La diagnosi microbiologica per *Neisseria meningitidis*

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La malattia meningococcica invasiva in Italia: le raccomandazioni del *position paper* SIMIT

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AULA MAGNA POLO LITA

Neisseria meningitidis - diagnosi microbiologica

- **Diagnosi di infezione invasiva**
- **Diagnosi *post-mortem***
- **Diagnosi portatore**

Neisseria meningitidis - diagnosi infezione invasiva

	Campione
Esame colturale	sedimento liquorale sangue *essudati siti sterili*
Esame batterioscopico	sedimento liquorale *essudati siti sterili*
Test molecolare	liquor *sangue* *essudati siti sterili*
Ricerca antigeni capsulari	surnatante liquor

Lab Methods for Diagnosis of Meningitis, CDC - WHO 2° Ed. 2011

Neisseria meningitidis – esame colturale per infezioni invasive

- Da sangue, sedimento liquorale, essudati da siti sterili

Se tempo di trasporto >1 h consigliabile uso di TI medium (incubaz. o.n. 35 °C)

- Isolamento su Agar Sangue, Agar Cioccolato (35 °C + 5% CO_2, ambiente umido)
  - ID biochimica
  - ID MALDI-TOF (possibile misidentificazione con *Neisseria polysaccharea*)
  - ID molecolare (sequenziamento 16S rDNA, PCR *sodC*, PCR *ctrA*)
  - Tipizzazione: agglutinaz. con antisieri o molecolare

Crescita in 24 - 48 h

Cunningham *et al*. – JCM 2014

Lab Methods for Diagnosis of Meningitis, CDC - WHO 2° Ed. 2011

Neisseria meningitidis – tipizzazione molecolare

Sero-group	Capsule type	Gene Target Name	Alternate Gene Names
A	(α1→6)-*N*-acetyl-D-mannosamine-1-phosphate	*sacB*	
B	(α2→8)- N-acetylneuraminic acid	*synD*	*siaD* *siaD* of B $siaD_B$
C	(α2→9)- N-acetylneuraminic acid	*synE*	*siaD* of C $siaD_C$
W135	6-D-Gal(α1→4)-N-acetylneuraminic acid(α2→6)	*synG*	*siaD* of W135 $siaD_W$
X	(α1→4)-*N*-acetyl-D-glucosamine-1-phosphate	*xcbB*	
Y	6-D-Glc(α1→4)-N-acetylneuraminic acid(α2→6)	*synF*	*siaD* of Y $siaD_Y$

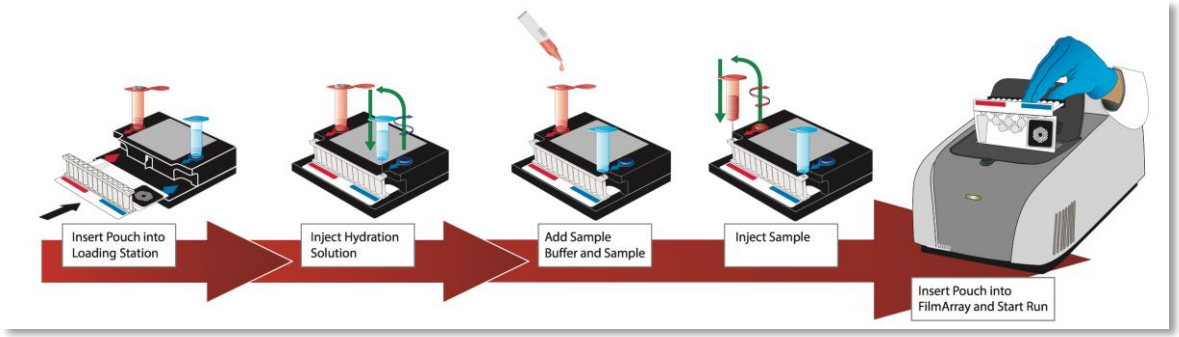
Lab Methods for Diagnosis of Meningitis, CDC - WHO 2° Ed. 2011

Neisseria meningitidis – test molecolari per infezioni invasive

- Vari formati: PCR, RT-PCR, NASBA, LAMP

- Materiali: liquor, *sangue, essudati da siti sterili*

- TTR rapido (1 - 4 h); possibilità POCT (es. Filmarray™)



- Maggiore sensibilità di esame colturale e batterioscopico

Lab Methods for Diagnosis of Meningitis, CDC - WHO 2° Ed. 2011

Neisseria meningitidis – ricerca Ag solubili per infezioni invasive

- Su liquor (surnatante), mediante test co-agglutinazione
  (su siero ed urine non affidabili)

- Rilevazione di sierogruppi: A, B/*E. coli* K1, C, Y/W-135

- Inizialmente considerato molto utile per diagnostica rapida ma problemi di sensibilità e specificità (legati a cross-reazioni con altri antigeni batterici)

- Ampiamente sostituito dai test molecolari

Nigrovic *et al* – PIDJ 2004
Brouwer *et al* - CMR 2010
Lab Methods for Diagnosis of Meningitis, CDC - WHO 2° Ed. 2011

Neisseria meningitidis - diagnosi *post-mortem*

- Possibile mediante ESAME COLTURALE e test molecolare

- Campioni esaminabili:
  - liquor, sangue, biopsia surrenalica, endocardio, lesioni cutanee, umor vitreo
  - secreto faringeo/nasale (possibile colonizzazione)
  - tessuti fissati e paraffinati (solo test molecolare)

- Positività colturale riportata fino a 5 giorni dopo la morte

Ploy *et al*. – DMID 2005
Fernandez-Rodriguez *et al*. – DMID 2008
Garland *et al*. – Am J Forens Med Pathol 2016

Neisseria meningitidis - diagnosi di portatore

Campione: tampone faringeo - parete posteriore (+ tonsille?)

Roberts *et al.* – J Infect 2009
Esposito *et al*. – EJCMID 2013
Basta *et al*. – PlosONE 2013

Semina *on-site* o terreno di trasporto (sensibilità può ridursi per tempi >5 h)

Terreno selettivo per meningococchi (Thayer-Martin modificato) + controllo crescita

- ID biochimica o con MALDI-TOF
- Conferma ID: PCR *sodC* / *ctrA*
- Tipizzazione: agglutinazione con antisieri o RT-PCR per loci capsulari

Test molecolare diretto (RT-PCR) per *N. meningitidis*
Target: *sodC* o *ctrA* (ceppi capsulati)

Tipizzazione: RT-PCR per loci capsulari

Possibile presenza di meningococchi non capsulati

STUDIO SUI PORTATORI SANI DI MENINGOCOCCO IN TOSCANA

Tamponi positivi per sierogruppo, metodo di analisi ed ex asl

Sierogruppo	Metodo colturale					PCR				
	FI	EMP	SI	GR	Tot	FI	EMP	SI	GR	Tot
A	0	0	0	0	0	0	0	0	0	0
B	6	9	5	4	24	10	10	11	6	37
C	0	0	0	0	0	0	3	0	1	4
W	0	0	0	0	0	0	0	0	0	0
Y	0	3	4	2	9	0	4	4	3	11
Z	0	1	0	0	1	0	1	0	0	1
29E	1	1	0	0	2	1	1	0	1	3
Non tipizzati	27	16	9	18	70 §	1*	1*	0	0	2
Totale positivi	**34**	**30**	**18**	**24**	**106**	**12**	**20**	**15**	**11**	**58**
Tamponi analizzati	597	595	494	601	2.287	597	595	494	601	2.287
Percentuale	**5,7**	**5,0**	**3,6**	**4,0**	**4,6**	**2,0**	**3,4**	**3,0**	**1,8**	**2,5**

§ Non capsulati

* Capsulati ma non tipizzabili

ESCMID guideline: diagnosis and treatment of acute bacterial meningitis

D. van de Beek[1], C. Cabellos[2], O. Dzupova[3], S. Esposito[4], M. Klein[5], A. T. Kloek[1], S. L. Leib[6], B. Mourvillier[7], C. Ostergaard[8], P. Pagliano[9], H. W. Pfister[5], R. C. Read[10], O. Resat Sipahi[11] and M. C. Brouwer[1], for the ESCMID Study Group for Infections of the Brain (ESGIB)

Level 2	CSF culture is positive in 60–90% of bacterial meningitis patients depending on the definition of bacterial meningitis. Pretreatment with antibiotics decreases the yield of CSF culture by 10–20%.
Level 2	CSF Gram stain has an excellent specificity and varying sensitivity, depending on the microorganism. The yield decreases slightly if the patient has been treated with antibiotics before lumbar puncture is performed.
Level 2	In patients with a negative CSF culture and CSF Gram stain, PCR has additive value in the identification of the pathogen.
Level 2	Latex agglutination testing has little incremental value in the diagnosis of bacterial meningitis.

What investigations should be done (suspected IMD)?

Test	Level of need	Timing	Notes
CSF culture	**mandatory**	**Urgent**	Sensitivity reduced by previous therapy
Blood culture	**mandatory**	**Urgent**	Sensitivity reduced by previous therapy
CSF Gram-stain	**mandatory**	**Urgent**	Low sensitivity
Molecular ID	**mandatory (if microscopy is not conclusive)**	**Urgent**	Higher sensitivity
Antigens	No longer recommended	**Urgent (if used)**	Consider only if molecular tests not available
Typing	Useful for epidemiological purposes	Not urgent	Serogroup but also clonality
Antibiogram	Useful for epidemiological purposes	Not urgent	Test drugs for treatment and prophylaxis

Urgent laboratory investigations **should be available** from Diagnostic Microbiology laboratories on a **24h/7d regimen**

Neisseria meningitidis – saggi di sensibilità agli antibiotici

Antibiotici da saggiare con MIC test (CLSI prevede anche DD)

- Per uso clinico
  - Penicillina
  - Ampicillina
  - Ceftriaxone
  - Meropenem
  - Cloramfenicolo

S (≤)	R (>)	note
0.06	**0.25**	
0.12	**1**	
0.12	**0.12**	
0.25	**0.25**	
2	***4***	*Solo CLSI*

- Per uso profilattico
  - Rifampicina
  - Ciprofloxacina
  - Minociclina
  - Azitromicina

S (≤)	R (>)	
0.25 *(0.5)*	**0.25 *(1)***	*(CLSI)*
0.03	**0.03 *(0.06)***	*(CLSI)*
1 *(2)*	**2**	*(CLSI)*
2		*Solo CLSI*

EUCAST breakpoints 7.1, 2017
CLSI M100-S27, 2017

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2005, p. 4811–4814 Vol. 43, No. 9
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.9.4811–4814.2005

Assessing the Risk of Laboratory-Acquired Meningococcal Disease

James J. Sejvar,[1]* David Johnson,[2] Tanja Popovic,[3] J. Michael Miller,[4] Frances Downes,[2] Patricia Somsel,[2] Robbin Weyant,[5] David S. Stephens,[4] Bradley A. Perkins,[3] and Nancy E. Rosenstein[3]

- 16 cases of probable laboratory-acquired Men disease reviewed (1996 – 2001); 50% fatal
- All cases among clinical microbiologists; 94% manipulation w/o respiratory protection
- Attack rate of 13 x 100,000 *vs*. 0.2 x 100,000 in the general population (USA)

What investigations should be done (suspected IMD)?

CSF culture: **mandatory** although this exam might be positive in 67-85% of cases, and the yield might decrease by about 20% in case of patients pre-treated with antibiotics.

Blood culture is mandatory, and whenever possible should be performed before starting antimicrobial chemotherapy.

Microscopic examination of Gram stained CSF sediment is mandatory. Microscopy has a good specificity but may suffer of low sensitivity. The value of this test could be related to microbiologist experience.

All the above laboratory investigations **should be available** from Diagnostic Microbiology laboratories on a **24h/7d regimen**.

Latex agglutination tests for the detection of meningococcal capsular antigens, performed on CSF supernatants, can provide rapid information but are affected by overall low sensitivity and specificity. These test have largely been abandoned in favor of molecular tests, but they might be used in settings where molecular tests are not readily available.

Molecular methods have high specificity and sensitivity, and should be done in all cases of suspected bacterial meningitis, especially when microscopy is not conclusive.
Systems to perform molecular analysis in less than 1 hour with minimal hands-on-time and minimal training requirements are commercially available and suitable for use also in smaller laboratories on a 24/7 regimen.
These methods should be evaluated in large prospective studies.

Procalcitonin may be useful to predict the bacterial etiology of meningitis. On the basis of the experience deriving from patients with sepsis, we could hypothesize that a high value could be predictive of severe disease.

Antimicrobial susceptibility testing of meningococcal isolates is important for epidemiological purposes and should be performed with all isolates by Diagnostic Microbiology laboratories. Antibiotics used for both treatment and prophylaxis should be tested.

Characterization of serogroup (either by serological methods on bacterial isolates or by molecular methods on bacterial isolates or DNA extracts from clinical specimens) and **genotyping** of the isolate to define the clonal lineage is important for epidemiological purposes and should be performed in all cases of invasive meningococcal disease.

These investigations, which are not urgent, should be performed by **experienced/reference laboratories**.

Whenever possible, therefore, **clinical isolates and aliquots of clinical specimens** (CSF and blood samples) should be **stored at -70 °C** for similar purposes.