LES TESTS BIOLOGIQUES NOUVEAUX ET FUTURS POUR LE DIAGNOSTIC DES MENINGITES « EN URGENCE »

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Principaux agents en cause

- Bactéries
  - *Neisseria meningitidis*
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae* ↘
  - *Listeria monocytogenes*, Mycobactéries …
- Virus
  - Enterovirus +++
  - Virus ourlien ↘
  - HSV-2, HIV, VZV
- Champignons
  - *Cryptococcus neoformans*

Impact « en urgence »

- Méningite ou méningo-encéphalite
  - ➔ sur clinique, imagerie ...
  - ➔ peu de valeur de la biologie
- Infection bactérienne ou infection virale
  - ➔ arguments cytologiques non spécifiques
  - ➔ arguments biochimiques non spécifiques
  - ➔ arguments microbiologiques spécifiques

Prélèvements

- Ponction lombaire +++
  - volume de LCR jamais suffisant (on peut prélever 10 ml et plus sans risque chez l'adulte, au moins 1,5 à 2 ml chez le nourrisson)
  - éviter de fractionner les tubes
    - examens biochimiques
    - examens cyto-bactériologiques et virologiques
  - LCR tardif essentiellement dans les ME
- Prélèvements périphériques (selles, gorge …) pour la recherche de virus
- Deux sérologies à 15 jours d'intervalle dans les ME

✂➔intérêt du concept de « trousse infections du SNC »

Tests non spécifiques « en urgence »

	Méningite bactérienne	Méningite virale
Taux de leucocytes (cellules / µl)	1000 – 10.000 < 100 à >10.000	< 300 < 100 à 1000
PN neutrophiles	> 80%	< 20%
Protéinorachie	Elevée	Normale
Glycorachie	Basse	Normale

NB :
Liquide clair
= < 500 / µl

💣 : **se méfier des algorithmes décisionnels trop simples, notamment chez l'adulte**

Fitch and van de Beek, Lancet, 2007, 7, 191-200

Protéines inflammatoires

(Mary *et al.*, 2007, ABC)

- Marqueurs disponibles en urgence :
  - CRP
  - récemment, procalcitonine (PCT) (Brahms Diagnostic, Berlin) ; seuil de 0,5 ng/ml
  - dans le futur : protéine sérique amyloïde A
- Sensibilité et spécificité de la PCT de 100% dans deux études françaises :
  - Gendrel et al. (1997, CID) chez l'enfant au seuil de 2 ng/ml
  - Viallon et al. (1999, CID) chez l'adulte au seuil de 0,93 ng/ml
- La supériorité de la PCT tient à sa cinétique plasmatique plus rapide ; l'élévation est d'autant plus importante que l'on a affaire à des bactéries capsulées

Méningites bactériennes « en urgence » (1)

- Problème du Gram en urgence (positif si concentration de germes > 105/ml)
- Colorations spécifiques des BK (+ dans 10 à 40% des cas selon Roos, Semin neurol, 2000, 20, 329-35)
- Antigènes solubles (PS de capsule) peu sensibles :
  - *E. coli* K1
  - méningocoques non B
  - streptocoques du groupe B
  - *Haemophilus inflenzae* b
  - pneumocoque +++ (LCR et urines)

Méningites bactériennes « en urgence » (2)

- Biologie moléculaire encore expérimentale
- Exemple : détection simultanée de *Neisseria meningitidis*, *Haemophilus influenzae* et *Streptococcus* sp. par PCR semi-nichée

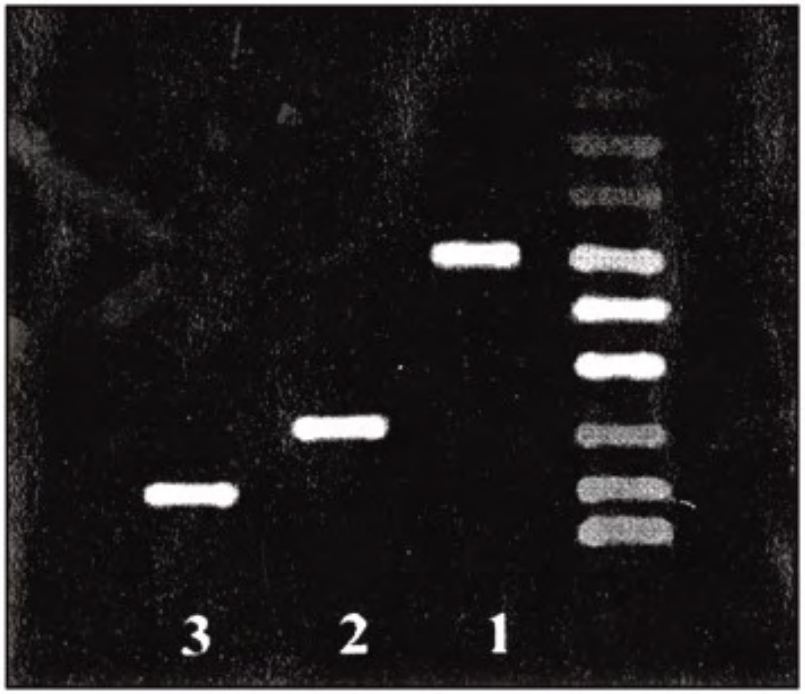


Fig. Electrophoresis of PCR amplification products in agarose gel (2%). Results are compared to molecular weight markers and DNA sequences of Streptococcus sp. (1), H. influenzae (2), and N. meningitidis (3).

Failace et al., Arq Neuro-Psiquiatria, 2005, 63

Méningites bactériennes « en urgence » (2)

Table 1. Clinical and laboratory characteristics of groups I, II, and III.

Variable	Group I (n=65)	Group II (n=19)	Group III (n=98)	p
Age (months)	12[a] (5-36)	45[b] (12-84)	48[b] (24-84)	< 0.001
Duration of hospital stay (days)	11[a] (10-16)	10[a] (10-11)	2[b] (2-3)	< 0.001
% of patients receiving antibiotics before spinal tap	14[a,b] (21.5%)	8[a] (42.1%)	13[b] (13.3%)	0.012
CSF leukocytes/mm^3	1320[a] (597-3400)	1200[a] (700-11700)	313[b] (85-604)	<0.001
% neutrophils	88[a] (77-93)	90[a] (81-93)	15[b] (4-64)	<0.001
CSF glucose (mg/dL)	13[a] (2-55)	48[a] (1-62)	59[b] (53-70)	<0.001
CSF protein (mg/dL)	200[a] (120-280)	150[a] (88-190)	43[b] (32-61)	<0.001

All values are expressed as medians (interquartile range), except for percentage of patients taking antibiotics. Superscript letters indicate statistically significant differences.

METHODE	Nbre tests	Sensibilité groupe I	Nbre tests	Sensibilité groupe I + II
PCR	65	92,3%	84	88,1%
Gram	65	81,5%	84	63,3%
Culture	65	81,5%	84	63,3%
Ags solubles	40	75,0%	50	60,0%

Failace et al., Arq Neuro-Psiquiatria, 2005, 63

Méningites virales « en urgence »

- **85 à 90% des méningites aseptiques sont dues aux entérovirus**
- Mode de transmission
  - surtout fécal-oral
  - mais aussi respiratoire, muqueuse
- Distribution mondiale
- Réservoir essentiellement humain
- Recrudescence estivo-automnale
- Petite prédominance masculine
- Surtout sujets jeunes

Epidémiologie des entéroviroses USA, 2003

Medscape® www.medscape.com

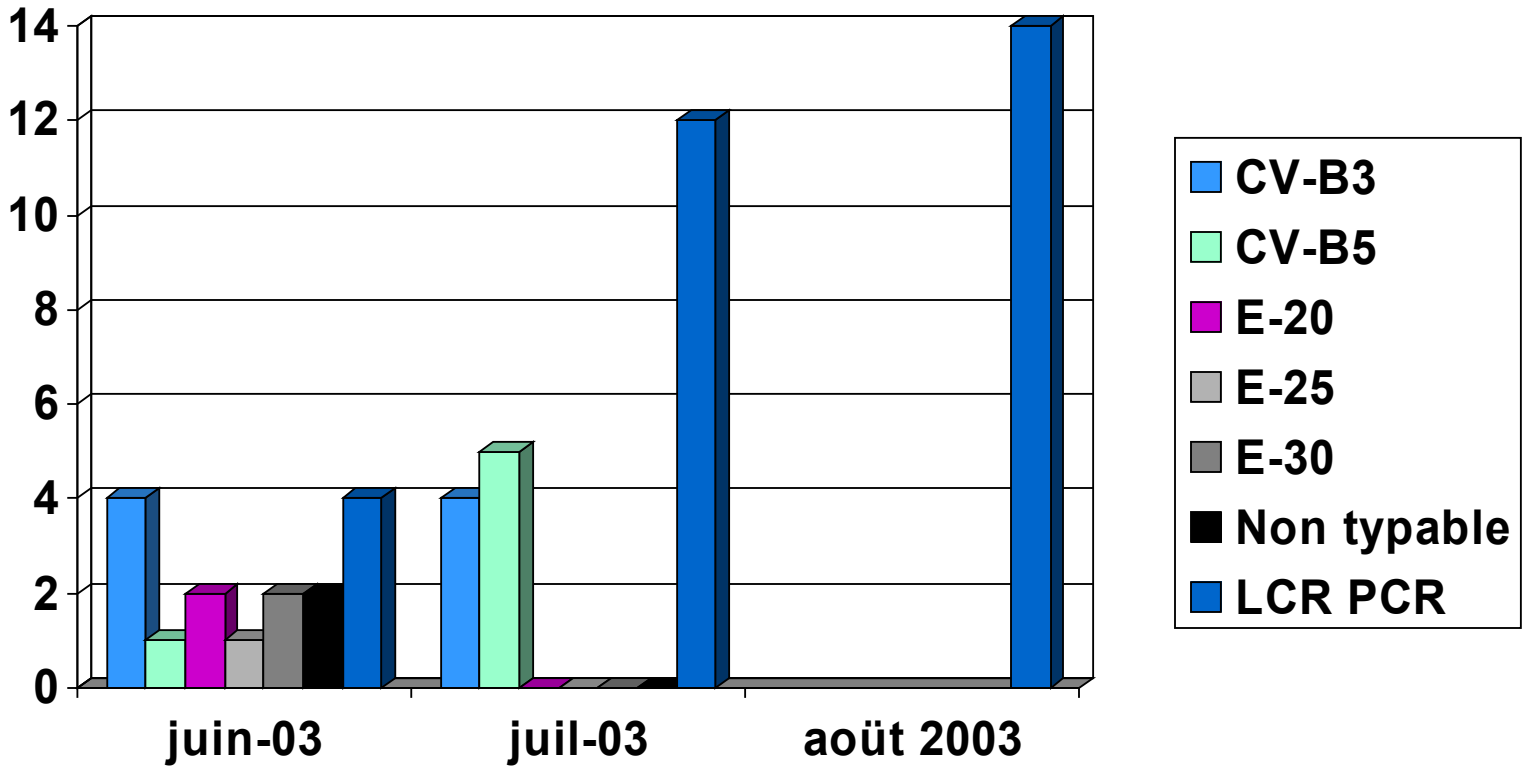
TABLE. Number and percentage of persons with aseptic meningitis, by demographic and clinical characteristics — Arizona, California, Georgia, Idaho, and South Carolina, 2003

Characteristic	Arizona January 1–July 31 (n = 465)*		California April 1–July 31 (n = 148)		Georgia March 10–July 23 (n = 320)†		Idaho May 21–July 17 (n = 38)§		South Carolina April 6–July 31 (n = 82)¶	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Sex										
Male	237	(51)	81	(55)	157	(60)	12	(32)	51	(62)
Female	227	(49)	67	(45)	104	(40)	26	(68)	31	(38)
Age group										
≤3 mos	—		6	(4)	39	(15)	0	—	1	(1)
4–11 mos	—		1	(1)	13	(5)	0	—	0	—
1–14 yrs	244	(52)	61	(41)	114	(44)	16	(42)	61	(78)
≥15 yrs	221	(48)	80	(54)	95	(36)	22	(58)	16	(21)
Clinical signs										
Fever	NA**		91	(61)	63	(80)	25	(78)	NA	
Headache	NA		NA		64	(64)	32	(100)	NA	
Stiff neck	NA		73	(49)	32	(41)	24	(75)	NA	
Photophobia	NA		NA		14	(18)	26	(81)	NA	
Nausea/Vomiting		NA		NA		41	(52)	29	(91)	NA

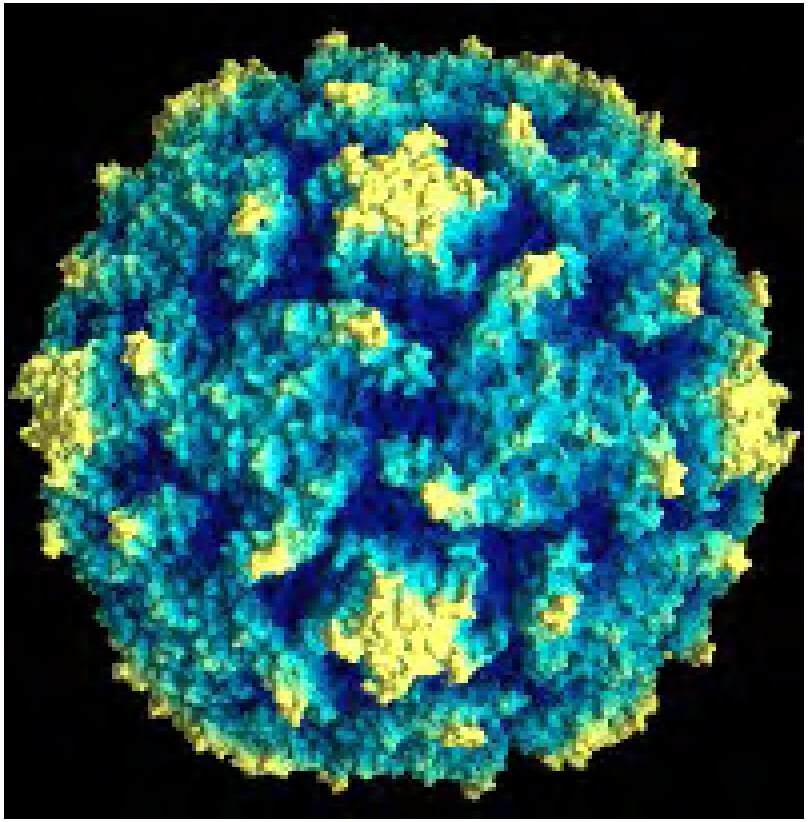
* Data for sex were unavailable for one person.
† Demographic information was available for 261 cases and clinical information for 79 cases.
§ Demographic information was available for 38 cases and clinical information for 32 cases.
¶ On the basis of the 82 cases reported in Aiken County. Information on age was available for 78 cases.
** Not available.

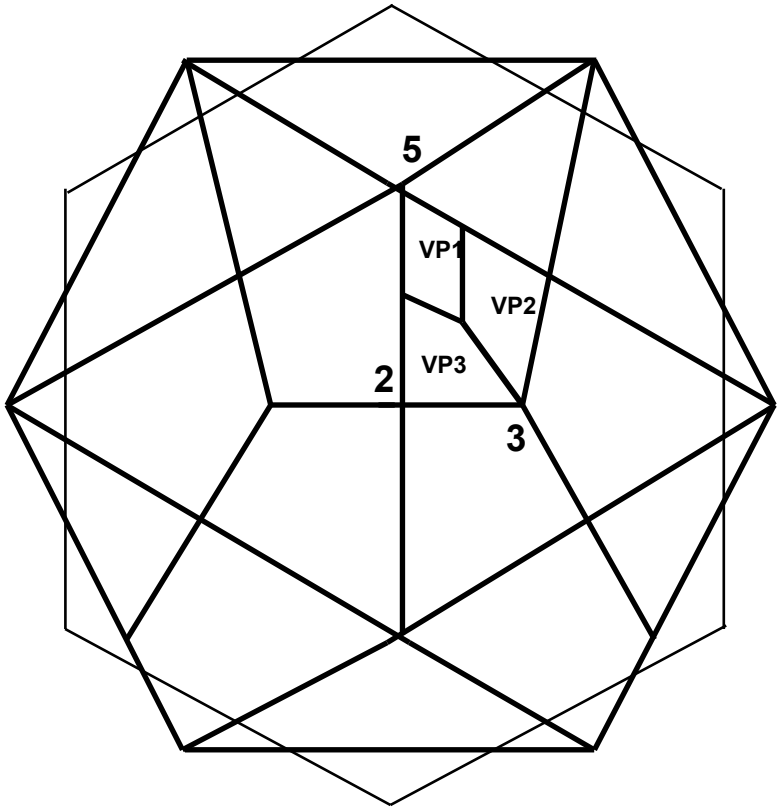
Source: MMWR © 2003 Centers for Disease Control and Prevention (CDC)

Epidémiologie des entéroviroses Saint-Etienne, été 2003

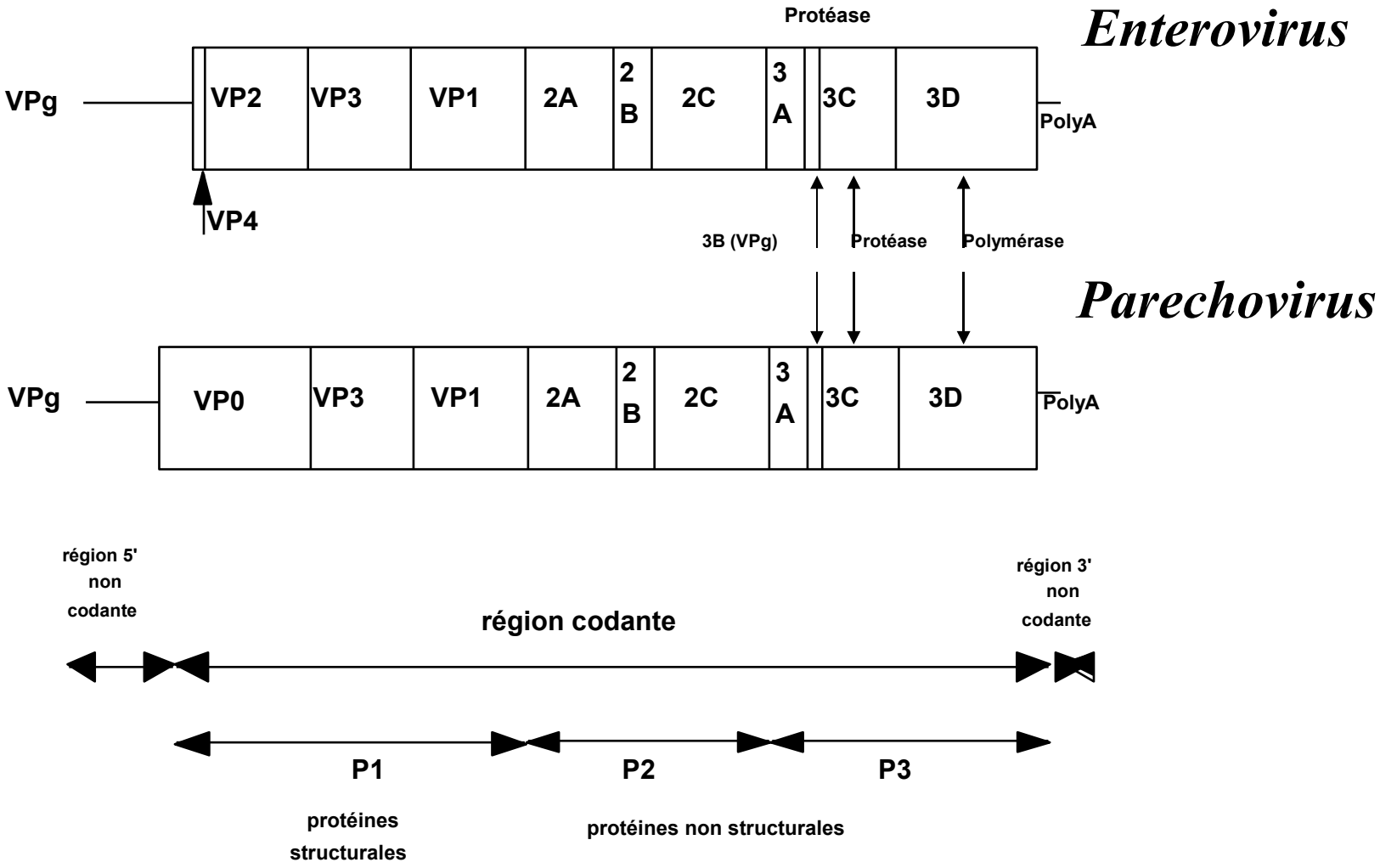


Structure des *Enterovirus*





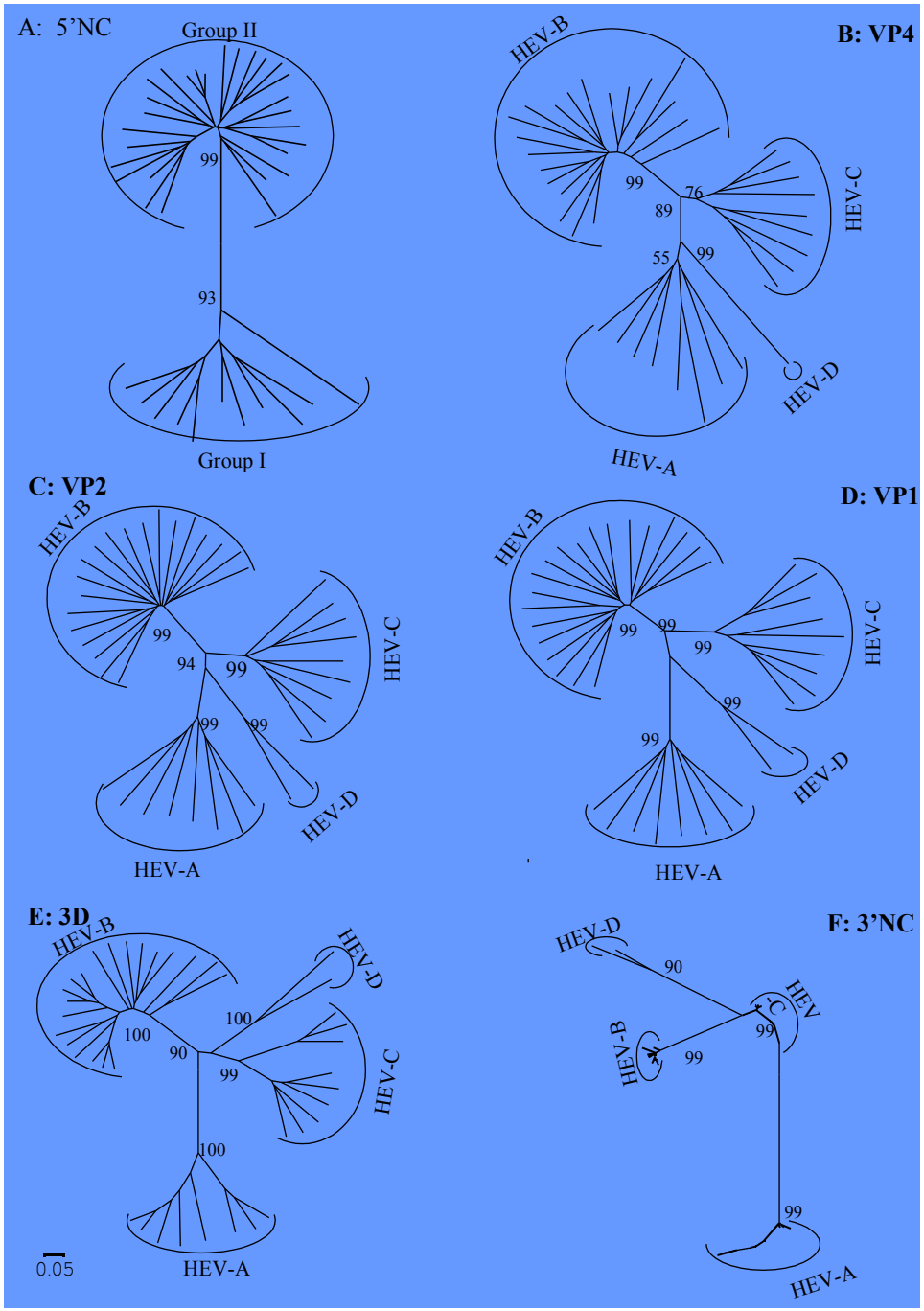
Organisation du génome



Nouvelle classification moléculaire des *Picornaviridae*

GENRE	ESPECE	Nbre de sérotypes	Nom et numéros des sérotypes
Enterovirus	*Human enterovirus A*	10	Coxsackievirus humains A2, 3, 5, 7, 8, 10, 12, 14, 16, Enterovirus humain 71
	Human enterovirus B	36	Coxsackievirus humains A9, B1-6, tous les 28 Echovirus humains, Enterovirus humain 69
	Human enterovirus C	11	Coxsackievirus humains A1, 11, 13, 15, 17-22, 24
	Human enterovirus D	2	Enterovirus humain 68, 70
	Poliovirus	3	Poliovirus 1-3
	Non classés	2	Coxsackievirus humains A4, 6
Parechovirus	*Human parechovirus*	2	Parechovirus humains 1 et 2 (ex-echovirus 22 et 23)
Rhinovirus	*Human rhinovirus A*	18	Rhinovirus humains 1, 2, 7, 9, 11, 15, 16, 21, 29, 36, 39, 49, 50, 58, 62, 65, 85, 89
	Human rhinovirus B	3	Rhinovirus humains 3, 14, 72
	Non classés	79	Autres sérotypes de rhinovirus humains
Hepatovirus	*Hepatitis A virus*	1	Virus humain de l'hépatite A

Quelle que soit la région considérée (à l'exception de la région 5 'NC), les entérovirus sont classés en 4 espèces.



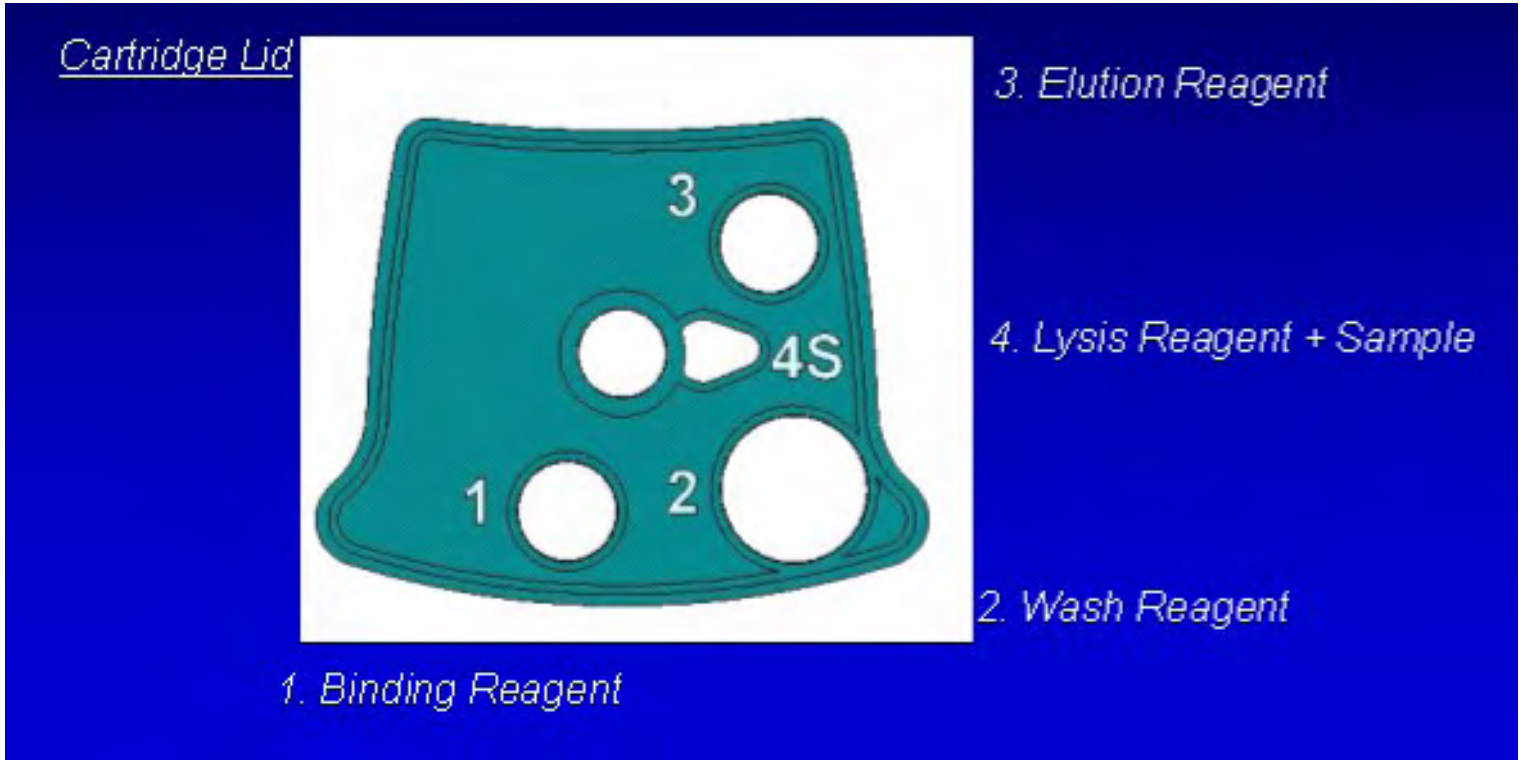
GeneXpert Dx

Biologie Moléculaire d'urgence

Xpert Enterovirus ™ : Products attributes

- ***140µL of CSF as sample***
- ***Two-step RT-PCR***
- ***Targets a 146nt sequence in the 5' NTR of enterovirus RNA***
- ***Internally controlled – Armored CIC RNA (Ambion) included in all runs as sample preparation/internal control***
- ***Time to result is 2h 29 minutes***

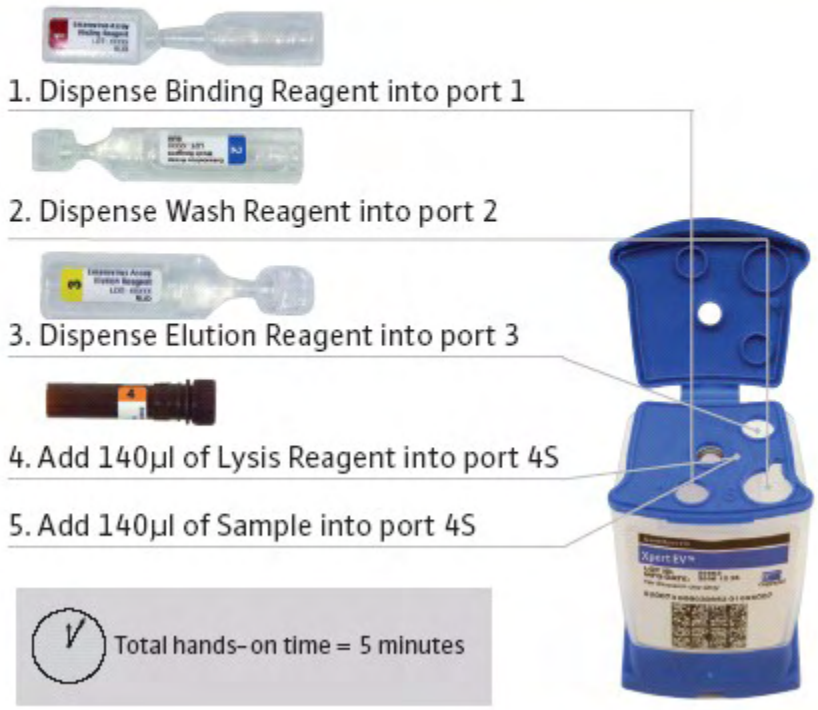
Xpert EV™ : cartridge



Xpert EV™ : protocol

The Xpert EV™ advantage: Simplicity

- Fully automated process reduces handling time to just minutes
- Random access for flexibility and workflow optimization
- Rapid results to improve patient management
- Fully integrated reagent and instrument system for accuracy and reproducibility



6. Insert cartridge and start assay

Cartridge protocol

CSF and Sample Preparation Reagents are Loaded into Cartridge

Sample organisms and Armored CIC are Lysed to release nucleic acids

Nucleic Acids Are Bound, and Washed on solid phase

Nucleic acids are Eluted and Concentrated

Nucleic acids (RNA) mixed with RT Lyophilized Beads

Mixture Delivered to Integrated Reaction Tube for RT

cDNA removed from tube, diluted then mixed with PCR Lyophilized Beads

Mixture Delivered to Integrated Reaction Tube for Real time PCR

Xpert EV™ : Tests results

- ***POS***
  - *EV - POS (valid Ct range 3-45)*
  - *Controls - NA*
  - *Probe Checks - PASS*

- ***NEG***
  - *EV - NEG*
  - *Controls - PASS*
  - *Probe Checks - PASS*

- ***INVALID***
  - *EV - INVALID*
  - *Controls – FAIL*
  - *Probe Checks - PASS*

FDA News – 16.03.2007

U.S. Food and Drug Administration

FDA Clears Rapid Test for Meningitis

The U.S. Food and Drug Administration (FDA) today cleared for marketing a test that uses molecular biology to quickly detect the presence of viral meningitis.

The Xpert EV test, when used in combination with other laboratory tests, will help physicians distinguish between viral meningitis and the less-common, but more severe, version of meningitis caused by bacteria.

Meningitis is an infection of the cerebrospinal fluid surrounding a person's spinal cord and brain, causing inflammation of the tissues in these areas. The illness is diagnosed by testing the fluid obtained from a patient during a spinal tap. Typically, diagnostic tests for meningitis can take up to a week to get results. But results from the Xpert EV test are available in two and one-half hours.

"Because this test is significantly faster than existing methods for diagnosing meningitis, it could minimize delays in treating patients. Swift recognition of the cause and appropriate treatment is critical to patient recovery," said Daniel Schultz, M.D., director of the Center for Devices and Radiological Health. "Since bacterial meningitis can be deadly within as little as two days, patients who have viral meningitis are frequently treated with antibiotics as a safeguard against the more dangerous bacterial meningitis. This test should help physicians manage patients appropriately and prevent unnecessary treatment with antibiotics."

Knowing whether the meningitis is viral or bacterial is imperative to early effective treatment. But distinguishing between the two types of infection is difficult because of similar symptoms. Patients with viral meningitis usually recover within two weeks without any medical intervention. Bacterial meningitis, however, can lead to brain damage, hearing loss and even death if not treated properly.

For patients over two years of age, symptoms of meningitis include fever, severe headache, stiff neck, nausea, sleepiness, confusion, and sensitivity to bright lights or seizures. These symptoms may be absent or difficult to detect in newborns and small infants who may only appear slow or inactive, or be irritable, have vomiting or feed poorly.

The Xpert EV test is the first fully-automated medical diagnostic test that isolates and amplifies viral genetic material present in a patient's cerebrospinal fluid by a process called reverse transcription-polymerase chain reaction. The test identifies infection resulting from a class of viruses known as Enterovirus, which are responsible for approximately 90 percent of all viral meningitis cases.

The Xpert EV test is performed by adding the sample directly to a disposable, single-use cartridge. The cartridge is loaded into the GeneXpert DX instrument which then conducts all the necessary laboratory procedures in a one-step, easy-to-use format that helps minimize errors.

The accuracy of the Xpert EV test was confirmed in a multi-site study at six institutions. A total of 255 patient samples were tested and demonstrated that 96 percent of patients who tested positive did have viral meningitis, and that 97 percent of patients who tested negative did not have viral meningitis.

The Xpert EV test was developed by Cepheid, a company located in Sunnyvale, Calif.

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Multicenter Beta Trial of the GeneXpert Enterovirus Assay▽

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Emory University School of Medicine, Atlanta, Georgia[1]; Children's Medical Center and the University of Texas Southwestern Medical Center, Dallas, Texas[2]; Centers for Disease Control and Prevention, Atlanta, Georgia[3]; The Children's Hospital, Denver, Colorado[4]; and Cepheid, Sunnyvale, California[5]

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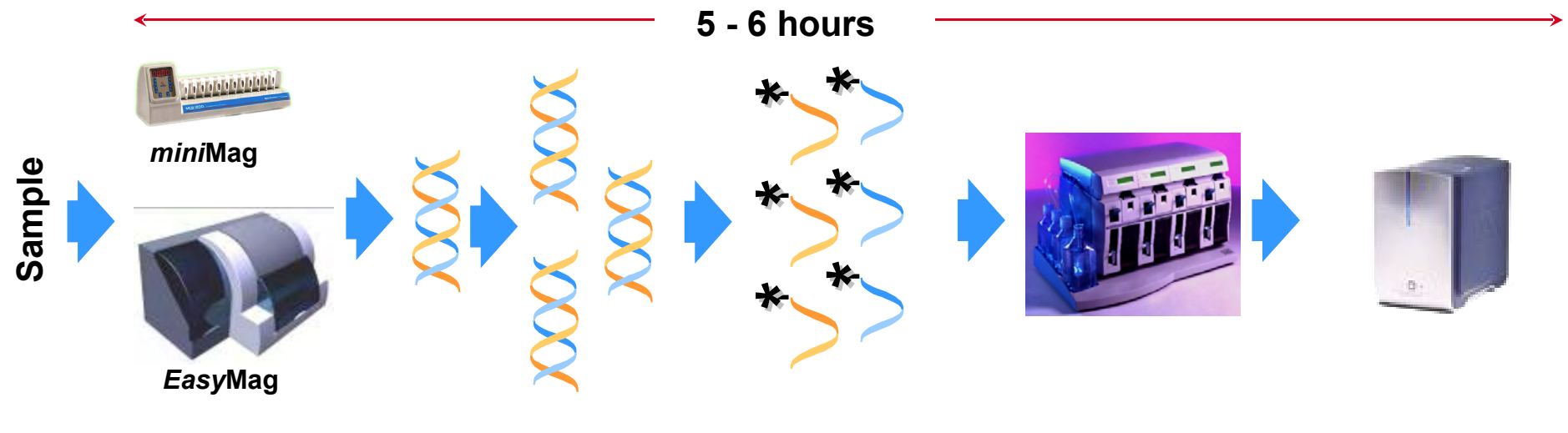
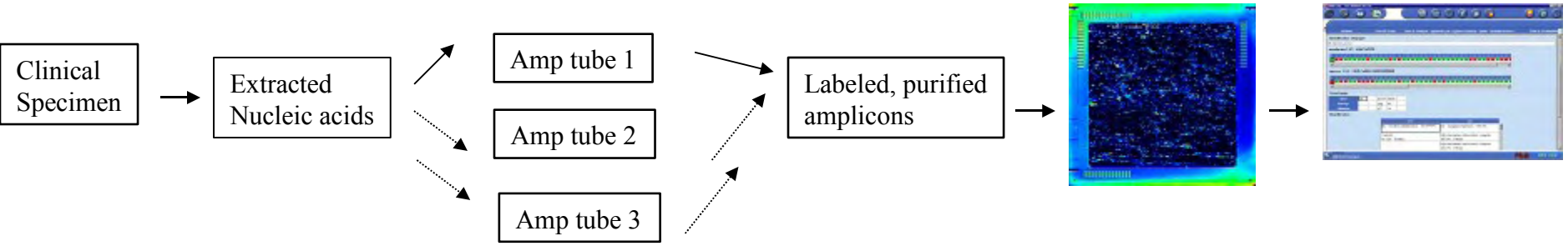
The GeneXpert Dx system (Cepheid, Sunnyvale, CA) is a fully integrated and automated nucleic acid sample preparation, amplification, and real-time detection system. It consists of an instrument, a personal computer, and disposable fluidic cartridges. The analytical sensitivity and specificity of the GeneXpert enterovirus assay (GXEA) were determined with a panel of 63 different enterovirus serotypes and 24 other microorganisms, respectively. The potential for blood, hemoglobin, white blood cells, and excess protein to interfere with the assay was also assessed. The performance parameters of the GXEA were determined at three sites with 102 cerebrospinal fluid (CSF) samples obtained from patients with suspected meningitis. All samples were tested for enterovirus RNA with locally developed reverse transcription-PCR (RT-PCR) assays at the trial sites and with a seminested RT-PCR and an analyte-specific reagent (Cepheid) at a reference laboratory. The 5′ nontranslated region was the target for all of the PCR assays except the seminested RT-PCR, which amplified a VP1 sequence. The VP1 amplicon was sequenced to identify the enterovirus types. Consensus reference laboratory RT-PCR results were used to classify cases of enteroviral meningitis. The GXEA detected all of the enterovirus serotypes and none of the other microorganisms tested except rhinovirus 16. The assay was unaffected by moderate amounts of blood or blood components. Thirty-six (35%) of the CSF samples tested had at least one positive PCR result. Eleven different enterovirus serotypes were identified in the positive samples. The GXEA had a sensitivity of 97.1% (95% confidence interval [CI], 84.7 to 99.9%) and a specificity of 100% (95% CI, 94.6 to 100%) for the diagnosis of enteroviral meningitis.

Futures approches

- Concept de diagnostic étiologique global prenant en compte tous les agents étiologiques potentiellement impliqués dans une infection neuro-méningées
  - PCR multiplex (projet ARGENE)
  - BIOPUCES ou MICROARRAYS (projet BIOMERIEUX-AFFYMETRIX)

bioMérieux microarrays

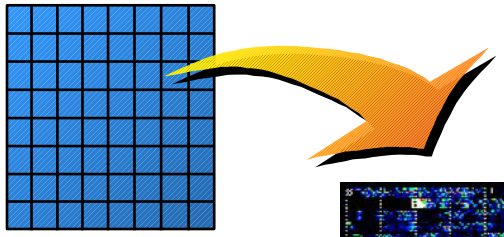




Affymetrix microarrays

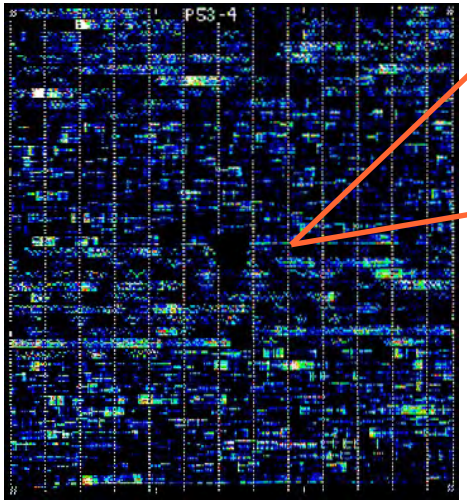
- *In-situ* synthesis of probes using photolithography
- 20-25 bases probes

Wafer and microarray format:



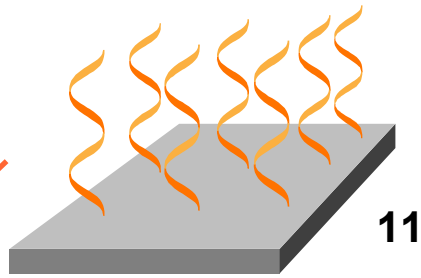
49 to 400 microarrays/wafer

0.5 to 1.3 cm



0.5 to 1.3 cm

>10 000 probes/microarray

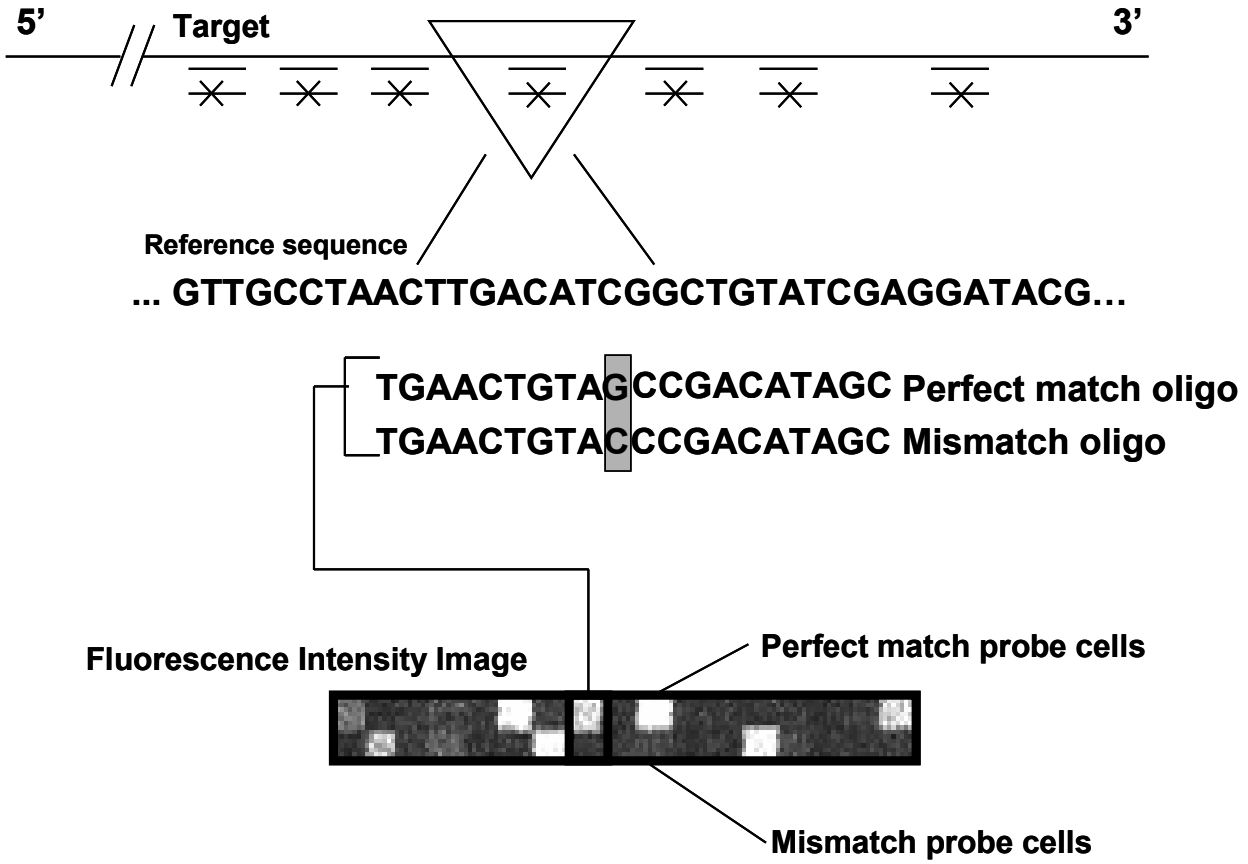


11µm

11µm

Millions of identical probes/feature

Signature sequences and probes



The high density of probes on the microarray allows to represent signature sequences of all viruses, genotypes, subtypes, variants.

Genus	Target	Pathogen	Probes
Herpesvirus	DNA polymerase	HSV 1 et 2, CMV, EBV, VZV et HHV6	4774
Flavivirus	NS5	YF, TBE, Dengue, Rio Bravo, WNV, JE, SLE, Powassan, KF, OHF, MV	9700
Enterovirus	5'NCR	All serotypes	1608
Enterovirus	VP1	Typing	10261
Polyomavirus	Large T-antigen	JC and BK	1240
Paramyxovirus	Nucleoprotein	Measles, mumps	3682
Parasite	B1	*Toxoplasma gondii*	502

In red: amplification optimised.

Genus	**Target**	**Pathogen**
Paramyxovirus	Nucleoprotein	Nipah, Hendra, Tioman, Menangle
Bunyavirus	S segment	Crimée Congo H fever virus and Rift Valley fever virus
Orthopoxvirus	14 kDa protein	Variola, Vaccinia, Camelpox, Cowpox and Monkeypox
Bacteria	16S gene	*Bacillus anthracis*, Y*ersinia pestis*, *Francisella tularensis*, *Clostridium botulinum*, *Chlamydophila psittaci*
Parasite	5S gene	*Pneumocystis carinii*

Specimen type: cerebrospinal fluid

- Extraction with Qiagen DNA Blood Mini kit (1 tube)
- RT-PCR (3 tubes)
  - Herpesviridae: 1 pair of primers for HSV 1, HSV 2 and CMV,
  - Enteroviruses: 1 pair of primers for all serotypes,
  - Flaviviruses: 1 pair of primers for all viruses.
- Labelling + Cleavage (1 tube)
- Hybridization (1 Chip)

Korimbocus et al., JCM, 2005, 43, 3779-87

CONCLUSIONS

- La cytologie (possiblement automatisable grâce à une microcaméra) et les données biochimiques (y compris la procalcitonine notamment) restent la clé pour orienter le diagnostic entre infection bactérienne et virale.
- La biologie moléculaire permet désormais une mise en évidence rapide (2h30) des entérovirus, cause la plus fréquente de méningite.
- La biologie moléculaire peut aider au diagnostic positif des infections bactériennes (mais nécessité de réorganiser les urgences biologiques).
- Dans le futur, possibilité de diagnostic global comportant l'identification, voire le typage de plusieurs agents de façon simultanée
  - par amplification multiplex (PCR, NASBA)
  - par amplification couplée aux biopuces

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