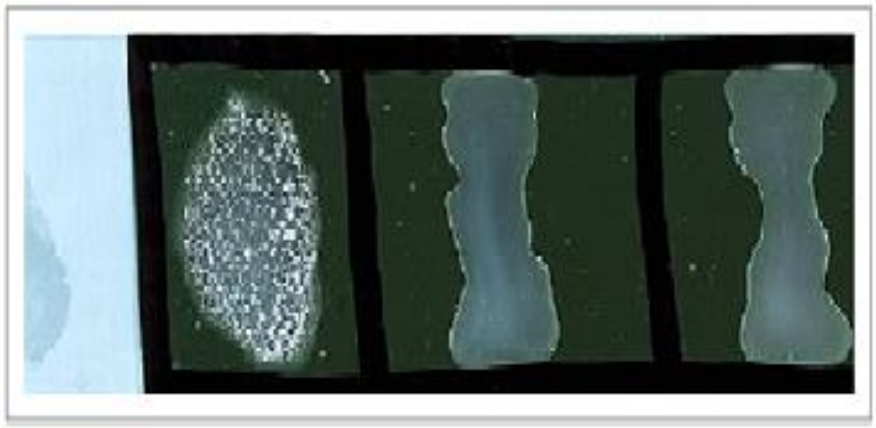
Serotyping of *Haemophilus influenzae*

The major antigenic determinant of capsulated isolates into six capsular types (a to f)

Determining the serotyping by agglutination reaction



Margareth Pittman, 1950



Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae.
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Serotype capsule type and gene targets for genotyping real-time PCR assays

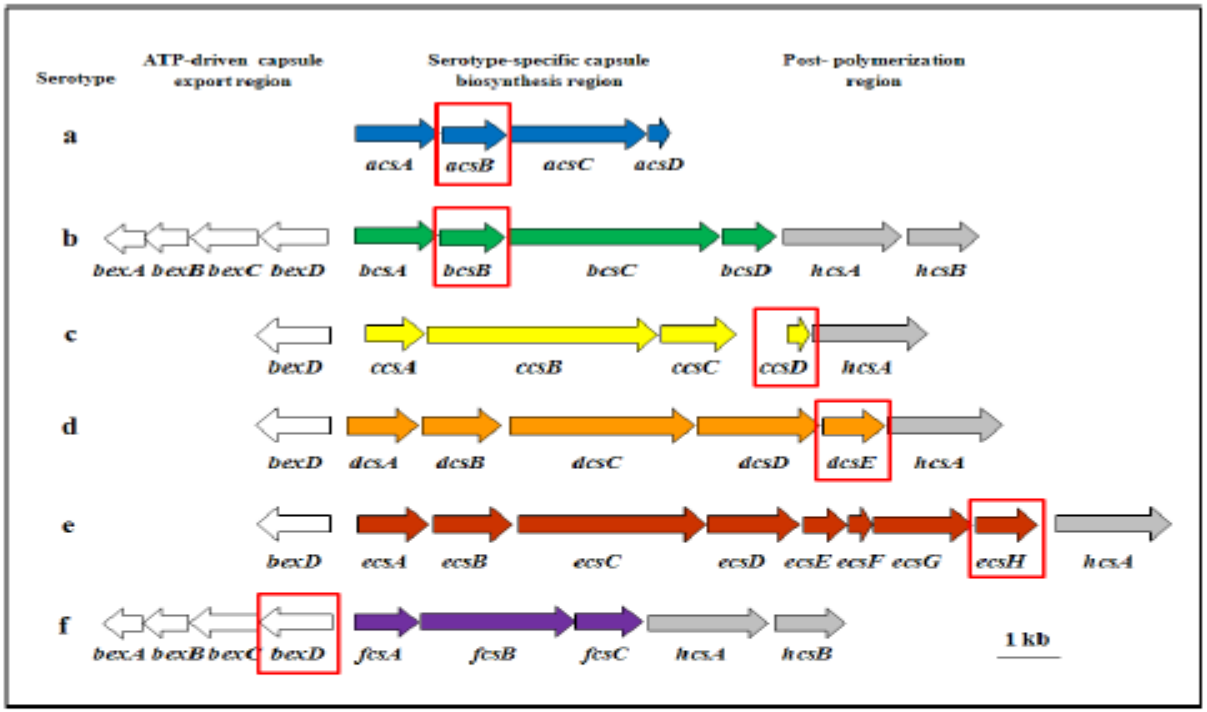

Figure 3. Capsule loci for *H. influenzae* serotypes a, b, c, d, e, and f, including the target genes for serotype-specific real-time PCR assays. The capsule locus of all six serotypes of *H. influenzae* (Hia-f) consists of three regions encoding functions for capsule polysaccharide synthesis, modification, and translocation. *bexDCBA* in the ATP-driven export region (white arrows) code for protein components of an ATP-driven polysaccharide export apparatus. *hcsA* and *hcsB* are in the post polymerization modification region (gray arrows) and may be involved in the modification and export of capsule polysaccharide. The serotype-specific region (colored arrows) contains genes for capsule synthesis and is unique to each serotype. The serotype-specific genes are named *acs*, *bcs*, etc. for "a capsule synthesis", "b capsule synthesis", and so on. With the exception of the Hif serotype-specific assay, the target genes for the serotype-specific assays can be found in this region and are highlighted by the red boxes.

Laboratory methods for the diagnosis of meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae.
WHO manual – 2nd Edition

Actualización sobre las meningitis bacterianas: diagnóstico, vigilancia, y tratamiento

Modulo 3. Diferenciación y caracterización de patógenos causantes de meningitis / Manejo clínico y estrategias de prevención y control de Nm

El laboratorio en el diagnóstico de los patógenos de meningitis: Pruebas de sensibilidad

Ana Paula S Lemos

Meningitis, Neumonía y Enfermedades Neumocócicas
Instituto Adolfo Lutz, São Paulo, Brasil

Recent IMD cases caused by a dual resistant serogroup Y suggest changing antimicrobial resistance patterns in the Latin America and the United States

J Antimicrob Chemother 2021; **76**: 1155–1159
doi:10.1093/jac/dkab010 Advance Access publication 29 January 2021

Journal of Antimicrobial Chemotherapy

Emergence of MDR invasive *Neisseria meningitidis* in El Salvador, 2017–19

José Eduardo Oliva Marín[1], Esmeralda Villatoro[2], María Jose Luna[2], Ana María Barrientos[3], Elmer Mendoza[3], Ana Paula S. Lemos[4]*, Carlos H. Camargo[4], Claudio T. Sacchi[5], Marcos Paulo V. Cunha[6], Marcelo Galas[7] and Jean-Marc Gabastou[8]

[1]Instituto Nacional de Salud, Ministerio de Salud, San Salvador, El Salvador; [2]Departamento de Laboratorio Nacional de Salud Pública, Ministerio de Salud, San Salvador, El Salvador; [3]Unidad de Investigación y Epidemiología de Campo, Ministerio de Salud, San Salvador, El Salvador; [4]Centro de Bacteriologia, Instituto Adolfo Lutz, São Paulo, Brazil; [5]Laboratório Estratégico, Instituto Adolfo Lutz, São Paulo, Brazil; [6]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil; [7]Servicio de Resistencia a los Antimicrobianos, Enfermedades Transmisibles y Determinantes Ambientales de la Salud, OPS, Washington, DC, USA; [8]Servicios de Laboratorio de Salud Pública y Redes, Emergencias de Salud de la OPS, Ciudad de México, México

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Clinical Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Acquisition of Ciprofloxacin Resistance Among an Expanding Clade of β-Lactamase–Positive, Serogroup Y *Neisseria meningitidis* in the United States

Caelin C. Potts,[1,a] Adam C. Retchless,[1,a] Lucy A. McNamara,[1,©] Daya Marasini,[2] Natashia Reese,[3] Stephanie Swint,[3] Fang Hu,[4] Shalabh Sharma,[4] Amy E. Blain,[1] David Lonsway,[3] Maria Karlsson,[3] Susan Hariri,[1] LeAnne M. Fox,[1] and Xin Wang[1]; the Antimicrobial-Resistant *Neisseria meningitidis* Team[b]

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Costa Rica National Reference Laboratory: reports of penicillin-resistant and cefotaxime non susceptible *Neisseria meningitidis*

INCIENSA
Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud

Centro Nacional de Referencia de Bacteriología

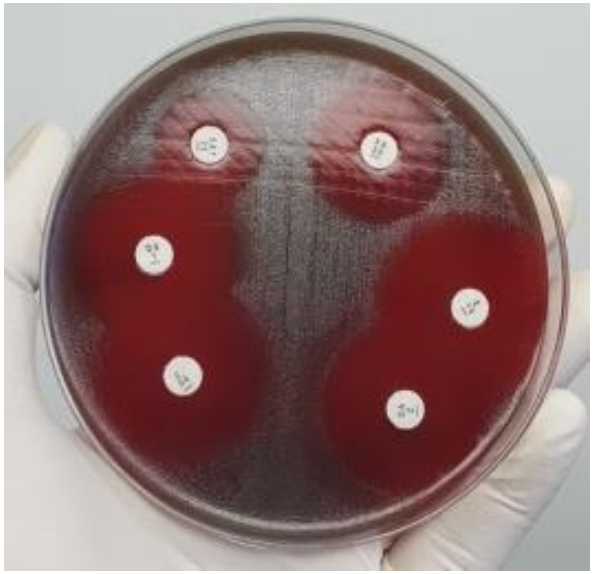
Alerta

Aislamientos de *Neisseria meningitis* serogrupo Y
resistentes a penicilina y no sensibles a cefotaxime en Costa Rica

Fecha: Febrero de 2020

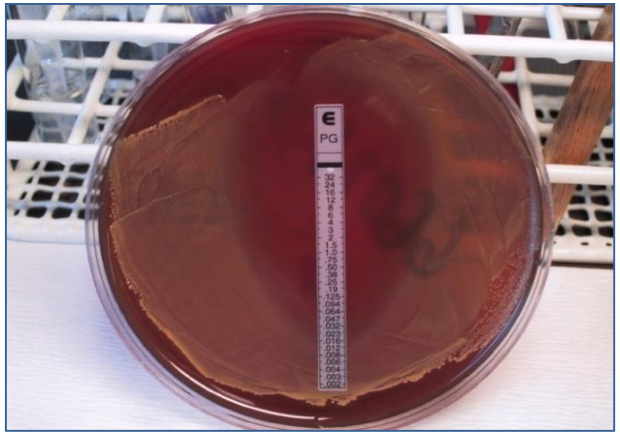
Antimicrobial Susceptibility Tests

Disk Diffusion
Qualitative: S, I, R



MIC - Broth Dilution
Quantitative: µg/mL

MIC – gradiente strip test
Quantitative: µg/mL



EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

CLSI: Clinical And Laboratory Standards Institute

Table 1
Key differences between the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) disk diffusion methodologies

Methodological discrepancy		CLSI	EUCAST
Incubation temperature		35 ± 2°C	35 ± 1°C
Duration of incubation		16–18 h for Enterobacteriaceae, *Staphylococcus* spp.[a] and *Pseudomonas aeruginosa*; 20–24 h for most other organisms	16–20 h
Media		MHA supplemented with 5% sheep blood for *Streptococcus* spp., *Campylobacter jejuni/coli, Pasteurella* spp. *and Neisseria meningitidis*; Haemophilus Test Medium for *Haemophilus* spp.; GC agar base with 1% defined growth supplement for *Neisseria gonorrhoeae*	MH-F agar for *Streptococcus* spp., *Campylobacter jejuni/coli, Pasteurella* spp., *H. influenzae, Moraxella catarrhalis, Listeria monocytogenes, Kingella kingae, Aerococcus* spp. and *Corynebacterium* spp.
Antimicrobial disk contents (μg unless otherwise stated)	Amoxicillin–clavulanic acid	20–10	20–10 and 2–1[b]
	Ampicillin	10	10 and 2[c]
	Cefotaxime	30	5
	Ceftaroline	30	5
	Ceftazidime	30	10
	Ceftazidime–avibactam	30–20	10–4
	Gentamicin[d]	120	30
	Linezolid	30	10
	Nitrofurantoin	300	100
	Penicillin G	10 units	1 unit
	Piperacillin	100	30
	Piperacillin–tazobactam	100–10	30–6
	Vancomycin	30	5
Zone diameter breakpoints		None for *Aerococcus* spp., *Kingella kingae, Listeria monocytogenes, Corynebacterium* spp. Rarely aligned with EUCAST	None for *N. gonorrhoeae, N. meningitidis, Burkholderia cepacia, Vibrio* spp. Fewer intermediate categories
ATCC strains for routine disk diffusion quality control		*Staphylococcus aureus* ATCC 25923, *H. influenzae* ATCC 49247	*S. aureus* ATCC 29213, *H. influenzae* ATCC 49766

MHA, Mueller–Hinton agar; MH-F agar, MHA supplemented with 5% horse blood and 20 mg/L β-NAD.
[a] Except when testing cefoxitin against coagulase-negative staphylococci which requires 24 h.
[b] For testing *H. influenzae*, *Pasteurella multocida*, *Moraxella catarrhalis*, and *Listeria monocytogenes*.
[c] For testing *H. influenzae*, viridans group streptococci, *Staphylococcus saprophyticus* and *Enterococcus* spp.
[d] For testing high-level resistance in *Enterococcus* spp.

Cusack et al. Time to switch form CLSI to EUCAST? A southeast Asian perspective. Clinical Microbiology and Infection 25 (2019) 782-785.

Disk Diffusion Susceptibility Testing Process

Reagents for the Disk Diffusion Test

Testing Strains that Fail to grow Satisfactorily

Antimicrobial Disks

Inoculum Preparation for Disk Diffusion Tests

Inoculation of Test Plates

Application of Disks to Inoculated Agar Plates

Reading plates and Interpreting Results

CLSI. *Performance standards for antimicrobial disk susceptibility tests* M02-A12. 2015

Broth or Agar Dilution Susceptibility Testing Process

Solvents and Diluents for Preparing Stock Solutions of Antimicrobial Agents

Preparing Solutions and Media Containing Combinations of Antimicrobial Agents

Preparing Stock Solutions for Antimicrobial Agents Provided With Activity Expressed as Units

Preparing Dilutions of Antimicrobial Agents to Be Used in Broth or agar Dilution Susceptibility Tests

CLSI. *Performance standards for antimicrobial susceptibility testing – Ed31:* M100. 2021

Neisseria meningitidis

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood
Broth microdilution: CAMHB supplemented with LHB (2.5% to 5% v/v) (see M07[1] for preparation of LHB)
Agar dilution: MHA supplemented with sheep blood (5% v/v)

Inoculum: Colony suspension from 20-24 hours growth from chocolate agar incubated at 35°C; 5% CO_2; equivalent to a 0.5 McFarland standard. Colonies grown on sheep blood agar may be used for inoculum preparation. However, the 0.5 McFarland suspension obtained from sheep blood agar will contain approximately 50% fewer CFU/mL. This must be considered when preparing the final dilution before panel inoculation, as guided by colony counts.

Incubation: 35°C±2°C; 5% CO_2; 20-24 hours

Routine QC Recommendations (See Tables 4A-1, 4B, 5A-1, and 5B for acceptable QC ranges.)

Streptococcus pneumoniae ATCC®a 49619:

Disk diffusion: incubate in 5% CO_2.

Broth microdilution: incubate in ambient air or CO_2 (except azithromycin QC tests that must be incubated in ambient air).

E. coli ATCC® 25922

Disk diffusion, broth microdilution or agar dilution for ciprofloxacin, nalidixic acid, minocycline, and sulfisoxazole: incubate in ambient air or CO_2.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

CLSI. *Performance standards for antimicrobial susceptibility testing – Ed31:* M100. 2021

Streptococcus pneumoniae

Testing Conditions

Medium: Disk diffusion: MHA with 5% sheep blood or MH-F agar (MHA with 5% defibrinated horse blood and 20 µg/mL NAD)
Broth dilution: CAMHB with LHB (2.5% to 5% v/v) (see M07[1] for instructions for preparation of LHB)
Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.

Inoculum: Colony suspension, equivalent to a 0.5 McFarland standard, prepared using colonies from an overnight (18- to 20-hour) sheep blood agar plate

Incubation: 35°C ± 2°C
Disk diffusion: 5% CO_2; 20-24 hours
Dilution methods: ambient air; 20-24 hours (CO_2 if necessary, for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC®a 49619

Disk diffusion: deterioration of oxacillin disk content is best assessed with *S. aureus* ATCC® 25923, with an acceptable range of 18-24 mm on unsupplemented MHA.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

CLSI. *Performance standards for antimicrobial susceptibility testing – Ed31:* M100. 2021

Haemophilus influenzae

Testing Conditions

Medium:	Disk diffusion: HTM Broth dilution: HTM broth
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard prepared using colonies from an overnight (preferably 20- to 24-hour) chocolate agar plate (see general comment [2])
Incubation:	35°C±2°C Disk diffusion: 5% CO_2; 16-18 hours Broth dilution: ambient air; 20-24 hours

Routine QC Recommendations (see Tables 4A-1, 4B, 5A-1, and 5B for acceptable QC ranges)

H. influenzae ATCC®a 49247
H. influenzae ATCC® 49766

Use either *H. influenzae* ATCC® 49247 or *H. influenzae* ATCC® 49766 or both of these strains, based on the antimicrobial agents to be tested. Neither strain has QC ranges for all agents that might be tested against *H. influenzae* or *H. parainfluenzae*.

E. coli ATCC® 35218 (when testing amoxicillin-clavulanate)

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

CLSI. *Performance standards for antimicrobial susceptibility testing – Ed31:* M100. 2021

Zona Diameter and MIC Breakpoints

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
PENICILLINS									
C	Penicillin		-	-	-	≤0.06	0.12-0.25	≥0.5	
C	Ampicillin		-	-	-	≤0.12	0.25-1	≥2	
CEPHEMS									
C	Cefotaxime or	30 µg	≥34	-	-	≤0.12	-	-	
C	ceftriaxone	30 µg	≥34	-	-	≤0.12	-	-	
CARBAPENEMS									
C	Meropenem	10 µg	≥30	-	-	≤0.25	-	-	
MACROLIDES									
C	Azithromycin	15 µg	≥20	-	-	≤2	-	-	See general comment (6). (7) May be appropriate only for prophylaxis of meningococcal case contacts. These breakpoints do not apply to therapy of patients with invasive meningococcal disease.
TETRACYCLINES									
C	Minocycline	30 µg	≥26	-	-	≤2	-	-	See comment (7).
FLUOROQUINOLONES									
(8) For surveillance purposes, a nalidixic acid MIC ≥8 µg/mL or a zone ≤25 mm may correlate with diminished fluoroquinolone susceptibility.									
C	Ciprofloxacin	5 µg	≥35	33-34	≤32	≤0.03	0.06	≥0.12	See comment (7).
C	Levofloxacin	-	-	-	-	≤0.03	0.06	≥0.12	

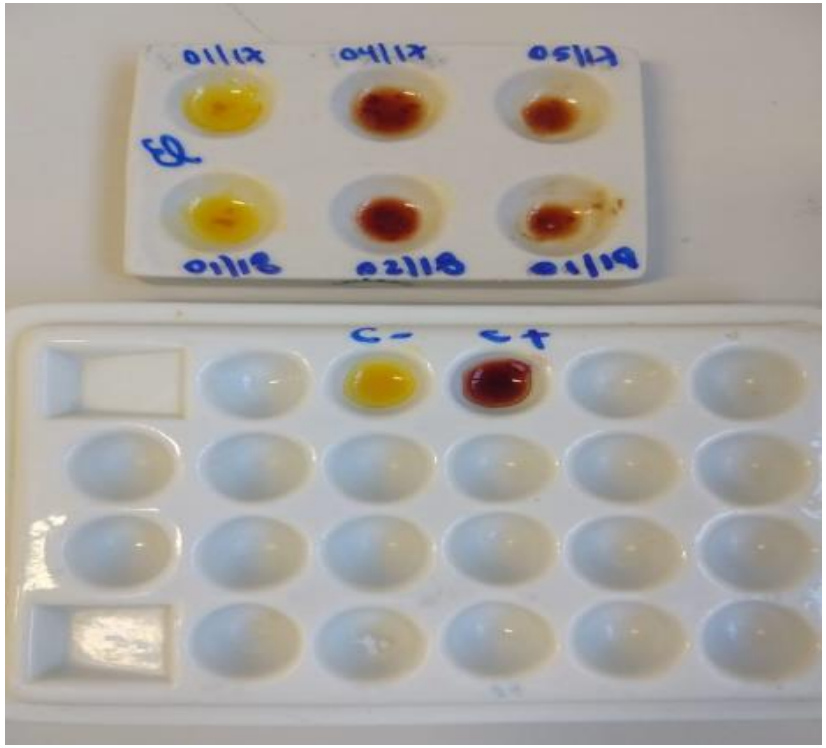
Table 2I. *Neisseria meningitidis* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
			S	I	R	S	I	R	
FOLATE PATHWAY ANTAGONISTS									
C	Sulfisoxazole	-	-	-	-	≤2	4	≥8	See comment (7).
C	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥30	26-29	≤25	≤0.12/2.4	0.25/4.75	≥0.5/9.5	(9) Trimethoprim-sulfamethoxazole is the preferred disk for detection of sulfonamide resistance. Trimethoprim-sulfamethoxazole testing predicts susceptibility and resistance to trimethoprim-sulfamethoxazole and sulfonamides. Sulfonamides may be appropriate only for prophylaxis of meningococcal case contacts.
PHENICOLS									
C	Chloramphenicol	30 µg	≥26	20-25	≤19	≤2	4	≥8	(10) Not routinely reported on isolates from the urinary tract.
ANSAMYCINS									
C	Rifampin	5 µg	≥25	20-24	≤19	≤0.5	1	≥2	See comment (7).

Abbreviations: AST, antimicrobial susceptibility testing; ATCC®, American Type Culture Collection; BSC, biological safety cabinet; BSL-2, biosafety level 2; BSL-3, biosafety level 3; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); I, intermediate; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; R, resistant; S, susceptible.

CLSI. *Performance standards for antimicrobial susceptibility testing – Ed31:* M100. 2021

Detection of Ciprofloxacin-resistant, β-lactamase-producing *Neisseria meningitidis*



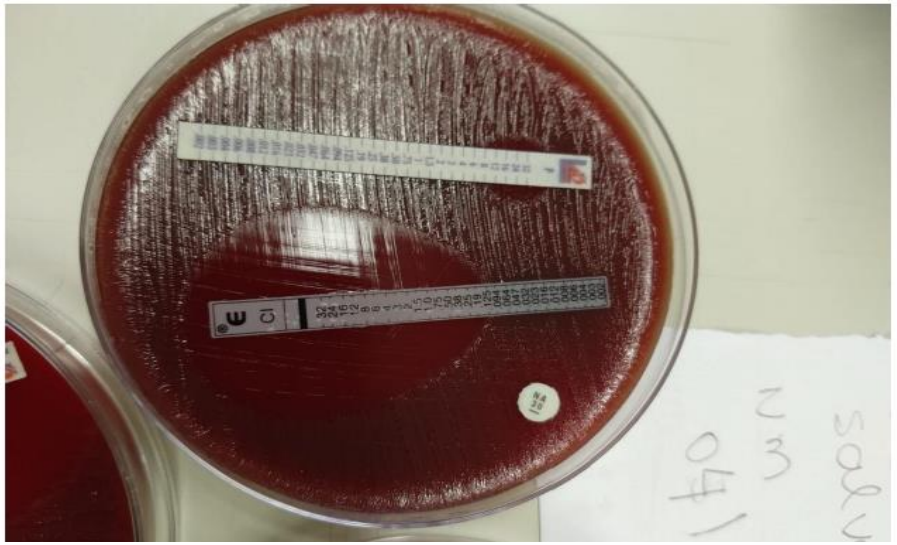
Cepa : Nm04-17

Etest®

Penicilina : 8 ug/mL

Ciprofloxacina : 0,125 ug/mL

Ácido Nalidíxico (30ug) ZERO mm



José Eduardo Oliva Marín, Esmeralda Villatoro, María Jose Luna, Ana María Barrientos, Elmer Mendoza, Ana Paula S Lemos, Carlos H Camargo, Claudio T Sacchi, Marcos Paulo V Cunha, Marcelo Galas, Jean-Marc Gabastou, Emergence of MDR invasive *Neisseria meningitidis* in El Salvador, 2017–19, *Journal of Antimicrobial Chemotherapy*, Volume 76, Issue 5, May 2021, Pages 1155–1159, https://doi.org/10.1093/jac/dkab010

muchas gracias