INTERET PRATIQUE DE LA PCR MULTIPLEX « HYBRISPOT HS12 auto »

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Laboratoire de Biologie Médicale
Clinique Internationale Hannibal

I- Les Infections sexuellement transmissibles

- ✓ HPV
- ✓ STD

II- Identification des bactéries et des gènes de résistance

- ✓ AMR
- ✓ MDR
- ✓ Sepsis

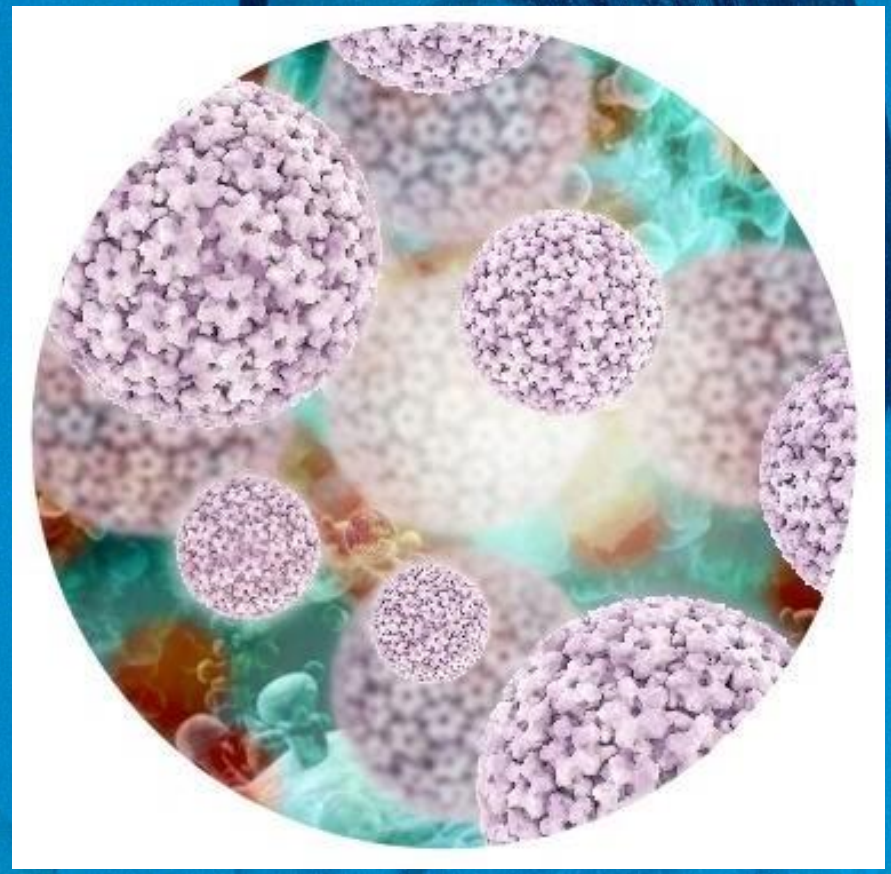
III- Les infections transmises par les tiques et Méningites

- ✓ Tick-born
- ✓ Bacterial CNS

HPV Direct Flow Chip Kit

dna Flow tecnology

Génotypage
De 35 types d'HPV



Organisation mondiale de la Santé

Cancer du col de l'utérus

5 mars 2024

Principaux faits

- **Le cancer du col de l'utérus est le quatrième cancer le plus courant chez la femme dans le monde, avec environ 660 000 nouveaux cas et 350 000 décès liés à cette maladie en 2022.**
- **Les taux d'incidence du cancer du col de l'utérus et la mortalité qui lui est imputable sont plus élevés dans les pays à revenu faible ou intermédiaire. Cette situation témoigne de graves inégalités qui s'expliquent par un accès insuffisant aux services nationaux de vaccination contre le papillomavirus humain (HPV), de dépistage et de traitement du cancer du col de l'utérus, ainsi que par des déterminants sociaux et économiques.**
- **Le cancer du col de l'utérus est causé par une infection persistante par le virus du papillome humain (HPV). Les femmes vivant avec le VIH ont six fois plus de risques de développer un cancer du col de l'utérus que les autres.**
- **La vaccination prophylactique contre le HPV, de même que le dépistage et le traitement des lésions précancéreuses, constituent des stratégies efficaces et bon marché pour prévenir le cancer du col de l'utérus.**
- **Le cancer du col de l'utérus peut être guéri s'il est diagnostiqué à un stade précoce et traité rapidement.**
- **Partout dans le monde, les pays s'attèlent à aboutir plus rapidement à l'élimination du cancer du col de l'utérus dans les prochaines décennies et sont convenus d'un ensemble de trois objectifs à atteindre d'ici 2030.**

Organisation mondiale de la Santé

Action de l'OMS

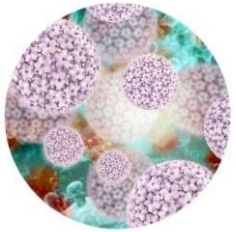
Face au problème de santé publique que constitue le cancer du col de l'utérus causé par une infection à HPV dans le monde, l'Assemblée mondiale de la Santé a adopté dans sa résolution WHA73.2 la Stratégie mondiale en vue d'accélérer l'élimination du cancer du col de l'utérus en tant que problème de santé publique afin que :

- 90 % des filles soient entièrement vaccinées contre le papillomavirus humain à l'âge de 15 ans ;
- 70 % des femmes bénéficient d'un dépistage réalisé à l'aide d'un test de haute performance à l'âge de 35 ans et à nouveau à 45 ans ; et
- 90 % des femmes chez qui une maladie du col de l'utérus a été diagnostiquée reçoivent un traitement (90 % des femmes atteintes de lésions précancéreuses sont traitées ; 90 % des femmes atteintes d'un cancer invasif sont prises en charge).

La prévention des lésions précancéreuses et des cancers associés à une infection à HPV est également un élément clé des Stratégies mondiales du secteur de la santé contre, respectivement, le VIH, l'hépatite virale et les infections sexuellement transmissibles pour la période 2022-2030 adoptées par l'OMS, ainsi que de la résolution WHA74.5 (2021) relative à la santé bucco-dentaire et aux mesures pour lutter contre les cancers de la bouche/gorge.

Le travail de l'OMS à l'échelle mondiale, régionale et nationale, en coopération avec d'autres organisations du système des Nations Unies, vise à :

1. renforcer la volonté politique en vue d'élaborer des politiques et d'appuyer leur mise en œuvre,
2. fournir une assistance technique en fonction du contexte, des enseignements tirés et des meilleures pratiques,
3. établir des normes et des critères fondés sur les données les plus récentes,
4. diriger l'écosystème mondial de la santé pour atteindre les cibles et améliorer la qualité des soins.



HPV Flow Chip
HPV Direct Flow Chip Kit

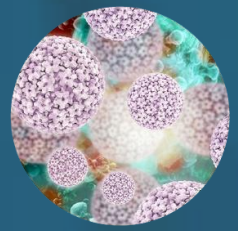
Détection simultanée et génotypage de **35 HPV**

B	33	58	42	71	16	52	B	
B	35	59	43	72	18	53	6	69
C	39	66	44/55		26	56	11	70
U	45	68	54	84	31	58	40	71
16	51	73	61	B	33	59	44/55	72
18	52	82	62/81	C	35	66	54	
26	53	6	67	U	39	68	61	84
31	56	11	69	42	45	73	62/81	
	B	40	70	43	51	82	67	

Haut-risque: 16, 18, 26, 31, 33, 35, **39**, 45, 51, 52, **53**, 56,58, 59, 66, 68, **73, 82.**

Bas-risque: 6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84.

- Amplification de l'ORF L1 of
- Controle B d'hybridation
- Controle C d'amplification
- Controle universel U (HPV specific) Présence du HPV DNA

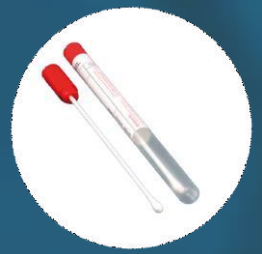


HPV Flow Chip

HPV Direct Flow Chip Kit

Broad range of validated **sample types**

dna Flow tecnology



Écouvillons

Cervicale

Et anale

Liquid-based cytology

Thinprep(Hologic)

Superpath (Becton Dickinson)

Novaprep (Novacyt)

CellPrep (Biodyne)

CY-PRER™ Pap Test (FJORD Diagnostics)

HURO PATH Cell-Preserve Solution (Celtrazone)

Tissu Paraffiné

VEIL

HPV Direct Flow Chip Kit

LOTS

PCR:	HPVP016AL-4	3/30/2024
Chips:	HPVH0106E	3/30/2024
Reagent:	HPVH0106E	3/30/2024

SAMPLE DETAILS

ID SAMPLE: SAMPLE4HHT **SAMPLE TYPE:**

ID PATIENT: **PATIENT:**

SEX: - **BIRTHDATE:** **AGE:**

REPORT

HPV POSITIVE

Positive sample for:

High-Risk:

53*

Low-Risk:

6

Note: Insufficient Material.

The sample is negative for the rest of genotypes included in the HPV direct flow chip test.

(*) Included when marking points manually.

PROTOCOL

Detection and genotyping of HPV viral DNA by PCR and reverse dot blot hybridization:

- High risk genotypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82.
- Low risk genotypes: 6, 11, 40, 42, 43, 44/55, 54, 61, 62/81, 67, 69, 70, 71, 72, 84.

Sample preparation/DNA purification

Add cell suspension/purified DNA for PCR amplification:

- PCR protocol (standard) HPV Direct Flow Chip: 1x 25°C 10 min, 1x 94°C 3min; 15x94-42-72°C (30"-30"-30"), 35x 94-60-72°C (30"-30"-30"), 1x 72°C 5 min.
- PCR protocol (lyophilized) HPV Direct Flow Chip: 1x 25°C 10 min, 1x 94°C 3min; 15x94-47-72°C (30"-30"-30"), 35x 94-65-72°C (30"-30"-30"), 1x 72°C 5 min.

REVERSE-DOT BLOT protocol:

- Hybridization of the biotinilated PCR products to the HPV CHIP.
- Post-hybridization washes.
- Streptavidin-Alkaline Phosphatase incubation.
- NBT-BCIP development.

Automatic analysis of results

HPV Direct Flow Chip Kit

LOTS

PCR:	HPVP016AL-4	3/30/2024
Chips:	HPVH0106E	3/30/2024
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ID PATIENT: **PATIENT:**

SEX: - **BIRTHDATE:** **AGE:**

REPORT

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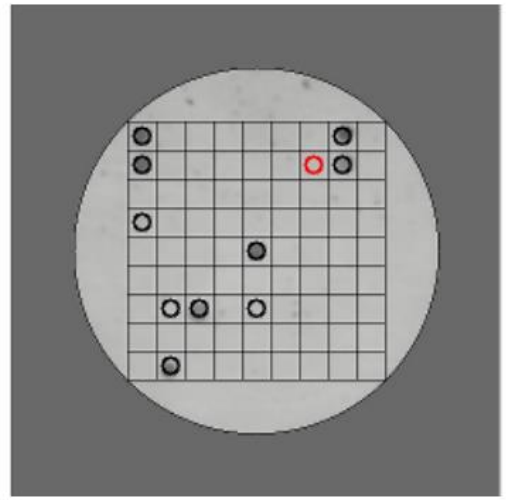

- Spot B: Hybridization control (5 signals to orientate the CHIP)
- Spot C: Internal DNA control (Genomic human DNA probe)
- Spot U: HPV Universal probe
- Spot #: Genotype specific probes

All the spots are printed in duplicate.

Exemple de Trouble shooting

vitro master diagnóstica®

HPV Direct Flow Chip Kit

LOTS

PCR:

Chips:

Reagent:

SAMPLE DETAILS

ID SAMPLE: nafissataessaie **SAMPLE TYPE:**

ID PATIENT: **PATIENT:**

SEX: - **BIRTHDATE:** **AGE:**

REPORT

BLANK

Inappropriate material.

Insufficient Material.

PCR inhibited.

- ✓ Matériel inapproprié
- ✓ Materiel insuffisant
- ✓ PCR inhibée

PROTOCOL

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- NBT-BCIP development.

Automatic analysis of results

vitro master diagnóstica®

HPV Direct Flow Chip Kit

LOTS

PCR:

Chips:

Reagent:

SAMPLE DETAILS

ID SAMPLE: nafissataessaie **SAMPLE TYPE:**

ID PATIENT: **PATIENT:**

SEX: - **BIRTHDATE:** **AGE:**

REPORT

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B	35	59	43	72	18	53	6	69
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16	51	73	61	B	33	59	44/55	72
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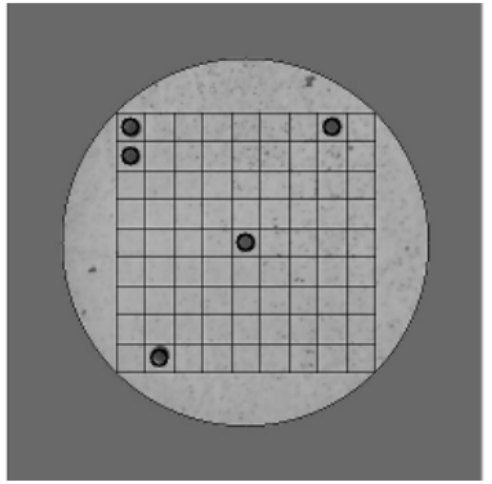

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- Spot C: Internal DNA control (Genomic human DNA probe)
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- Spot #: Genotype specific probes

All the spots are printed in duplicate.

ANALYSIS INFORMATION

Journal of Virological Methods 193 (2013) 9–17

Contents lists available at SciVerse ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

HPV Direct Flow CHIP: A new human papillomavirus genotyping method based on direct PCR from crude-cell extracts☆

Elsa Herraez-Hernandez[a,*], Martina Alvarez-Perez[b], Gloria Navarro-Bustos[c], Javier Esquivias[d], Sonia Alonso[e], Jose Aneiros-Fernandez[f], Cesar Lacruz-Pelea[g], Magdalena Sanchez-Aguera[h], Javier Saenz Santamaria[i], Jesus Chacon de Antonio[j], Jose Luis Rodriguez-Peralto[k]

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In conclusion, comparative results obtained in this pilot study demonstrated that the performance of HPV Direct Flow CHIP is similar to that of LA, CLART, and HC2. Given that it offers direct PCR from clinical specimens without a DNA purification step, this novel test may be a valuable tool for automated, rapid, and sensitive HPV genotyping, especially in large-scale vaccine surveillance and epidemiology studies.

International Journal of Infectious Diseases 111 (2021) 295–302

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Prevalence and Genotype Distribution of Human Papillomavirus Infection among 12 076 Iranian Women ☆

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Soheil
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[4] Tehran U
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ARTI

Article his
Received
Revised 2
Accepted

Keywords:
Cervical c
Human p
Iranian p
Genotype

Conclusions

It has been suggested that HPV can become a dynamic threat. In the context of protective and preventive methods for HPV infection, the present study highlights the genotype distribution of this infection in the Iranian population. Determining the HPV prevalence and the distribution of specific genotypes in a large population of Iranian people can improve health policies implemented by government and health agencies. The results obtained from the present study may be useful for policymakers to specify cost-effective interventions and recommendations to improve national immunization against HPV and CC.

STD Direct Flow Chip Kit

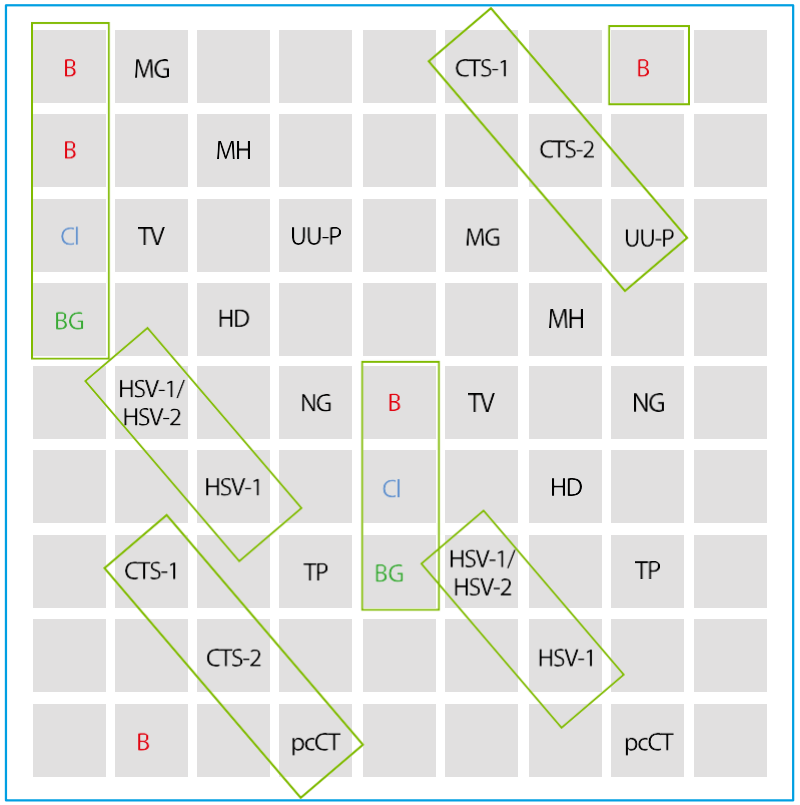
dna Flow tecnology

Détection de 11 pathogènes

STD Flow Chip - 11 STD related pathogens

ISTs–

Détection simultanée de 11 agents pathogènes



pcCT	*Chlamydia trachomatis*	CT-S1: Biovar Trachoma: Serovars A-K
		CT-S2: Biovar LGV: Serovars L1-L3
HD	*Haemophilus ducreyi*	
HSV-1/HSV-2 + HSV-1	Herpes simplex virus 1	
HSV-1/HSV-2	Herpes simplex virus 2	
MG	*Mycoplasma genitalium*	
MH	*Mycoplasma hominis*	
NG	*Neisseria gonorrhoeae*	
TP	*Treponema pallidum*	
TV	*Trichomonas vaginalis*	
UU-P	*Ureaplasma (urealyticum/parvum)*	

STD Flow Chip - 11 STD related pathogens

STIs – scope of the problem

11 pathogènes

Écouvillonage
Urètre
Endocervical
Vaginal

Anale et gorge

Cytologie endocervicale en milieu liquide

sperme Urine

VEIL

> Eur J Clin Microbiol Infect Dis. 2020 Feb;39(2):235-241. doi: 10.1007/s10096-019-03686-w. Epub 2020 Jan 4.

Detection of sexually transmitted disease–causing pathogens from direct clinical specimens with the multiplex PCR–based STD Direct Flow Chip Kit

Antonio Barrientos-Durán [#1], Adolfo de Salazar [#1], Marta Alvarez-Estévez [1], Ana Fuentes-López [1], Beatriz Espadafor [2], Federico Garcia [3]

Affiliations + expand

PMID: 31902016 DOI: 10.1007/s10096-019-03686-w

Abstract

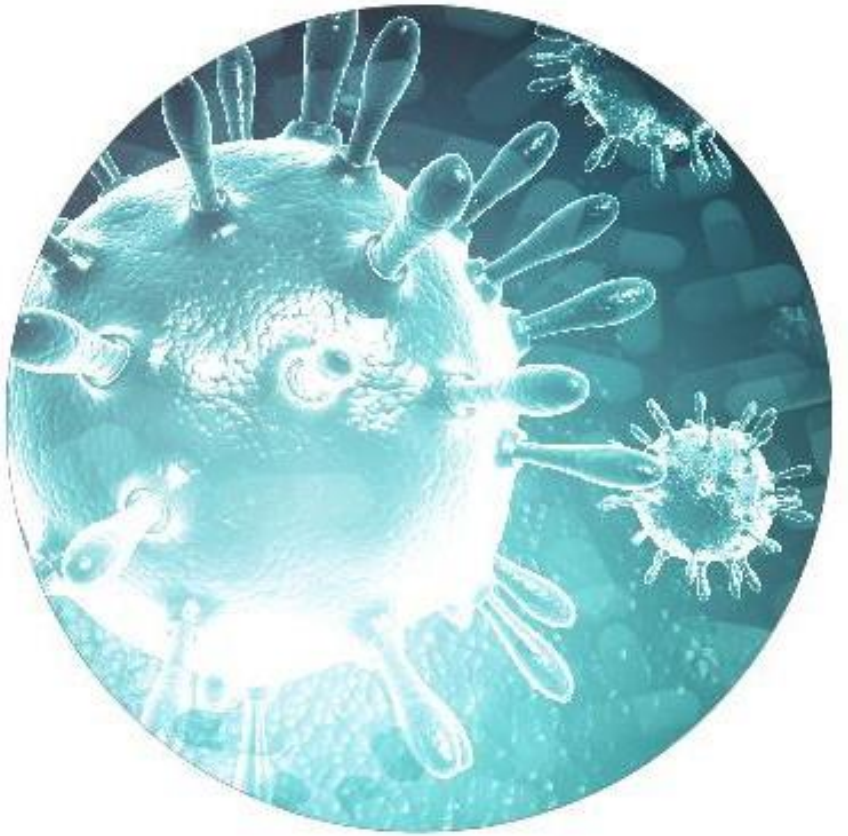
Pathogens causing sexually transmitted diseases (STDs) include viruses, bacteria, and parasites. The ability to rapidly and efficiently detect these pathogens in a single reaction still remains a health challenge. The aim of this study was to evaluate the clinical reliability and accuracy of the STD Direct Flow Chip Kit (Vitro, IVD-EC approved), which can simultaneously detect up to 9 different species of STD pathogens at once. This kit enables direct analysis-direct-PCR-of clinical specimens (urine, semen, endocervical, urethral, nasopharyngeal, and perianal swabs) without DNA purification for the following pathogens: Chlamydia trachomatis (serovars A-K and L1-L3), Haemophilus ducreyi, Herpes Simplex Virus (Types I and II), Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Treponema pallidum, Trichomonas vaginalis, and Ureaplasma. The Anyplex™ II STI-7 Detection Kit (Seegene, IVD-EC) was used as the reference's method. Existing discordances were resolved using either a third molecular assay or DNA sequencing. Clinical performance was evaluated at two different stages: (i) from purified DNA of three hundred and fifty-eight clinical specimens with a diagnostic sensitivity (SE) and specificity (SP) of 99.4% and 100%, respectively, and an agreement of 99% (kappa index, κ = 0.97) with the reference's method and; (ii) by direct-PCR from six hundred and thirty-three specimens rendering SE, SP, and agreement values of 98.4%, 99.9%, and 98.0% (κ = 0.95), respectively. The STD Direct Flow Chip Kit constitutes a promising alternative to routine procedures in diagnostic, allowing direct analysis of specimens and enabling the detection of a broad panel of pathogens.

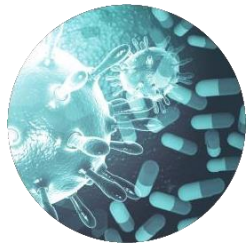
Keywords: Clinical specimens; DNA: DNA hybridization; Direct analysis; Multiplex-PCR based; Sexually transmitted diseases.

AMR Direct Flow Chip Kit

dna Flow tecnology

Détéction de 20 gènes de résistance aux ATB





AMR Flow Chip - 20 antibiotic resistance genes

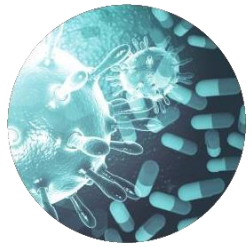
AMR Flow Chip Kit

Détection simultanée de **20 gènes de résistances aux ATB** présents chez les bactéries Gram-positif et Gram-négatif

B			kpc	spm			vanB	blaSHV-S	B
B			sme	ndm			vanA	ges	oxa23 _like
CI			nmc/imi	sim			mecA	vim	oxa24 _like
BG				imp_like				gim	oxa48 _like
			blaSHV	blaSHV-S				kpc	oxa51 _like
	SA		blaCTX	blaSHV -SK	B			spm	oxa58 _like
			ges	oxa23 _like	CI			sme	ndm
			vim	oxa24 _like	BG			nmc/imi	sim
		mecA	gim	oxa48 _like				blaSHV -SK	imp_like
		vanA		oxa51 _like		SA		blaSHV	
	B	vanB		oxa58 _like				blaCTX	

- SA: Staphylococcus aureus
- mecA: Methicillin resistance gene
- vanA: Vancomycin resistance gene
- vanB: Vancomycin resistance gene
- KPC: Class A carbapenemase
- SME: Class A carbapenemase
- NMC/IMI: Class A carbapenemase
- blaSHV: extended-spectrum ß-lactamase CTX-M
- GES: Class A carbapenemase
- VIM: Class B carbapenemase
- GIM: Class B carbapenemase
- SMP: Class B carbapenemase
- NDM: Class B carbapenemase
- SIM: Class B carbapenemase
- IMP: Class B carbapenemase IMP3, 15, 19_like
- Oxa 23: Class D carbapenemase OXA23_like
- Oxa 24: Class D carbapenemase OXA24_like
- Oxa 48: Class D carbapenemase OXA48_like
- Oxa 51: Class D carbapenemase OXA51_like
- Oxa 58: Class A carbapenemase OXA58_like

B: Hybridization control
CI: Exogenous amplification control
BG: Endogenous amplification control (ß-globin human fragment)



AMR Flow Chip - 20 antibiotic resistance genes

AMR Flow Chip Kit

Détection simultanée de **20 gènes de résistances aux ATB** présents chez les bactéries Gram-positif et Gram-négatif

Carbapenemase class	Gene	Detected Allelic variant
A	ges	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26
	sme	1, 2, 3, 4 and 5
	kpc	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 and 23
	nmc/imi	1, 2, 3, 4, 5, 6, 7, 8 and 9
B	sim	sim
	gim	1 and 2
	spm	spm
	ndm	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16
	vim	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 and 46
	imp	1, 2, 3, 5, 6, 8, 9, 10, 11, 15, 19, 20, 21, 24, 25, 28, 29, 30, 40, 41, 42 and 47
D	oxa-23-like	23, 27, 49, 73, 133, 146, 165, 166, 167, 168, 169, 170, 171 and 225
	oxa-24-like	24, 25, 26, 40, 72, 139 and 160
	oxa-48-like	48, 162, 163 and 181
	oxa-51-like	51, 60, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 82, 83, 84, 88, 89, 90, 91, 92, 93, 94, 95, 98, 99, 106, 107, 108, 109, 110, 111, 112, 113, 115, 116, 117, 128, 130, 131, 132, 138, 144, 148, 149, 150, 172, 173, 174, 175, 176, 177, 178, 179, 180, 195, 196, 197, 194, 200, 201 202, 203, 206, 208 and 223
	oxa-58-like	58, 96, 97 and 164

- Panel de carbapénèmases le plus étendu
- Identification de 15 gènes et détection de plus de 240 variants alléliques

AMR Flow Chip - 20 antibiotic resistance genes

AMR Flow Chip Kit

dna Flow tecnology

Hémocultures positives

Colonies bactériennes

Exsudat
Rectale
Nasopharyngée

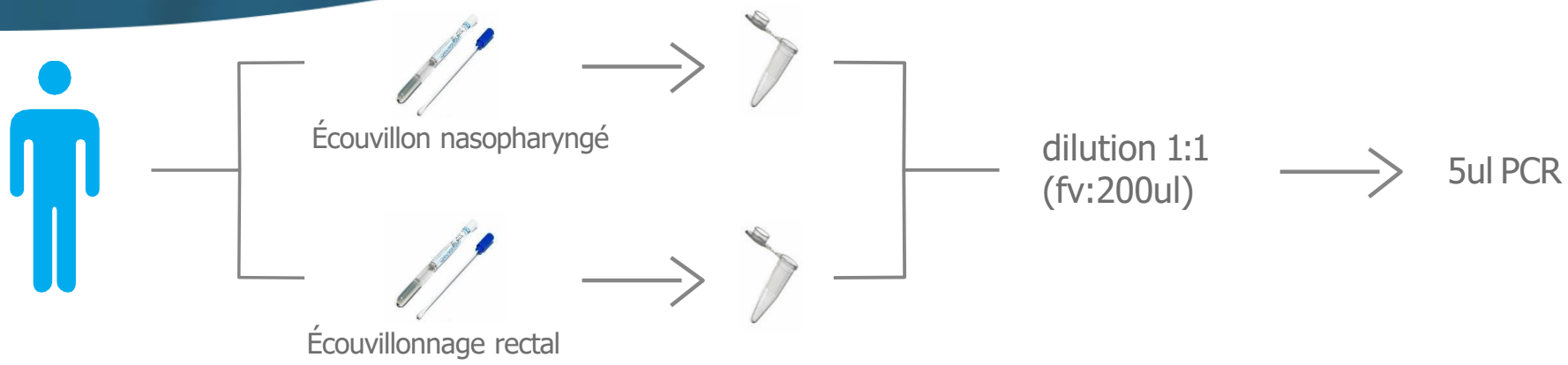
AMR Flow Chip - 20 antibiotic resistance genes

AMR Flow Chip Kit

Ecouvillons nasopharyngés et rectaux dans un seul tube/puce PCR

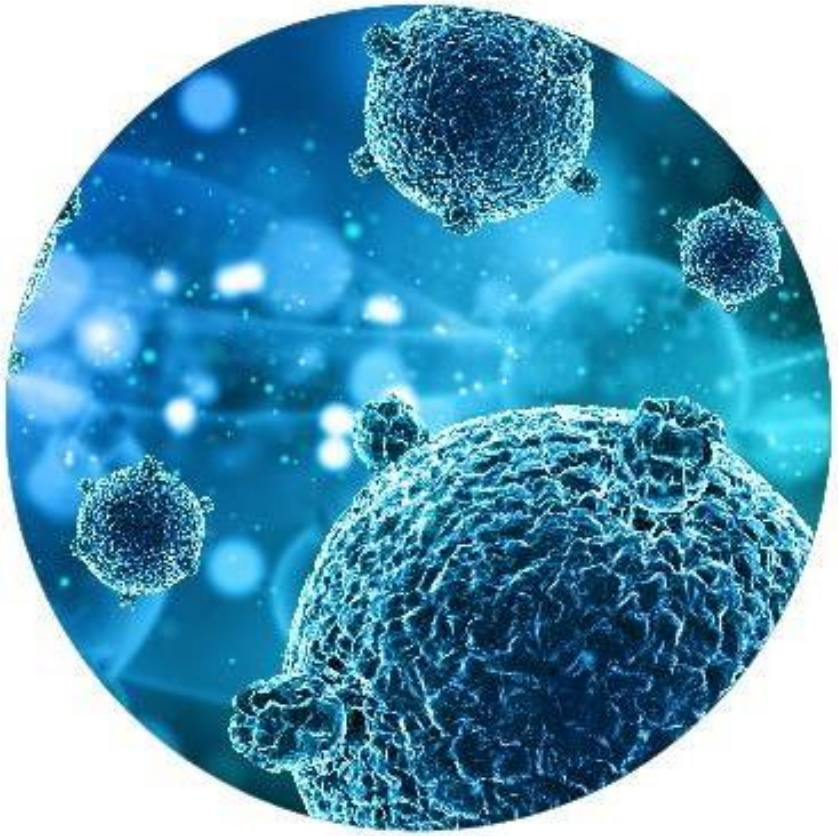
Exsudats rectaux

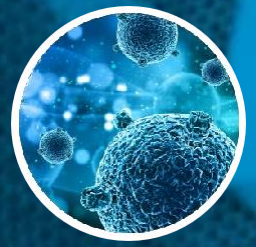
- Nasopharyngée



MDR Direct Flow Chip Kit

dna Flow tecnology





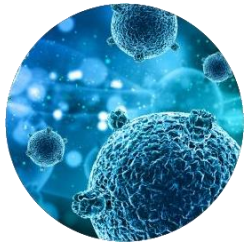
MDR Flow Chip |5 bacterial species +56 resistance markers

MDR Flow Chip Kit

5 espèces bactériennes + 56 mécanismes de résistance

- Staphylococcus aureus
- Escherichia coli
- Klebsiella pneumoniae
- Pseudomonas aeruginosa
- Acinetobacter baumannii

dna Flow tecnology



MDR Flow Chip |5 bacterial species +56 resistance markers

MDR Flow Chip Kit

5 espèces bactériennes + 56 mécanismes de résistance

aac (6´)-Ib	Aminoglycosides
arma	
rmtB	
rmtC	
rmtF	
blaCMY	β-lactam antibiotics
blaDHA	
blaSHV-SK	Cephalosporins
blaSHV-S	
catB3	Chloramphenicol
Mcr1	Colistin
Mcr2	

gyrE-S83L	Quinolones
gyrE-S83L-D87G	
gyrE-S83L-D87G, parES80I	
gyrE-S83L-D87N	
gyrE-S83W-D87G	
gyrP-T83I	
gyrP-T83I-D87G	
gyrP-T83I-D87N	
parE-S80I	
cfr	Macrolides / lincosamide / streptogramin
ermA	Macrolides
ermB	
ermC	
mefA/E	
msrA	

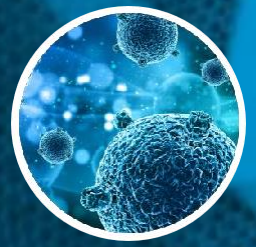
Oxacilina-mecA
Vancomicina
- vanA
- vanB

β-lactam antibiotic resistance
- blaSHV
- blaCTX-M

Carbapenems
- kpc
- sme
- nmc/imi
- ges
- vim
- gim
- spm

- ndm
- sim
- imp
- oxa23_like
- oxa24_like
- oxa48_like
- oxa51_like
- oxa58_like

oqxA	Phenicol/quinolone
oqxB	
qnrA	
qnrB	Quinolones
qnrS	
sul1	Sulfonamides
sul2	
sul3	

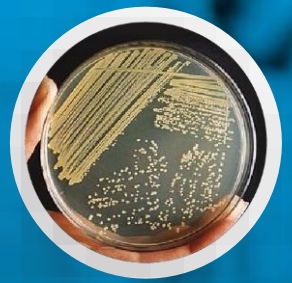


MDR Flow Chip |5 bacterial species +56 resistance markers

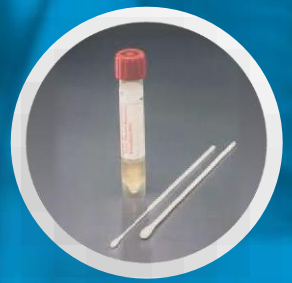
MDR Flow Chip Kit

5 espèces bactériennes+ 56 marqueurs de résistance

Hémoculture positives



Colonies bactériennes



Rectal Exudates

Rectal

Nasopharyngeal

› Enferm Infecc Microbiol Clin (Engl Ed). 2021 Jun-Jul;39(6):276-278. doi: 10.1016/j.eimce.2020.05.014.

Evaluation of the "AMR Direct Flow Chip Kit" DNA microarray for detecting antimicrobial resistance genes directly from rectal and nasopharyngeal clinical samples upon ICU admission

Efthymia Protonotariou [1], Georgios Meletis [2], Dimitra Papadopoulou [2], Melania Kachrimanidou [2], Lilian Toptsi [2], Lemonia Skoura [2]

respectively).

Conclusion: The AMR Direct Flow Chip Kit is a useful alternative to phenotypic testing for rapid detection of resistance markers.

Keywords: ADN micromatriz; Carbapenemasas; Carbapenemases; DNA microarray; ESBLs; Genotypic resistance; MRSA; Resistencia genotípica; mecA.

Enferm Infecc Microbiol Clin. 2019;37(7):454–457

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Brief report

Evaluation of the DNA microarray "AMR Direct Flow Chip Kit" for detection of antimicrobial resistance genes from Gram-positive and Gram-negative bacterial isolated colonies

Ignacio Torres Fink[a], Nuria Tormo Palop[b], Rafael Borrás Salvador[a,c], Javier Buesa Gómez[a,c], Concepción Gimeno Cardona[b,c], David Navarro Ortega[a,c,*]

[a] Microbiology Service, Hospital Clínico Universitario, Fundación INCLIVA, Valencia, Spain
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ARTICLE INFO

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Keywords:
DNA microarray
Genotypic resistance
Carbapenemases
Extended-spectrum β-lactamases
mecA
vanA
vanB

ABSTRACT

Introduction: The AMR Direct Flow Chip assay allows the simultaneous detection of a large variety of antibiotic resistance genetic markers. To assess this kit's performance, we use isolated colonies as starting material. The assay has been approved by the European Economic Area as a suitable device for *in vitro* diagnosis (CE IVD) using clinical specimens.
Methods: A total of 210 bacterial isolates harbouring either one or more antimicrobial resistance genes including plasmid-encoded extended-spectrum β-lactamases (SHV, CTX-M) and carbapenemases (GES, SME, KPC, NMC/IMI, SIM, GIM, SPM, NDM, VIM, IMP, and OXA), *mecA*, *vanA* and *vanB*, and 30 controls were included.
Results: The assay displayed a sensitivity and specificity of 100% for all target genes included in the array.
Conclusion: The AMR Direct Flow Chip Kit is an accurate assay for detecting genes which commonly confer resistance to β-lactams and vancomycin from isolated colonies in culture of Gram-positive and Gram-negative bacteria.

vitro
master diagnóstica®

MDR Flow Chip Kit

LOTS

PCR:

Chips:

Reagent:

SAMPLE DETAILS

vitro
master diagnóstica®

MDR Flow Chip Kit

LOTS

PCR:

Chips:

Reagent:

SAMPLE DETAILS

ID SAMPLE: hicharf2 SAMPLE TYPE:

MDR POSITIVE

Positive sample for:

Bacteria:

Klebsiella pneumoniae, Acinetobacter baumannii

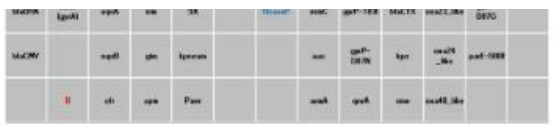
Antibiotic Resistance:

Methicillin resistance gene (mecA), ß-lactamase SHV, Extended-spectrum ß-lactamase CTX-M, Carbapenemase NDM, Carbapenemase OXA23_like, Carbapenemase OXA24_like, Carbapenemase OXA48_like, Carbapenemase OXA51_like, Sulfonamides resistance gene (sul-1), Macrolides resistance gene (msrA), Macrolides resistance gene (ermA), Aminoglycosides resistance gene (aac(6´)-Ib), Aminoglycosides resistance gene (armA), Quinolones or fluoroquinolones resistance gene (qnrB), Olaquindox resistance gene (oqxA)

mut. gyrE-S83L, mut. gyrE-S83L-D87G, mut. gyrE-S83L-D87N, mut. gyrE-S83W-D87G, mut. gyrP-T83I, mut. gyrP-T83I-D87N, mut. gyrP-T83I-D87G, mut. parE-S80I, qnrA, qnrB, qnrS, oqxA, oqxB, cfr, catB3, mecA, mecC, vanA, vanB, blaSHV, blaCTX-M, KPC, SME, NMC-IMI, GES, VIM, GIM, SPM, NDM, SIM, IMP, blaSHV-S (mut. G238S), blaSHV-SK (mut. G238S y E240K), OXA23, OXA24, OXA48, OXA51, OXA58, mcr-2.

- Sample preparation/DNA purification:
- Add suspension of DNA (prepared according manufacturer´s instructions) for PCR amplification.
- PCR protocol: 1x [25°C, 10 min]; 1x [95°C, 3 min]; 40x [95°C, 10 s -55°C, 30 s - 72°C, 30min]; 1x [8°C, ∞].
- REVERSE-DOT BLOT protocol:

Hybridization of the biotinylated PCR products to the MDR CHIP, Post-hybridization washes, Streptavidin-Alkaline Phosphatase incubation, NBT-BCIP development and Automatic analysis of results.



*Spot"B": Hybridization control (5 signals to orientate the CHIP)
*Spot "CI-1": Amplification control for reaction mixture Mix-1.
*Spot "CI-2": Amplification control for reaction mixture Mix-2.
*Spot "RNaseP": DNA Control for reaction mixture Mix-1.
*Spot "BG": DNA Control for reaction mixture Mix-2.
*Spot "#":Pathogen specific probes.

MICROBIAL DRUG RESISTANCE
Volume 00, Number 00, 2023

DOI: 10.1089/mdr.2023.0019

Colonization of Residents and Staff of an Italian Long-Term Care Facility and an Adjacent Acute Care Hospital Geriatrics Unit by Multidrug-Resistant Bacteria

Maria Teresa Nitti,[1] Ferisa Sleghel,[1] Malgorzata Kaczor,[1] Richard Aschbacher,[2] Elena Moroder,[2] Angela Maria Di Pierro,[2] Francesca Piscopiello,[3] Melissa Spalla,[3] Aurora Piazza,[3] Roberta Migliavacca,[3] and Elisabetta Pagani[2]

In 2022, we undertook a point prevalence screening study for *Enterobacterales* with extended-spectrum β-lactamases (ESBLs), high-level AmpC cephalosporinases and carbapenemases, and also methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) in a long-term care facility (LTCF) and the associated acute-care hospital Geriatrics unit in Bolzano, Northern Italy. Urine samples and rectal, inguinal, oropharyngeal, and nasal swabs were plated on selective agar plates. Metadata of the patients, including demographic data, were collected, and risk factors for colonization were determined. ESBL, AmpC, carbapenemase, and quinolone resistance genes were investigated by the HybriSpot 12 PCR AUTO System. The following colonization percentages by multidrug-resistant (MDR) bacteria have been found in LTCF residents: all MDR organisms, 59.5%; ESBL producers, 46.0% (mainly CTX-M-type enzymes); carbapenemase producers, 1.1% (one *Klebsiella pneumoniae* with KPC-type); MRSA, 4.5%; VRE, 6.7%. Colonization by MDR bacteria was 18.9% for LTCF staff and 45.0% for Geriatrics unit patients. Peripheral vascular disease, the presence of any medical device, cancer, and a Katz Index of 0 were significant risk factors for colonization of LTCF residents by MDR bacteria in univariate and/or multivariate regression analysis. To conclude, the ongoing widespread diffusion of MDR bacteria in the LTCF suggests that efforts should be strengthened on MDR screening, implementation of infection control strategies, and antibiotic stewardship programs targeting the unique aspects of LTCFs. ClinicalTrials.gov ID: 0530250-BZ Reg01 30/08/2022.

Keywords: long-term care facility, AmpC, ESBLs, carbapenemases, MRSA, VRE, *Enterobacterales*

MICROBIAL DRUG RESISTANCE
Volume 00, Number 00, 2023

DOI: 10.1089/mdr.2022.0264

Evaluation of the MDR Direct Flow Chip Kit for the Detection of Multiple Antimicrobial Resistance Determinants

Ángel Rodríguez-Villodres,[1–3] Antonio Galiana-Cabrera,[4] Ignacio Torres Fink,[5] Rosario Duran Jiménez,[1] José Miguel Cisneros,[1–3,6] and José Antonio Lepe[1–3,7]

The objective of this study was to evaluate the accuracy of the MDR Direct Flow Chip Kit for the detection of antimicrobial resistance (AMR) determinants from bacterial colonies. Ninety-two clinical isolates with known AMR determinants genotypically characterized were used. The MDR Direct Flow Chip Kit is a microarray-based assay that included 55 AMR determinants for beta-lactams (23), quinolones (13), aminoglycosides (5), macrolides (5), sulfonamides (3), colistin (2), vancomycin (2), chloramphenicol (1), and linezolid (1). The MDR Direct Flow Chip Kit correctly detects 52 of 53 AMR determinants tested. The *cfr* gene (linezolid resistance) was not detected. The global sensibility, specificity, positive predictive value, and the negative predictive value calculated were 98%, 100%, 100%, and 97%. The Cohen's Kappa coefficient calculated was 0.97 [95% Confidence Interval (0.90–1.03)]. In conclusion, the MDR Direct Flow Chip is an accurate assay for the detection of multiple AMR determinants in one simple reaction.

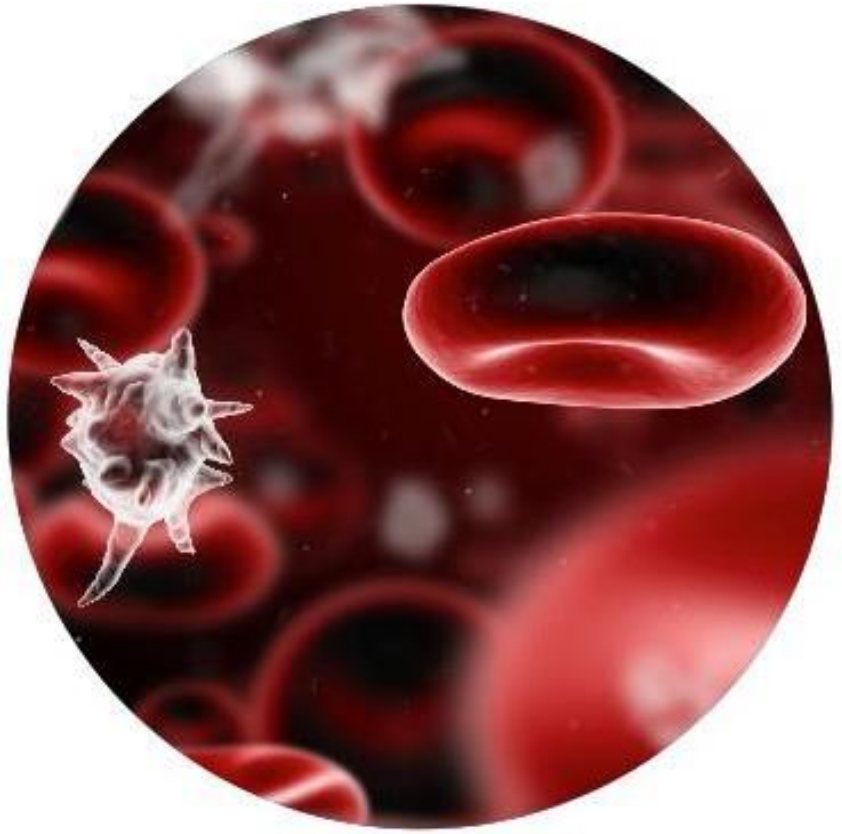
Keywords: antimicrobial resistance, rapid detection, microarray

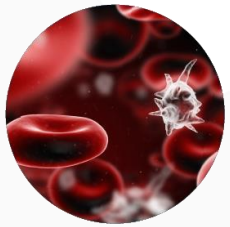
Sepsis Direct Flow Chip Kit

dna Flow tecnology

Détection simultanée de plus de

- ✓ 36 espèces de bactéries
- ✓ de 20 gènes de résistance





Sepsis Flow Chip |36 pathogens +20 antibiotic resistance genes

Sepsis Direct Flow Chip Kit

dna Flow tecnology

Bactéries Gram-positif

Staphylococcus Coagulase-Negative
- S. epidermidis
- S. haemolyticus
- S. capitis
- S. hominis-hominis
- S. intermedius

Staphylococcus aureus

Streptococcus spp.
- S. pasteurianus
- S. dysgalactiae
- S. gallolyticus
- S. macedonicus
- S. mitis/oralis
- S. salivarius
- S. infantarium
- S. pyogenes
- S. intermedius

Streptococcus pneumoniae

Streptococcus agalactiae

Streptococcus pyogenes

Listeria monocytogenes

Enterococcus spp.
- E. faecalis
- E. faecium

Bactéries Gram-négatif

Pseudomonas aeruginosa
Acinetobacter baumannii
Neisseria meningitidis
Stenotrophomonas maltophilia
Escherichia coli
Klebsiella pneumoniae
Serratia marcescens
Enterobacteriaceae
E. aerogenes
E. cloacae
K. oxytoca
K. pneumoniae
Morganella morganii
E. coli
S. marcescens
Citrobacter
Salmonella enterica
Proteus spp./Morganella

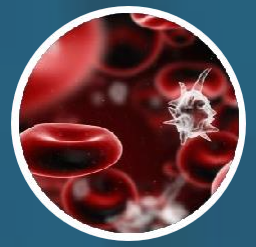
champignons

Candida albicans

Candida spp
- C. tropicalis
- C. parapsilosis
- C. krusei

Marqueurs de résistance aux antibiotiques

Oxacilina-mecA	β-lactam antibiotic resistance	Carbapenems	ges	ndm	oxa24_like
Vancomicina		kpc	vim	sim	oxa48_like
vanA	blaSHV	sme	gim	imp	oxa51_like
vanB	blaCTX-M	nmc/imi	spm	oxa23_like	oxa58_like



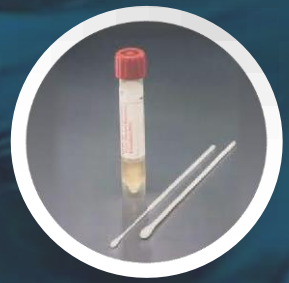
Sepsis Flow Chip |36 pathogens +20 antibiotic resistance genes

Sepsis Direct Flow Chip Kit

Large gamme de types d'échantillons

Hémocultures positives

Colonies bactériennes



Rectal Exudates
Exsudats rectaux

PLOS ONE

RESEARCH ARTICLE

Evaluation of the Sepsis Flow Chip assay for the diagnosis of blood infections

Antonio Galiana[1], Javier Coy[2], Adelina Gimeno[2], Noemi Marco Guzman[2], Francisco Rosales[2], Esperanza Merino[3], Gloria Royo[1], Juan Carlos Rodriguez[2]*

1 Department of Microbiology, Hospital General Universitario de Elche, Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunidad Valenciana (FISABIO) Elche, Spain, 2 Department of Microbiology, Hospital General Universitario de Alicante, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL - FISABIO), Alicante, Spain, 3 Department of Infectious Diseases, Hospital General Universitario de Alicante, Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL - FISABIO), Alicante, Spain

* rodriguez_juadia@gva.es

OPEN ACCESS

Citation: Galiana A, Coy J, Gimeno A, Guzman MM, Rosales F, Merino E, et al. (2017) Evaluation of the Sepsis Flow Chip assay for the diagnosis of blood

Abstract

Background

Blood infections are serious complex conditions that generally require rapid diagnosis and treatment. The big challenge is to reduce the time necessary to make a diagnosis with current clinical microbiological methods so as to improve the treatment given to patients.

Conclusions

This is the first evaluation of SFC assay in clinical samples. This new method appears to be very promising by combining the high number of distinct pathogens and genetic resistance determinants identified in a single assay. Further investigations should be done to evaluate the usefulness of this assay in combination with clinical multidisciplinary groups

Funding: This work was supported by Hospital General Universitario de Alicante (UGP-14-270) http://alicante.san.gva.es/; Fundación Soria Melguizo (no number) http://www.f-soria.es/; and FISABIO (UGP-14-216) http://fisabio.san.gva.es/.

Competing interests: The authors have declared that no competing interests exist.

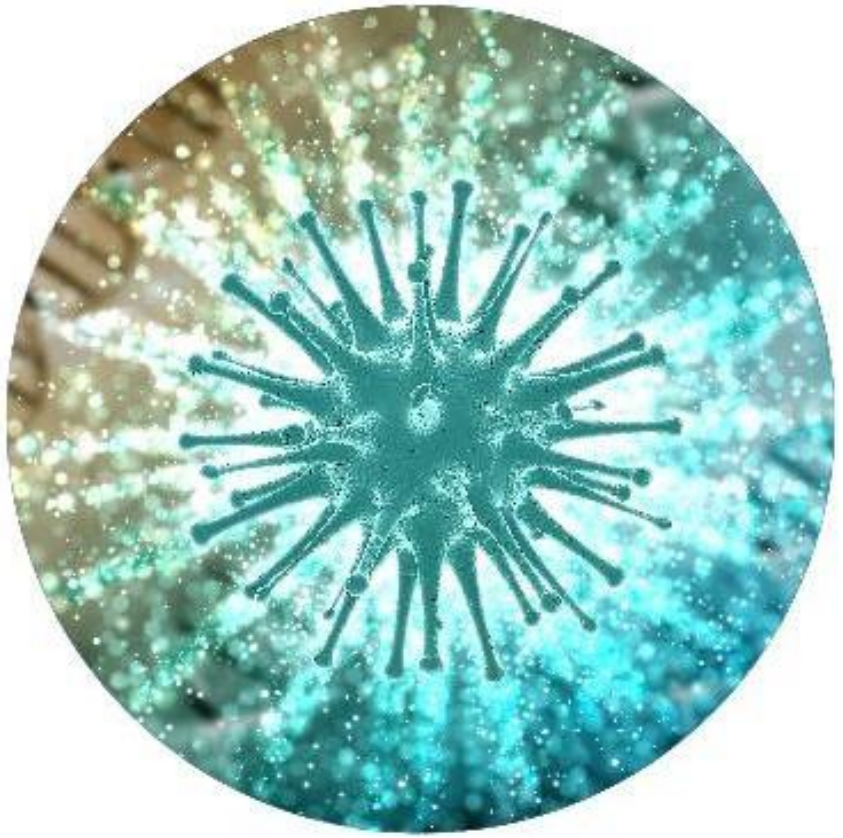
Conclusions

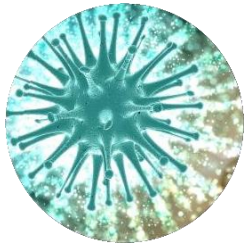
This is the first evaluation of SFC assay in clinical samples. This new method appears to be very promising by combining the high number of distinct pathogens and genetic resistance determinants identified in a single assay. Further investigations should be done to evaluate the usefulness of this assay in combination with clinical multidisciplinary groups

Respiratory Flow Chip Kit

dna Flow tecnology

Détection des principaux agents pathogènes à l'origine d'infections aiguës des voies respiratoires





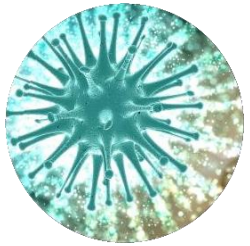
Respiratory Flow Chip |23 main infectious agents, respiratory diseases

Respiratory Flow Chip Kit

B	FluA	PIV-1	CoV-OC43		RNaseP	RSV-A	B	
B	FluA-H1N1	PIV-2	BP		BG	RSV-B	CoV-229E	
Cl-1	FluA-H3	PIV-3	BPP			RhV	CoV-HKU1	
Cl-2	FluB	PIV-4	MP			PIV-1	CoV-NL63	
RNaseP	MPV	AdV	EV	B	FluA	PIV-2	BPP	
BG	RSV-A	Bov	CoV-2	Cl-1	FluA-H1N1	PIV-3	MP	
	RSV-B	CoV-229E	SARS	Cl-2	FluA-H3	PIV-4	EV	
	RhV	CoV-HKU1		CoV-OC43	FluB	AdV	CoV-2	
	B	CoV-NL63		BP	MPV	Bov	SARS	

Identification des principaux agents infectieux **des maladies respiratoires**

- Adenovirus
- Influenza Type A: subtype H3 and subtype H1N1 (pandemic 2009)
- Influenza Type B
- Coronavirus 229E
- Coronavirus HKU-1
- Coronavirus NL63
- Coronavirus OC43
- Coronavirus SARS-CoV2: RdRP (spefici of SARS-CoV-2) and E (generic for all Sarbecovirus)
- Parainfluenza type 1
- Parainfluenza type 2
- Parainfluenza type 3
- Parainfluenza type 4
- Bocavirus
- Metapneumovirus
- Sincitial Respiratory virus type A
- Sincitial Respiratory virus type B
- Rihnovirus
- Enterovirus (EV-A, EV-B, EV-D)
- Bordetella pertussis
- Bordetella parapertusis
- Mycoplasma pneumoniae



Respiratory Flow Chip |23 main infectious agents, respiratory diseases

Respiratory Flow Chip Kit

B	FluA	PIV-1	CoV-OC43		RNaseP	RSV-A	B	
B	FluA-H1N1	PIV-2	BP		BG	RSV-B	CoV-229E	
Cl-1	FluA-H3	PIV-3	BPP			RhV	CoV-HKU1	
Cl-2	FluB	PIV-4	MP			PIV-1	CoV-NL63	
RNaseP	MPV	AdV	EV	B	FluA	PIV-2	BPP	
BG	RSV-A	Bov	CoV-2	Cl-1	FluA-H1N1	PIV-3	MP	
	RSV-B	CoV-229E	SARS	Cl-2	FluA-H3	PIV-4	EV	
	RhV	CoV-HKU1		CoV-OC43	FluB	AdV	CoV-2	
	B	CoV-NL63		BP	MPV	Bov	SARS	

Identification des principaux agents infectieux des **maladies respiratoires**

Validé à partir de matériel génétique purifié provenant de différents types d'échantillons clinique

- Prélèvements nasopharyngés, oropharyngés
- aspiration, lavage nasopharyngé
- LBA

frontiers in Microbiology

ORIGINAL RESEARCH
published: 24 March 2022
doi: 10.3389/fmicb.2022.804700

Microbia
Multiplex

The identifi
infections wa
reverse dot bl
based on DI
Diagnostica,
multiplex PC
5 µl of gen
primers follo
the membra
most import
the respirat
colorimetric
by the Hybri
Seville, Spain
by a camera and analyzed by the Hybri-Soft software reporting

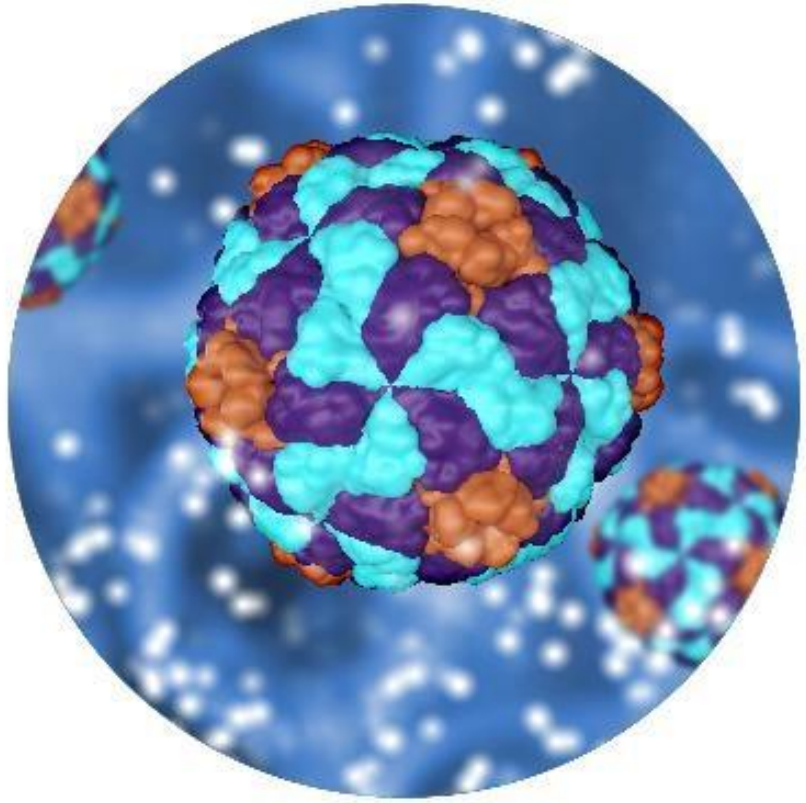
In conclusion, our data demonstrated a profound change in the typical epidemiology of pediatric respiratory pathogens during 2020–2021 winter season in a large cohort of children in northeast Italy. Influenza and RSV infections were not detected, whereas HRV was the main pathogen during winter. Social distancing measures, in particular face masks use and school closure, did have an impact on the circulation of common respiratory pathogens. The use of a multiplex PCR allowed a rapid and useful differential diagnosis of common respiratory infections in children during COVID-19 pandemic. Given the novelty of these findings, continuing surveillance for a delayed spread, in particular of RSV and influenza, seems mandatory.

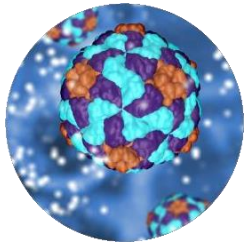
94, $p < 0.001$). Adenovirus, observed in 11.6%
sk factor (RRR = 6.44, $p < 0.001$). Bocavirus
clusion, our results showed that social isolation

Tick Borne Bacteria Flow Chip Kit

dna Flow tecnology

Identification des agents pathogènes bactériens transmis par les arthropodes





Tick-borne Flow Chip |7 tick-borne bacteria genera

Tick-borne Direct Flow Chip Kit

Identification des agents pathogènes bactériens transmis par les arthropodes

B			FR				B	
B	GR				EH		BOR	
CI	TG		BAR		AN			
BG	SFG						COX	
			BAR-2	B				FR
				CI				
	EH		BOR	BG	GR			BAR
	AN				TG			BAR-2
	B		COX		SFG			

AN	*Anaplasma*	*Anaplasma spp.*
		A. phagocytophilum
		Bovis
		A. equi
EH	*Ehrlichia*	*Ehrlichia chaffeensis*
		E. ewingii
		Candidatus Neoehrlichia mikurensis
BOR	*Borrelia spp.*	
BAR +BAR-2	*Bartonella spp.*	
COX	*Coxiella burnetii*	
GR	*Rickettsia*	*Rickettsia spp*
GR +TG		Rickettsia typhus group
GR +SFG		Rickettsia spotted fever group
FR	*Francisella spp*	

Bacterial CNS Flow Chip Kit

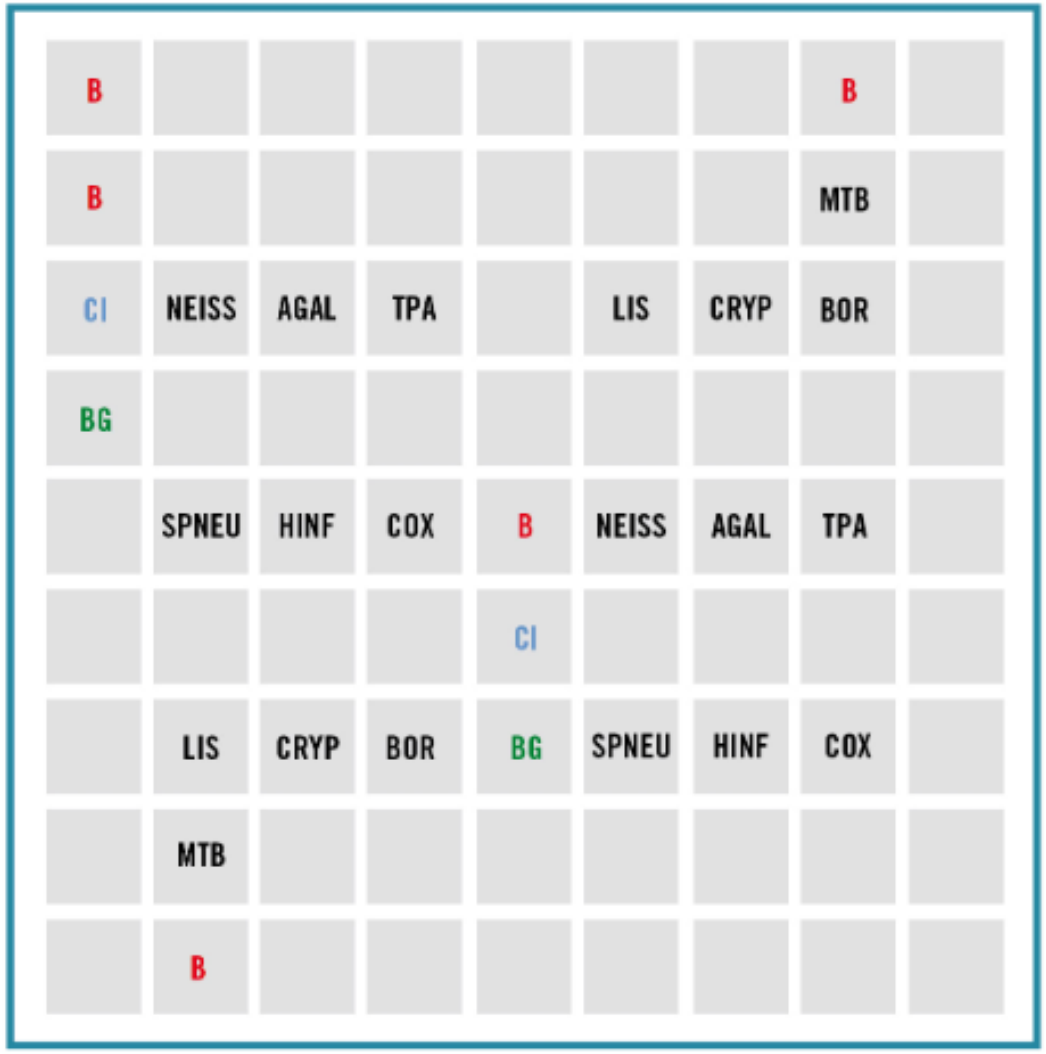
dna Flow tecnology

Kit de diagnostic des bactéries et des champignons responsables de méningites

Bacterial CNS Flow Chip Kit

9 bacteria and 1 fungus CNS infections

dna Flow Tecnology



Organism
Mycobacterium tuberculosis complex
Streptococcus pneumoniae
Streptococcus agalactiae
Haemophilus influenzae
Listeria monocytogenes
Treponema pallidum
Neisseria meningitidis
Coxiella burnetii
Borrelia burgdorferi
Cryptococcus neoformans (fungus)

Merci de votre attention