

X REUNIÓN
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PCR y diagnóstico molecular en la rutina clínica: ¿Es el presente?

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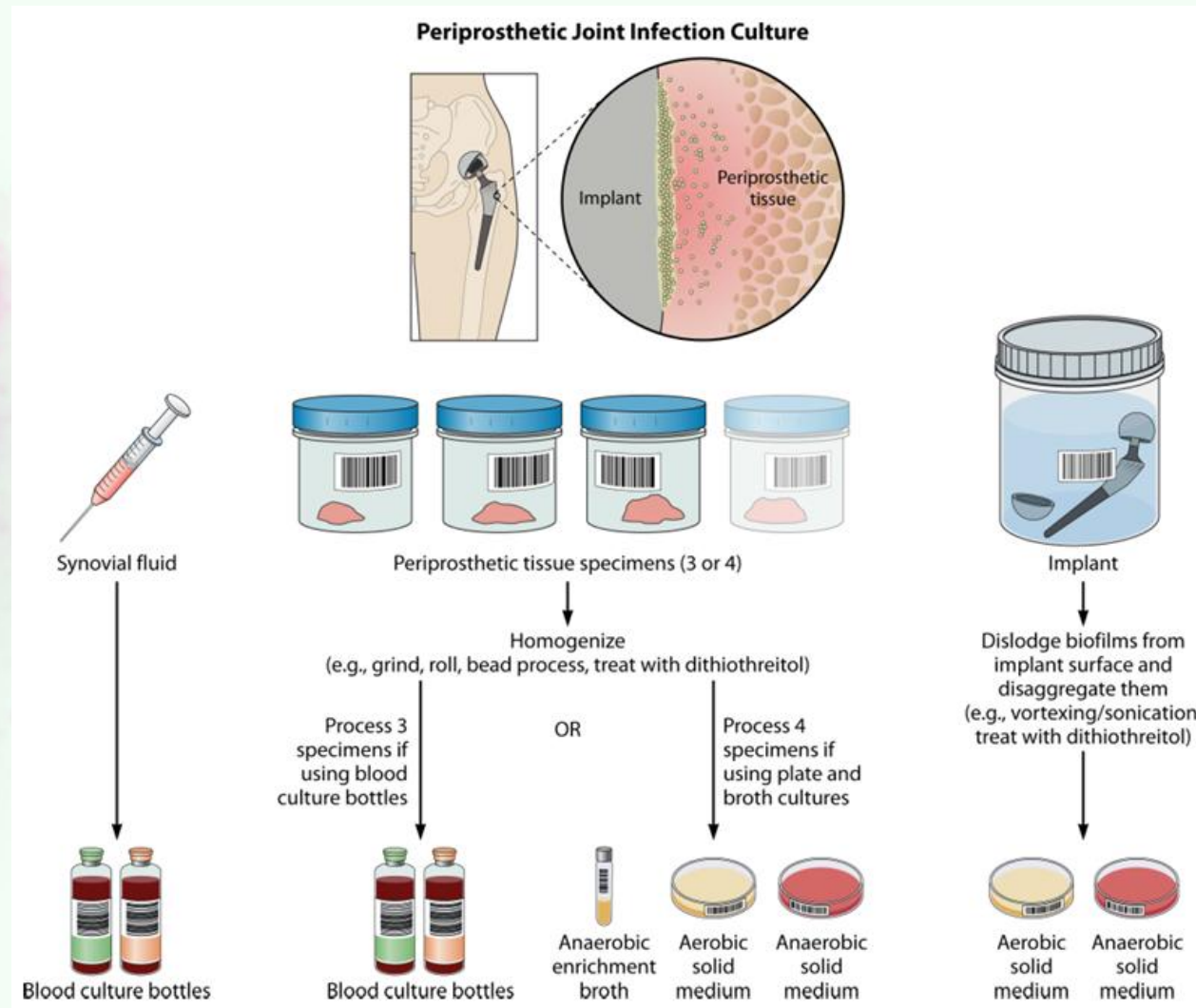
Agenda

- 01 – Escenario actual**
- 02 – Técnicas de PCR**
- 03 – Secuenciación de nueva generación NGS**
- 04 – Aplicación en vida real**
- 05 – Rol técnicas moleculares en los criterios diagnósticos**
- 06 – Conclusiones**

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Escenario actual



Rol del cultivo en las definiciones de IPA

Organization (reference)	Year	Role of culture in PJI diagnosis
Musculoskeletal Infection Society (MSIS) (1)	2011	PJI is confirmed when a pathogen is isolated by culture from at least two separate tissue or fluid specimens obtained from the affected joint; ^a isolation of a microorganism in one culture of periprosthetic tissue or fluid is a minor criterion ^a
Infectious Diseases Society of America (IDSA) (2)	2013	Two or more intraoperative cultures or a combination of preoperative aspiration and intraoperative cultures that yield the same organism (indistinguishable based on common laboratory tests, including genus and species identification or results of antimicrobial susceptibility testing) may be considered definitive evidence of PJI; growth of a virulent microorganism (e.g., <i>Staphylococcus aureus</i>) in a single specimen of a tissue biopsy or synovial fluid may also represent PJI; one of multiple tissue cultures or a single aspiration culture that yields an organism that is a common contaminant (e.g., coagulase-negative staphylococci, <i>Cutibacterium acnes</i>) should not necessarily be considered evidence of definite PJI and should be evaluated in the context of other available evidence
International Consensus Meeting (ICM) (3)	2018	Two positive growths of the same organism using standard culture methods confirm PJI; ^b a single positive culture is a minor criterion assigned two points in a scoring system that determines the likelihood of PJI (infected, inconclusive, non-infected) ^b
European Bone and Joint Infection Society (EBJIS) (4)	2021	PJI is confirmed with two or more positive specimens with the same microorganism; ^c if no other standalone criteria are met, a single positive aspiration or intraoperative tissue or fluid culture may be considered along with at least one other suggestive diagnostic study to contribute to a "likely PJI" diagnosis ^c

Microbiological diagnosis of PJI

Culture based methods

Non culture based methods

Tissue cultures

Sonication fluid culture

Vortexing culture

Synovial fluid culture

Blood culture bottles

DTT

MALDI TOF MS

PCR

NGS

Broad range PCR

Targeted PCR

Multiplex PCR

tNGS

sNGS

Specimens: Type, location and number of samples

Type of recipients

Understanding the etiology

Previous antimicrobial treatment effect

Incubation time


Recognition of Small Colony Variants

Culture interpretation

Multidisciplinary diagnosis




- NGS la mayor precisión diagnóstica.
- Gran heterogeneidad entre estudios.



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com



Systematic review

Diagnostic accuracy of 16S rDNA PCR, multiplex PCR and metagenomic next-generation sequencing in periprosthetic joint infections: a systematic review and meta-analysis

Flaminia Olearo ¹, Said El Zein ², Maria Eugenia Portillo ³, Antonia Zapf ⁴, Holger Rohde ¹, Elie F. Berbari ², Marjan Wouthuyzen-Bakker ^{5,*}, on behalf of the ESCMID study group on implant-associated infections (ESGIAI) and the molecular working group for the unified PJI definition task force

TÉCNICA	SENSIBILIDAD	ESPECIFICIDAD
PCR	62%	96%
16S rRNA	80%	94%
mNGS	88%	93%



Study characteristics by index tests

Study characteristics	16S rDNA PCR (n of studies = 31 ^a ; total patient = 3031) N (%)	mPCR (n of studies = 29 ^a ; total patient = 3316) N (%)	mNGS (n of studies = 22 ^a ; total patient = 2446) N (%)
Years of publication			
2005–2009	8 (25.8)	0	0
2010–2014	7 (22.6)	5 (17.2)	0
2015–2019	11 (35.5)	13 (44.8)	4 (18.2)
2020–2024	5 (16.1)	11 (37.9)	18 (8.2)
Subgroup molecular tests	Conventional PCR: 18 (58.1) Real-time PCR: 12 (38.7) Not specified: 1 (3.2)	Biofire JI: 4 (13.8) Other mPCR: 7 (24.1) SeptiFast: 2 (6.9) Unyvero III: 16 (55.2)	Shotgun NGS: 18 (81.8) Targeted NGS: 4 (18.2)
Study design			
Retrospective	4 (12.9)	9 (31)	10 (45.5)
Prospective	26 (83.9)	19 (65.5)	12 (54.5)
Unclear	1 (3.2)	1 (3.5)	
PJI reference	EBJIS: 1 (3.2) ICM: 1 (3.2) IDSA: 1 (3.2) MSIS: 10 (32.3) Clinical criteria: 18 (58.1)	EBJIS: 4 (13.8) ICM: 1 (3.4) IDSA: 3 (10.3) MSIS: 12 (41.4) Clinical criteria: 9 (31.1)	ICM: 1 (4.6) IDSA: 6 (27.3) MSIS: 15 (68.2)
GRADE	A: 4 (12.9) B: 10 (32.3) C: 8 (25.8) D: 9 (29.19)	A: 3 (10.3) B: 12 (41.4) C: 4 (13.8) D: 10 (34.5)	A: 0 B: 19 (86.4) C: 2 (9.1) D: 1 (4.6)
Specimens used	PPT: 7 (22.6) PPT + any fluid: 8 (25.8) SNV fluid: 10 (32.3) SON + SNV fluid: 1 (3.2) SON fluid: 5 (16.1)	PPT: 2 (6.9) PPT + any fluid: 7 (24.1) SNV fluid: 14 (48.3) SON + SNV fluid: 1 (3.5) SON fluid: 5 (17.2)	PPT: 1 (4.6) PPT + any fluid: 7 (31.8) SNV fluid: 9 (40.9) SON + SNV fluid: 1 (4.6) SON fluid: 4 (18.2)
Previous antibiotics	Yes: 12 (38.7) Unknown: 12 (38.7) No: 7 (22.6)	Yes: 15 (51.7) Unknown: 7 (24.2) No: 7 (24.1)	Yes: 14 (63.6) Unknown: 4 (18.2) No: 4 (18.2)

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Diagnóstico molecular: PCR panbacterianas

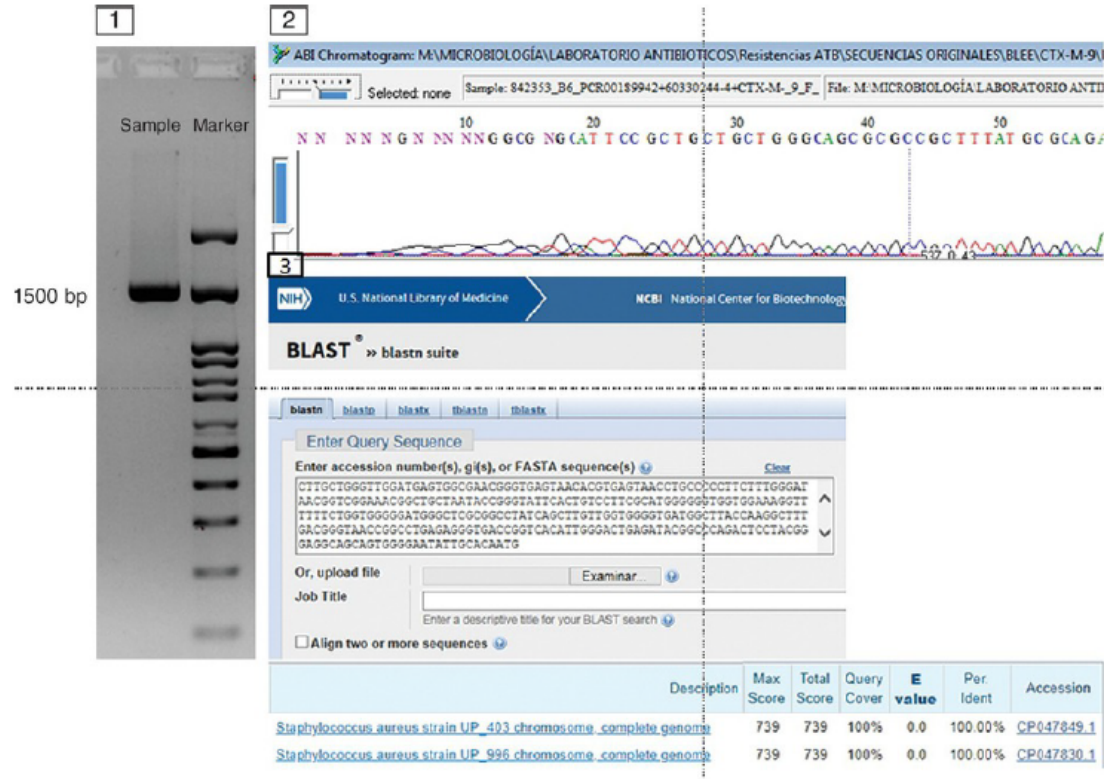
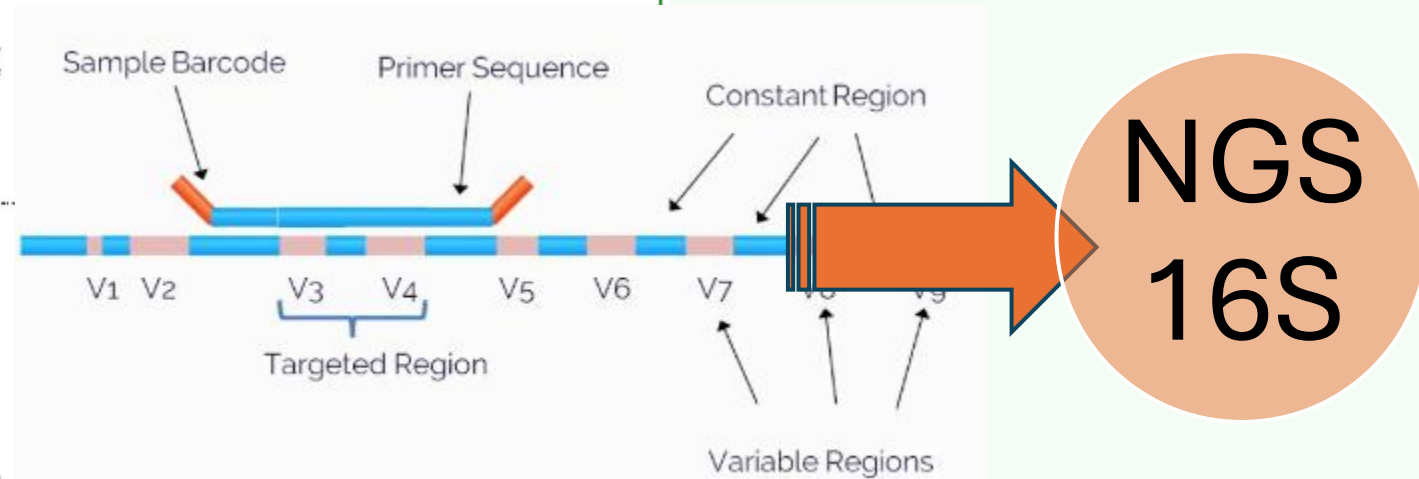


Figure 4.1. Broad-range PCR scheme for bacteria and fungi. From the corresponding genome region: 1) a DNA fragment is amplified using primers targeting conserved regions present in all (universal) bacteria or fungi and 2) polymorphic regions are sequenced 3) to identify the species.

- PCR tiempo real: 83%
- PCR convencional: 78%
- Especificidad: 94–95%.



16S rRNA:

- ✓ Ribosomal RNA
- ✓ Phylogenetic markers
- ✓ 1542 bp

Plataformas de Diagnóstico Molecular

Platform	Manufacturer	Technology	Sample type	Microorganisms identified	Approx TAT (h) ^a	FDA cleared for use in BJI
Unyvero i60	Curetis	Cartridge multiplex PCR assay	Joint aspirate, sonication fluid	114 bacterial and fungal DNA targets and antibiotic resistance markers	5 (30)	No
GeneXpert	Cepheid	Modular RT-PCR with fluorescent-probe-based detection in closed system	Skin and soft tissue infection swabs	Skin and soft tissue infection panel for MSSA, MRSA	1 (5)	No
Septifast	Roche	Multiplex RT-PCR and specific melting point analysis software	FDA cleared for blood	25 most common bacteria and fungi known to cause bloodstream infections	6	No
FilmArray	BioFire	Two-stage nested PCR with fluorescent-probe-based detection	Synovial fluid	BJI panel in development	1 (5)	No
IRIDICA/Plex-ID	Abbott	PCR with ESI-MS	Sterile fluids and tissues (IRIDICA BAC SFT Assay)	800 bacterial and fungal targets and 4 antimicrobial resistance markers	6	No
Metagenomic sequencing	Research laboratory only					No

^aTAT, turnaround time. Values in parentheses are hands-on times in minutes.



Diagnóstico molecular: plataformas

SeptiFast (Roche)

Table 1 List of detectable microorganism by multiplex PCR (SeptiFast®).

Type of microorganisms

Gram-negative

Escherichia coli
Klebsiella pneumoniae/oxytoca
Serratia marcescens
Enterobacter cloacae/aerogenes
Proteus mirabilis
Pseudomonas aeruginosa
Stenotrophomonas maltophilia
Acinetobacter baumannii

Gram-positive

Staphylococcus aureus
Coagulase-negative staphylococci
Enterococcus faecalis/faecium
Streptococcus pneumoniae
Streptococcus spp.

Fungi

Candida albicans
Candida glabrata
Candida krusei
Candida tropicalis
Candida parapsilosis
Aspergillus fumigatus



Journal of Infection (2012) xx, 1–8



BIAM
British Infection Association

www.elsevierhealth.com/journals/jinf

Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure

María Eugenia Portillo ^{a,*}, Margarita Salvadó ^a, Lluïsa Sorli ^c, Albert Alier ^b, Santos Martínez ^b, Andrej Trampuz ^d, Julià Gómez ^a, Lluïsa Puig ^b, Juan Pablo Horcajada ^c

- Roche Diagnostics ha descontinuado la prueba LightCycler SeptiFast (SeptiFast) en 2019. Ya no se encuentra en el mercado



Diagnóstico molecular: plataformas

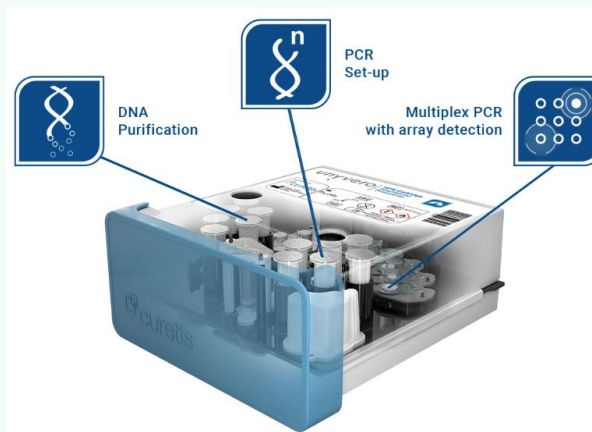
Unyvero i60 (Curetis)

Detected pathogens

Staphylococcus aureus
Staphylococcus epidermidis
*Coagulase-negative Staphylococcus*¹
Streptococcus mitis group²
Streptococcus anginosus group³
Streptococcus salivarius group⁴
Streptococcus agalactiae
*Streptococcus pyogenes*⁵
Enterococcus faecalis
Enterococcus sp.⁶
Granulicatella adjacens
Abiotrophia defectiva
Corynebacterium sp.⁷
Escherichia coli
Enterobacter cloacae complex
Enterobacter aerogenes
Proteus sp.⁸
*Klebsiella pneumoniae*⁹
Pseudomonas aeruginosa
Acinetobacter baumannii
Propionibacterium acnes
Propionibacterium avidum/granulosum
Finegoldia magna
Bacteroides fragilis group¹⁰
Candida sp.¹¹
Candida parapsilosis
Candida albicans

Antibiotic resistance markers

mec A
mec C (LGA251)
aac(6')/aph(2'')
ermA
ermC
vanA
vanB
rpoB
ctx-M
vim
imp
kpc
ndm
aacA4
gyrA
oxa-23
oxa-24
oxa-48
oxa-58



- Múltiples tipos de muestras
- Identifica SCN y *C. acnes*
- Sensibilidad baja (entre 60% y 70%)
- Tiempo resultado 4 a 5 h.
- No detecta *K.kingae*

A pesar de que el Unyvero i60IT1 está diseñado específicamente para infecciones de huesos y articulaciones, su baja sensibilidad limita su utilidad sólo a resultados positivos.



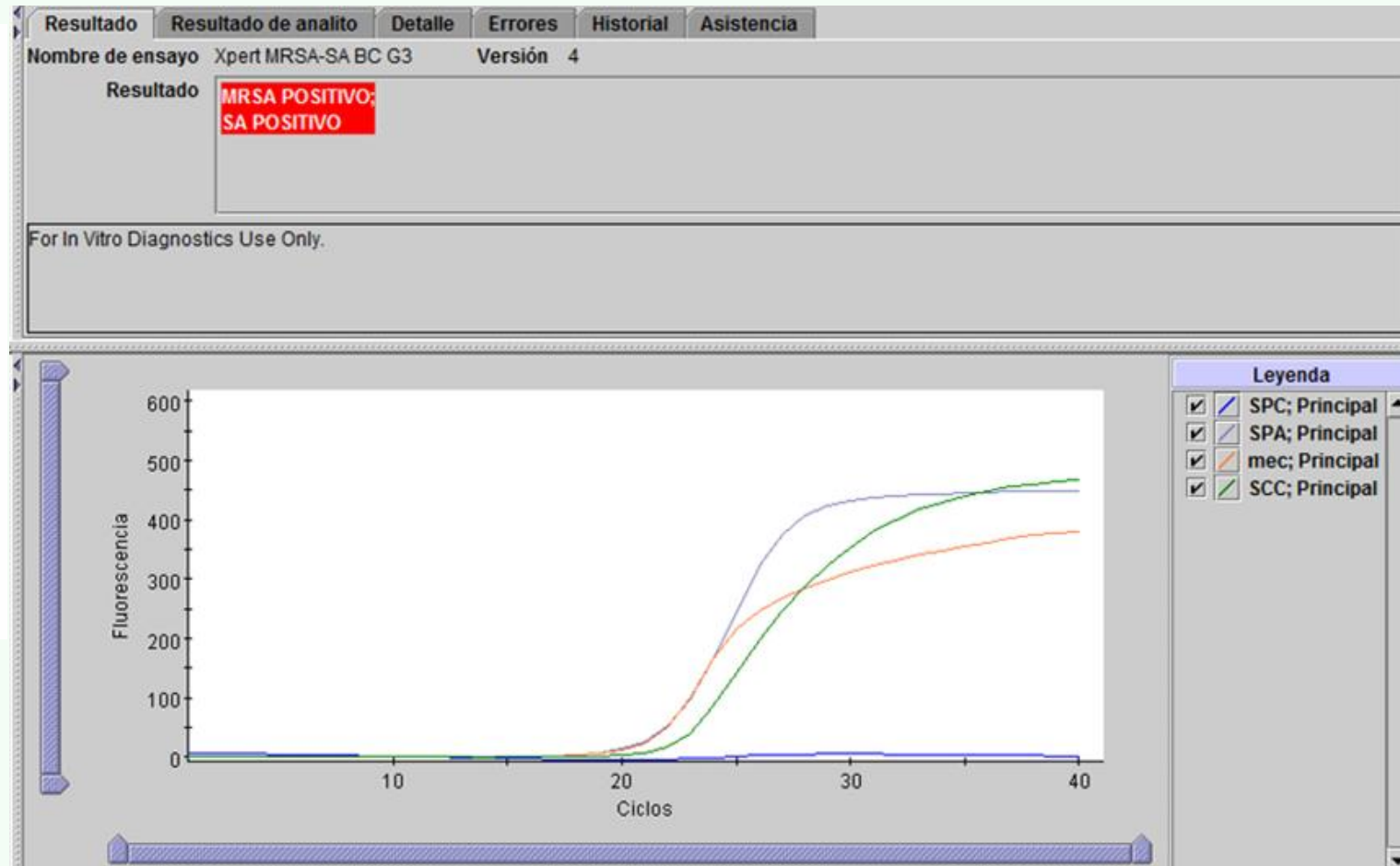
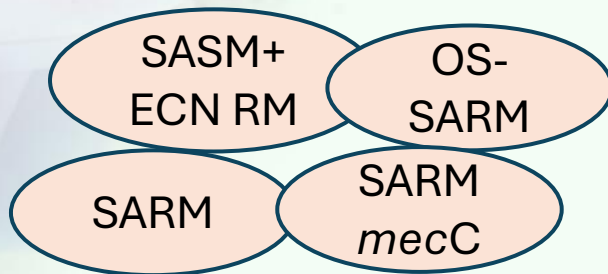
Diagnóstico molecular: plataformas

GeneXpert (Cepheid)



- PCR a tiempo real comercial
- Hisopos IPPB
- *spa*, *mecA*, *SCCmec*
- Técnica rápida (1h)
- Muestra directa
- Sensible y específica
- Fácil de realizar

Microorganismo	Sensibilidad (%)	Especificidad (%)
MSSA	97.6	97.3
MRSA	90.9	100



Diagnóstico molecular: plataformas

Iridica (Abbot)



- PCR/ESI-MS, comercializada como el sistema IRIDICA (Abbott) y anteriormente PLEX-ID, llevaba más de una década en desarrollo y obtuvo el mercado CE y se comercializó en Europa en 2014.
- Capaz de detectar una gran cantidad de microorganismos, se encontraba bajo revisión en la FDA cuando, en abril de 2017, Abbott lo suspendió.



Diagnóstico molecular: plataformas

FimArray BJI (Biofire)

GRAM-POSITIVE BACTERIA

Anaerococcus prevotii/vaginalis
Clostridium perfringens
Cutibacterium avidum/granulosum
Enterococcus faecalis
Enterococcus faecium
Finexdolia magna
Parvimonas micra
Peptoniphilus
Peptostreptococcus anaerobius
Staphylococcus aureus
Staphylococcus lugdunensis
Streptococcus spp.
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

GRAM-NEGATIVE BACTERIA

Bacteroides fragilis
Citrobacter
Enterobacter cloacae complex
Escherichia coli
Haemophilus influenzae
Kingella kingae
Klebsiella aerogenes
Klebsiella pneumoniae group
Morganella morganii
Neisseria gonorrhoeae
Proteus spp.
Pseudomonas aeruginosa
Salmonella spp.
Serratia marcescens

YEAST

Candida spp.
Candida albicans

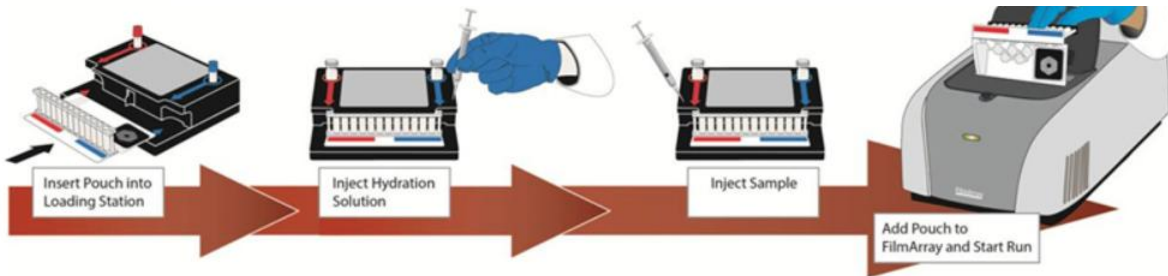
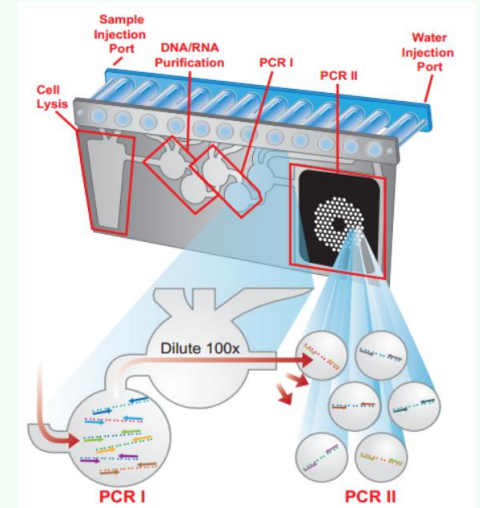
ANTIMICROBIAL RESISTANCE GENES

Carbapenemases
 IMP
 KPC
 NDM
 OXA-48-like
 VIM

 ESBL
 CTX-M

 Methicillin Resistance
mecA/C and MREJ (MRSA)

 Vancomycin Resistance
vanA/B



Simple:
 Only 2 minutes of hands-on time

Easy:
 No precise pipetting required

Fast:
 Run time of about 1 hour



Journal of Clinical Microbiology



Clinical Microbiology | Full-Length Text

Multicenter evaluation of the BIOFIRE Joint Infection Panel for the detection of bacteria, yeast, and AMR genes in synovial fluid samples

Jaime Esteban,¹ Llanos Salar-Vidal,¹ Bryan H. Schmitt,² Amy Waggoner,² Frédéric Laurent,³ Lelia Abad,³ Thomas W. Bauer,⁴ Irving Mazariegos,⁴ Joan-Miquel Balada-Llasat,⁵ Jared Horn,⁵ Donna M. Wolk,⁶ Alexa Jefferis,⁶ Mirjam Hermans,⁷ Irma Verhoofstad,⁷ Susan M. Butler-Wu,⁸ Minette Umali-Wilcox,⁸ Caitlin Murphy,⁹ Barbara Cabrera,⁹ David Craft,¹⁰ Benjamin von Bredow,¹⁰ Amy Leber,¹¹ Kathy Everhart,¹¹ Jennifer Dien Bard,¹² Irvin Ibarra Flores,¹² Judy Daly,¹² Rebecca Barr,¹² Kristen Holmberg,¹⁴ Corrin Graue,¹⁴ Bart Kensingler¹⁴



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Commentary

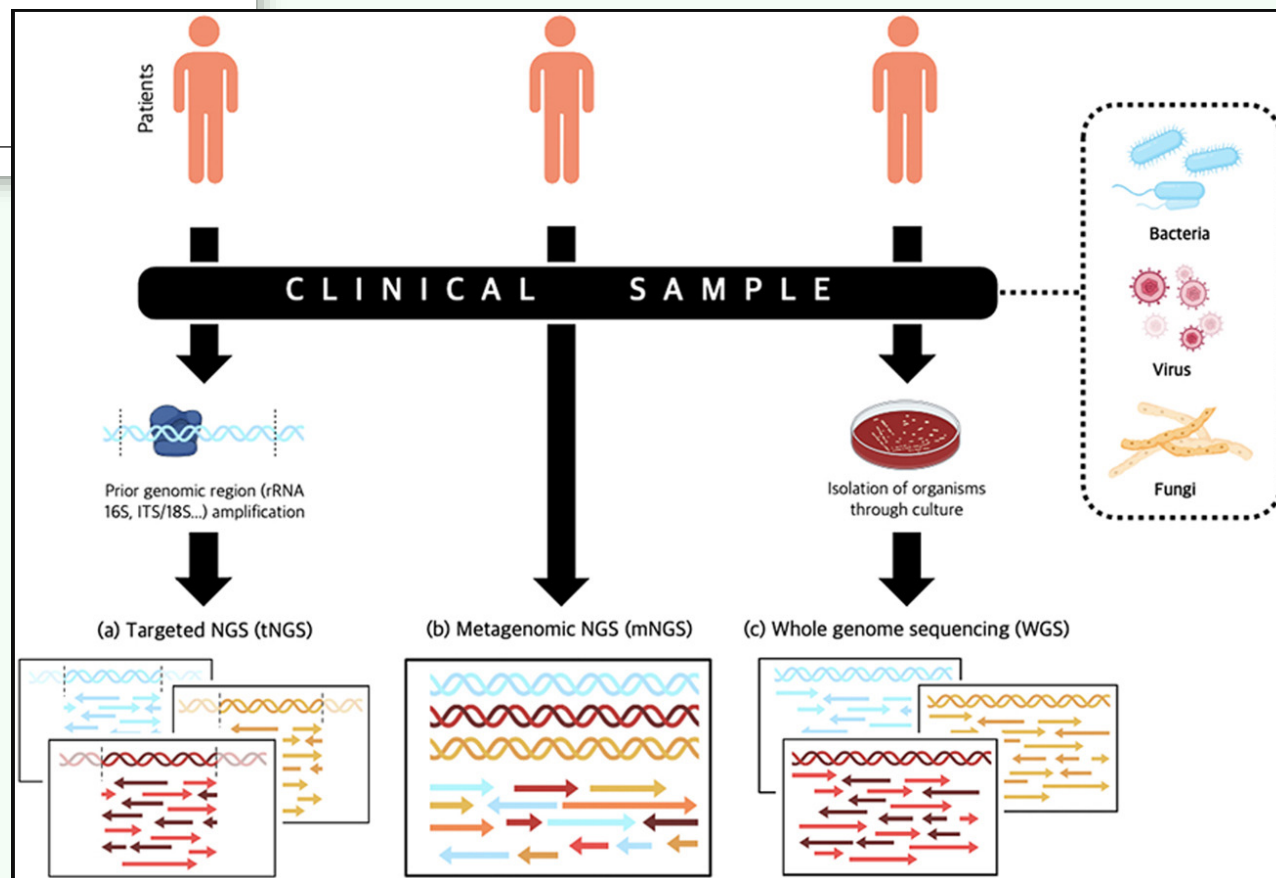
Revisiting diagnostics: practical application of next-generation sequencing technologies for infectious diseases

Lucía Henríquez^{1,2,*}, Ander Uribarri^{1,2}, Maria Eugenia Portillo^{1,2,3}

¹ Department of Clinical Microbiology, University Hospital of Navarra, Pamplona, Navarra, Spain

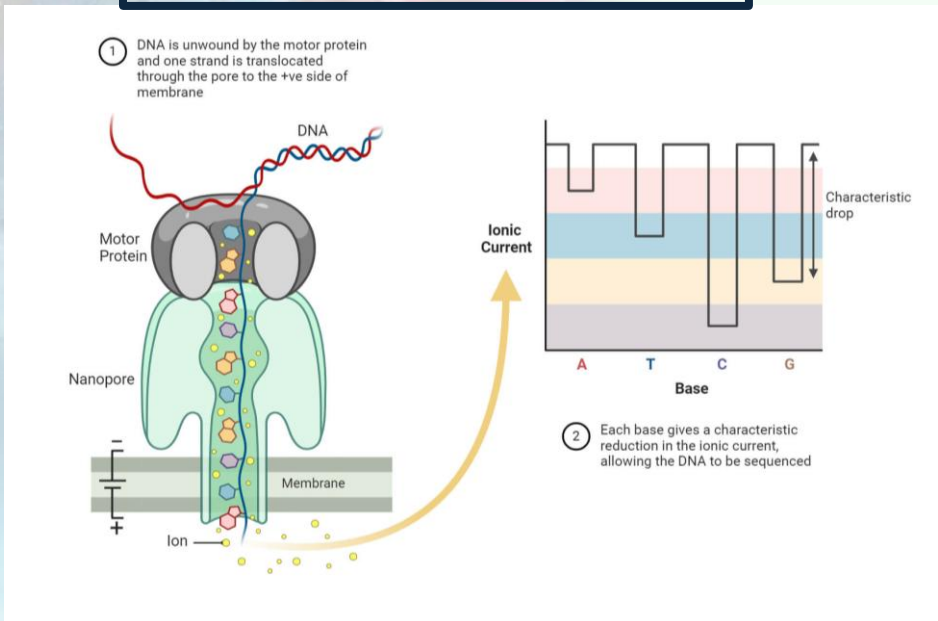
² Institute of Healthcare Research of Navarra (IdISNa), Pamplona, Navarra, Spain

³ CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

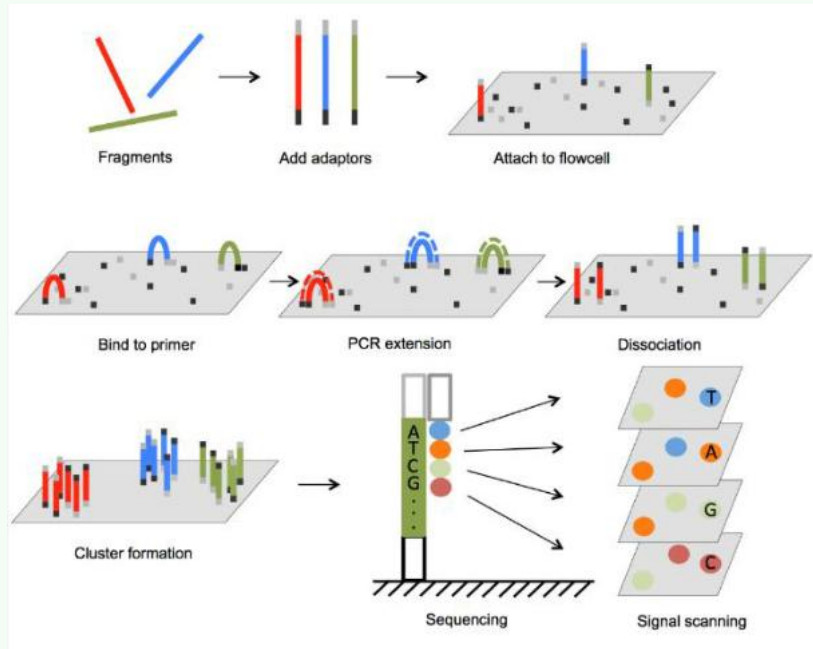


Sequencing plataforma	Advantages	Applications
<i>Illumina</i>	<ul style="list-style-type: none"> - Very low error rate (0.1-0.2%) - High accuracy for mutation detection (SNPS...) 	<ul style="list-style-type: none"> - In-depth genomic analysis - Antimicrobial resistance studies - Epidemiological surveillance
<i>Oxford Nanopore</i>	<ul style="list-style-type: none"> - Real-time data collection - Rapid diagnosis (within hours) - Ability to read long DNA/RNA fragments 	<ul style="list-style-type: none"> - Point-of-care testing - Rapid clinical diagnostics - Field-based sequencing

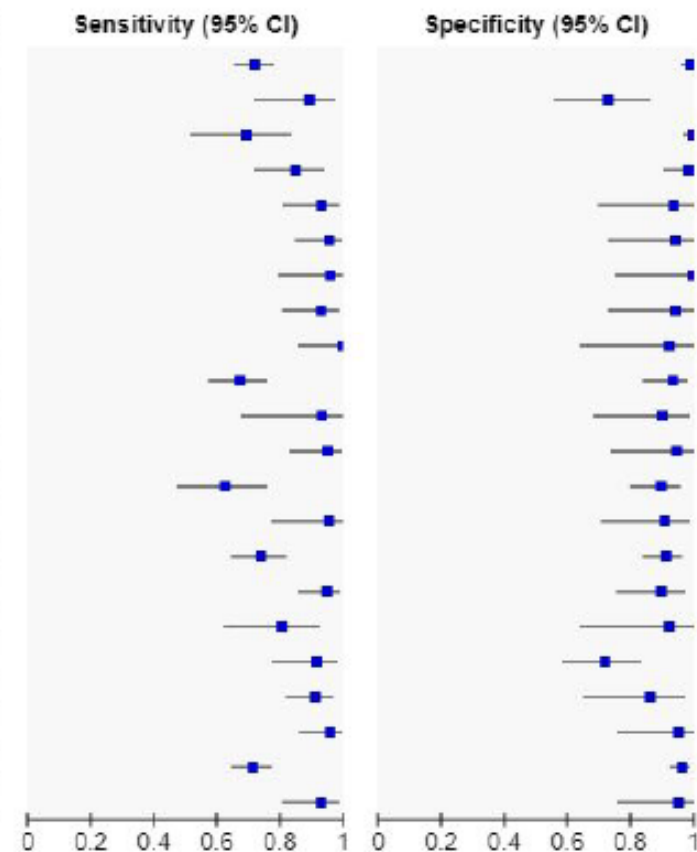
Nanoporos (ONT)



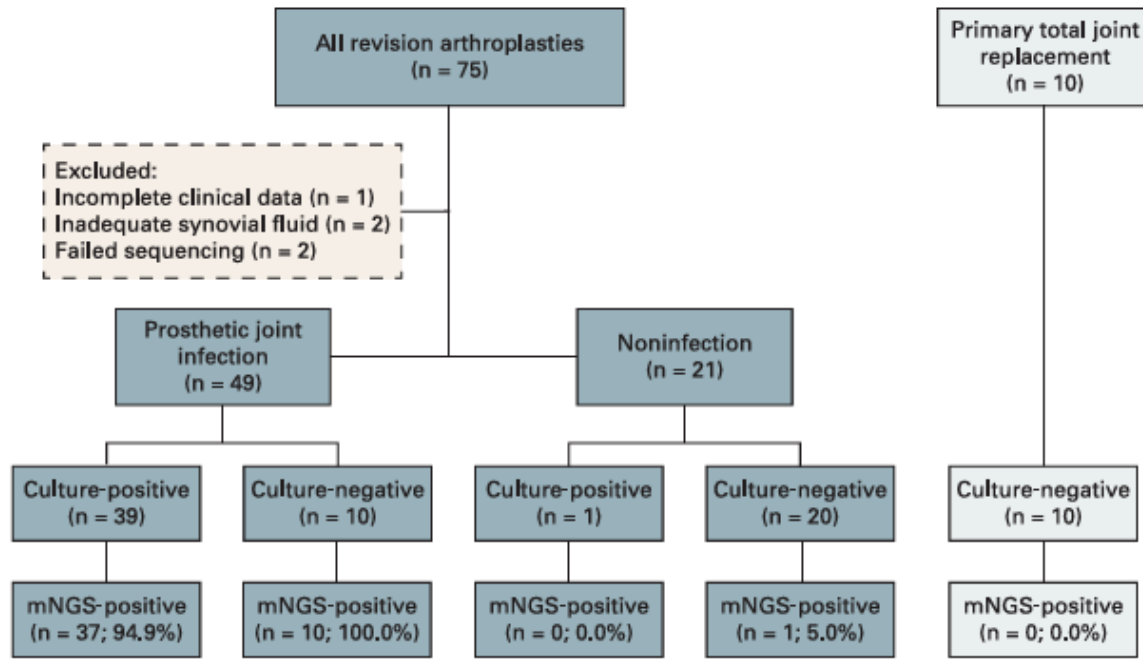
Síntesis (Illumina)



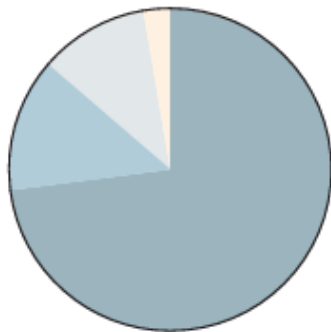
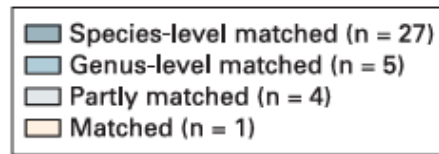
Study	TP	FP	FN	TN	PCR details	Samples used	Sensitivity (95% CI)	Specificity (95% CI)
Hong 2023	150	2	58	185	tNGS	SON fluid	0.72 [0.65, 0.78]	0.99 [0.96, 1.00]
Tarabichi 2018	25	10	3	27	tNGS	PPT + any fluid	0.89 [0.72, 0.98]	0.73 [0.56, 0.86]
Flurin 2022	25	0	11	118	tNGS	SNV fluid	0.69 [0.52, 0.84]	1.00 [0.97, 1.00]
Flurin 2021	40	1	7	57	tNGS	SON fluid	0.85 [0.72, 0.94]	0.98 [0.91, 1.00]
Azad 2022b	41	1	3	15	tNGS	SNV fluid	0.93 [0.81, 0.99]	0.94 [0.70, 1.00]
Wang 2020a	43	1	2	17	Shotgun NGS	SON + SNV fluid	0.96 [0.85, 0.99]	0.94 [0.73, 1.00]
Fang 2020	24	0	1	13	Shotgun NGS	SNV fluid	0.96 [0.80, 1.00]	1.00 [0.75, 1.00]
Tan 2024	40	1	3	17	Shotgun NGS	PPT + any fluid	0.93 [0.81, 0.99]	0.94 [0.73, 1.00]
Zhang 2019	24	1	0	12	Shotgun NGS	SON fluid	1.00 [0.86, 1.00]	0.92 [0.64, 1.00]
Ivy 2018	72	4	35	57	Shotgun NGS	SNV fluid	0.67 [0.58, 0.76]	0.93 [0.84, 0.98]
Yin 2021	14	2	1	18	Shotgun NGS	SNV fluid	0.93 [0.68, 1.00]	0.90 [0.68, 0.99]
He 2021	38	1	2	18	Shotgun NGS	PPT + any fluid	0.95 [0.83, 0.99]	0.95 [0.74, 1.00]
Kildow 2021	30	7	18	61	Shotgun NGS	SNV fluid	0.63 [0.47, 0.76]	0.90 [0.80, 0.96]
Cai 2020	21	2	1	20	Shotgun NGS	PPT	0.95 [0.77, 1.00]	0.91 [0.71, 0.99]
Li 2023	79	8	28	86	Shotgun NGS	SNV fluid	0.74 [0.64, 0.82]	0.91 [0.84, 0.96]
Hao 2023	55	4	3	35	Shotgun NGS	PPT + any fluid	0.95 [0.86, 0.99]	0.90 [0.76, 0.97]
Yu 2023	25	1	6	12	Shotgun NGS	PPT + any fluid	0.81 [0.63, 0.93]	0.92 [0.64, 1.00]
Zhang 2023	33	16	3	41	Shotgun NGS	SNV fluid	0.92 [0.78, 0.98]	0.72 [0.58, 0.83]
Mei 2023	63	3	6	19	Shotgun NGS	PPT + any fluid	0.91 [0.82, 0.97]	0.86 [0.65, 0.97]
Huang 2020	47	1	2	20	Shotgun NGS	PPT + any fluid	0.96 [0.86, 1.00]	0.95 [0.76, 1.00]
Thoendel 2018	152	7	61	188	Shotgun NGS	SON fluid	0.71 [0.65, 0.77]	0.96 [0.93, 0.99]
Huang 2023	40	1	3	20	Shotgun NGS	SNV fluid	0.93 [0.81, 0.99]	0.95 [0.76, 1.00]



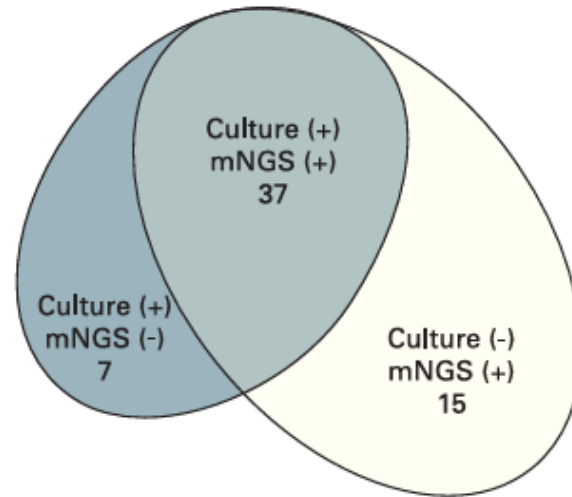
Aproximación de NGS



a



b



c

Fig. 3

- Shotgun NGS de líquido sinovial sensibilidad (95,9 %) de la NGS era significativamente mayor que la del cultivo microbiano (79,6 %).
- Especificidad sNGS (95,2 %) como cultivo



Aproximación de NGS

Diagnostic test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PCR	18.40	100.00	100.00	62.60
NGS	60.90	89.90	80.00	77.50
Culture	76.90	95.30	91.80	82.90
Parvizi et al ²¹	100	98.60	97.80	100.00

NGS, next-generation sequencing; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

ence. For PCR/NGS, samples were collected according to the protocol from the testing kit provided by Microgen Dx. This included placing four sterile tissue swabs invasively to soak up fluid by placing them over an area on the prosthesis most susceptible to infection/biofilm formation. For aspirated samples, fluid

MICRO MENU

MICROBIOLOGY

BENEFITS

Clinical Microbiology
Microbiology Lab Services
Reading Report

NGS TECHNOLOGY

MicroGeNDX NGS Process
Nucleic Acid Extraction

Illumina MiSeq Sequencing

Bioinformatic Pipeline
Diagnostic Technology
Curated Microbial Database

RESULTS

DNA Diagnostic Case Studies
Published Research

Illumina MiSeq Sequencing

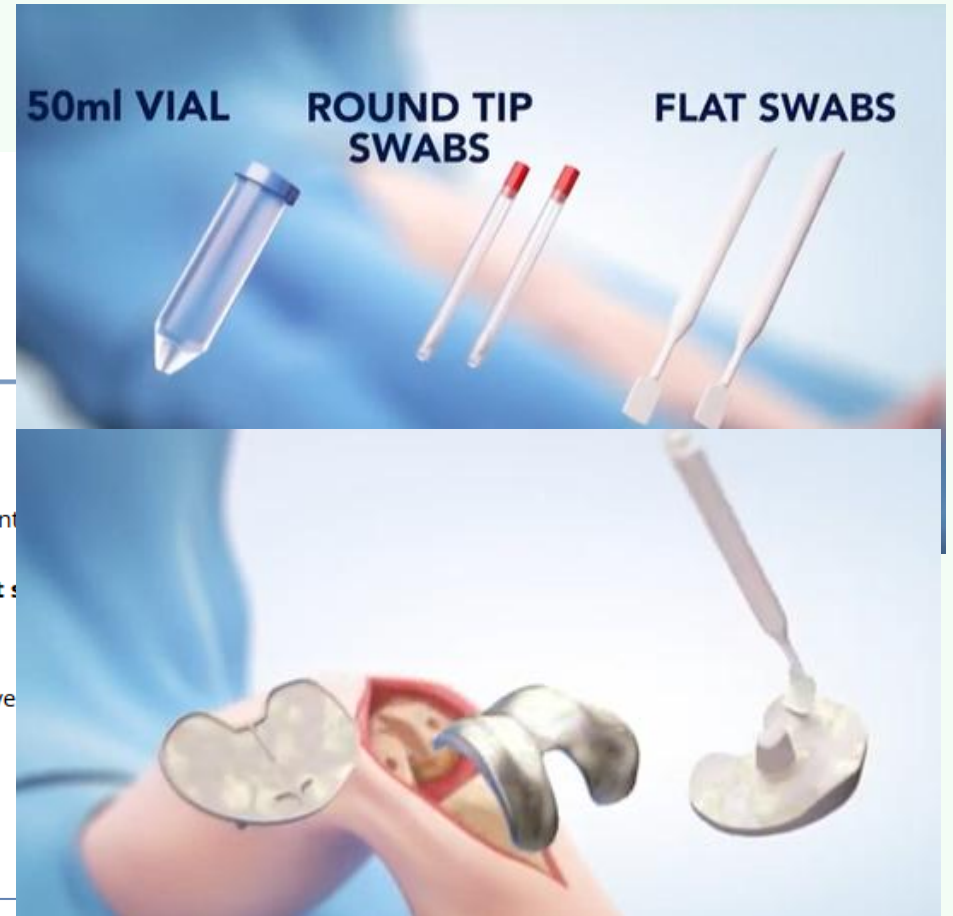
Work flow



Library preparation



- Adaptors added to DNA fragments
- Amplification of the universal genes 16S and ITS**
- Sequencing of binding sites, indexes, and regions complementary oligos
- Each plate has three positive amplification controls that span the analytic range, and a negative control for each assay**
- Specimens are combined based on qualitative measures
- Fragment size selection and clean-up is performed to remove dimers, salts, and short fragments



Tipo de muestras

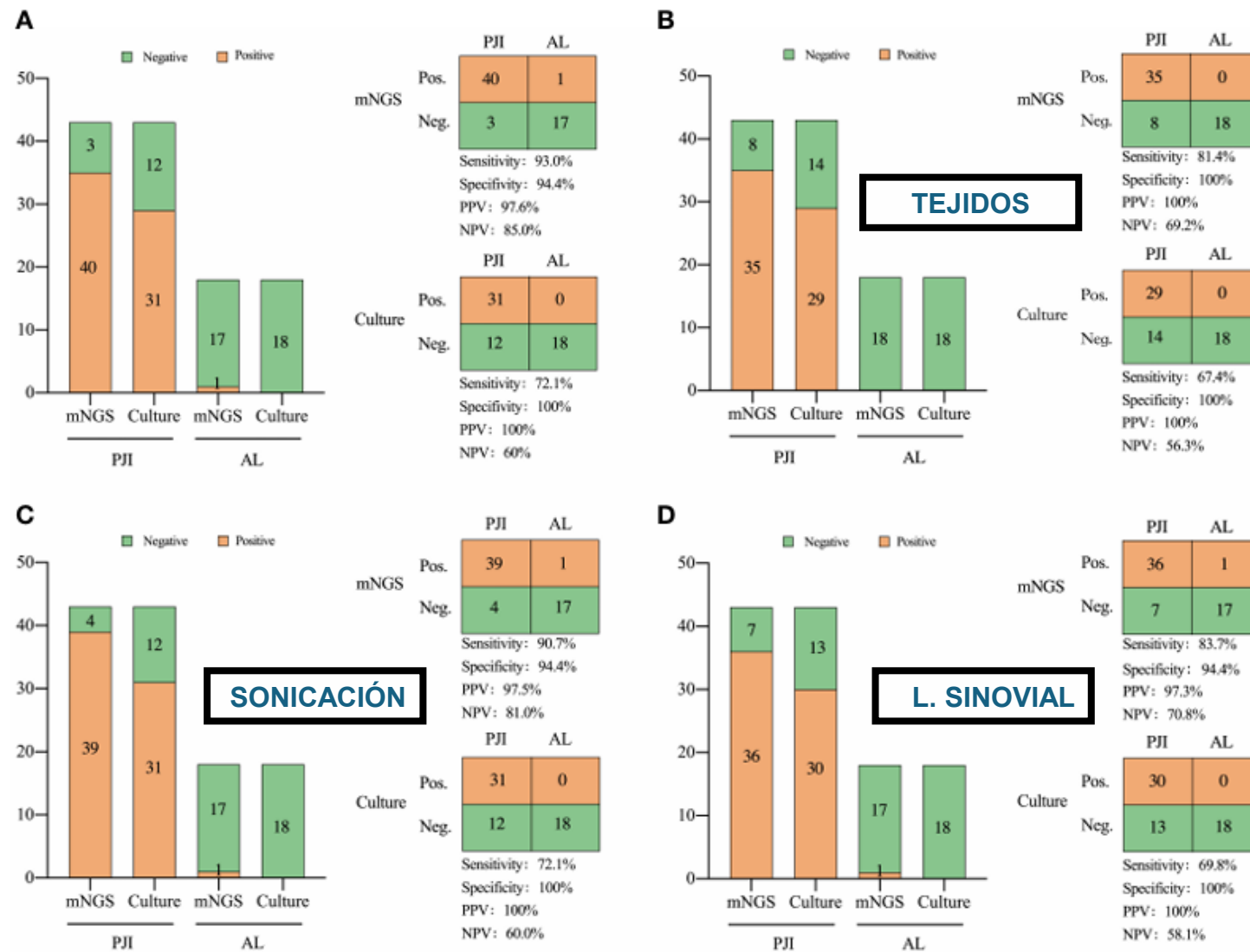
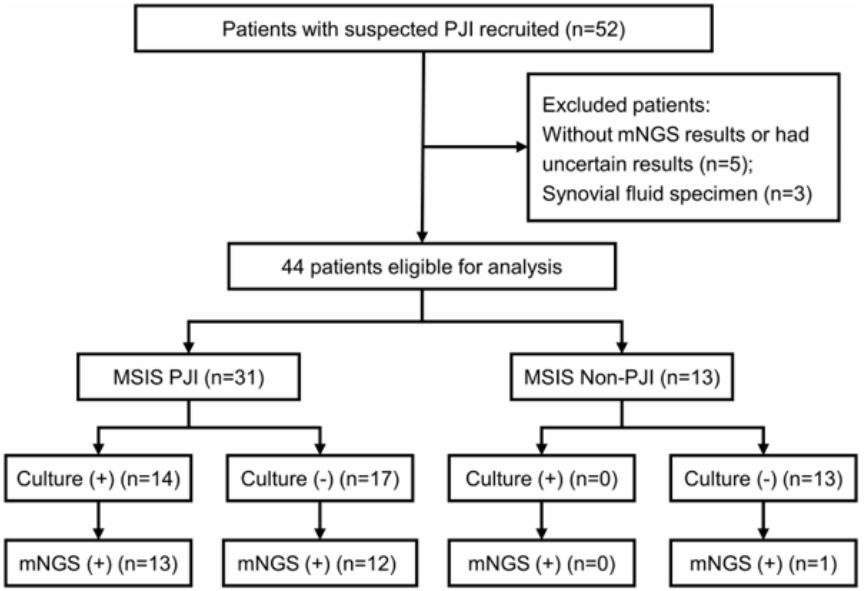


FIGURE 3

Comparison of the diagnostic efficiency of metagenomic next-generation sequencing and culture for PJI in: (A) all types of specimens, (B) periprosthetic tissue, (C) prosthetic sonication fluid, and (D) synovial fluid. NPV, negative predictive value; PPV, positive predictive value. Neg., negative; Pos., positive.



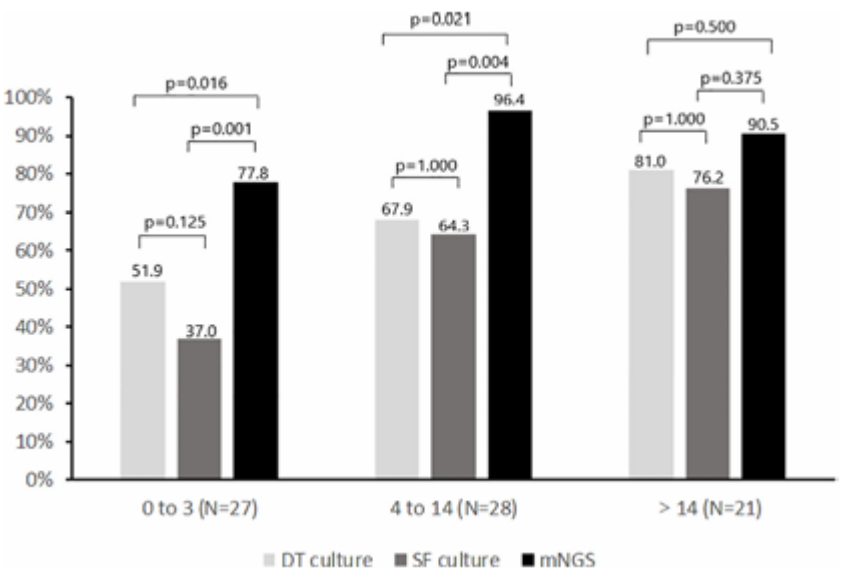
Efecto antibiótico



Gold Standard	Methods	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	PLR (95% CI)	NLR (95% CI)	AUC (95% CI)
MSIS	mNGS	80.6 (71.9–91.8)	84.6 (73.7–97.9)	92.6 (84.2–98.7)	64.7 (58.6–74.7)	5.241 (4.081–6.693)	0.229 (0.108–0.482)	0.826 (0.786–0.967)
	Culture	45.2 (40.8–51.5)	100 (100.0–100.0)	100 (100.0–100.0)	43.3 (39.1–49.5)	+∞	0.548 (0.396–0.617)	0.726 (0.621–0.864)

Specimen Type	Cases	Culture		mNGS		P value
		Positive Cases	Positive Rate	Positive Cases	Positive Rate	
Antibiotic use	13	3	23.1%	9	69.5%	0.000
Without antibiotic use	18	11	61.1%	10	55.6%	0.766

Y. Yu et al. IDR 2023.



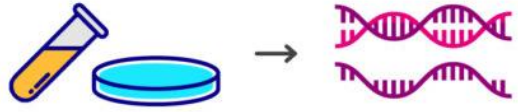
- La mNGS se ve menos afectada por la exposición previa a antibióticos
- Podría ser necesario suspender los agentes antimicrobianos más de 3 días antes del muestreo para aumentar aún más la tasa de positividad de la mNGS.



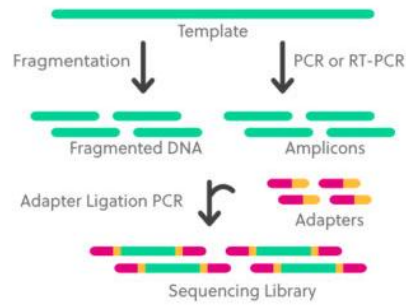
Agenda

- 01** – Escenario actual
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STEP 1:
Extraction



STEP 2:
Library
Prep



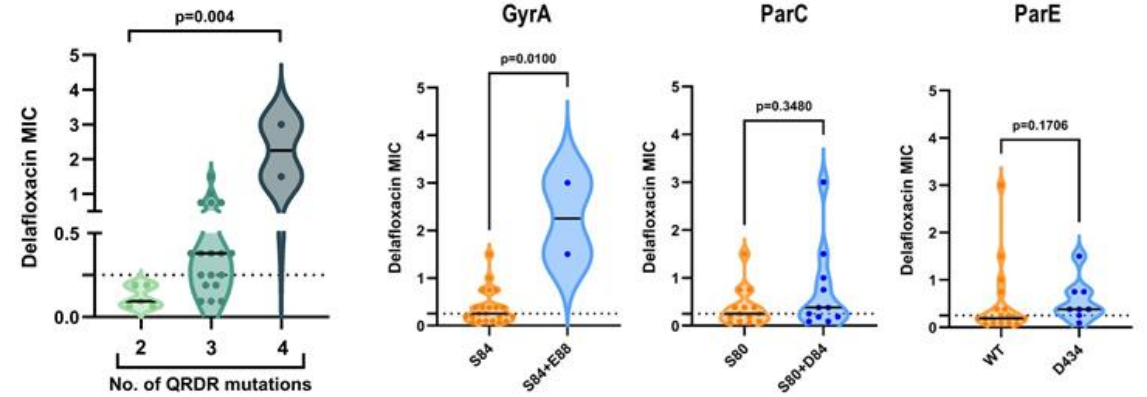
STEP 3:
Sequencing



STEP 4:
Analysis



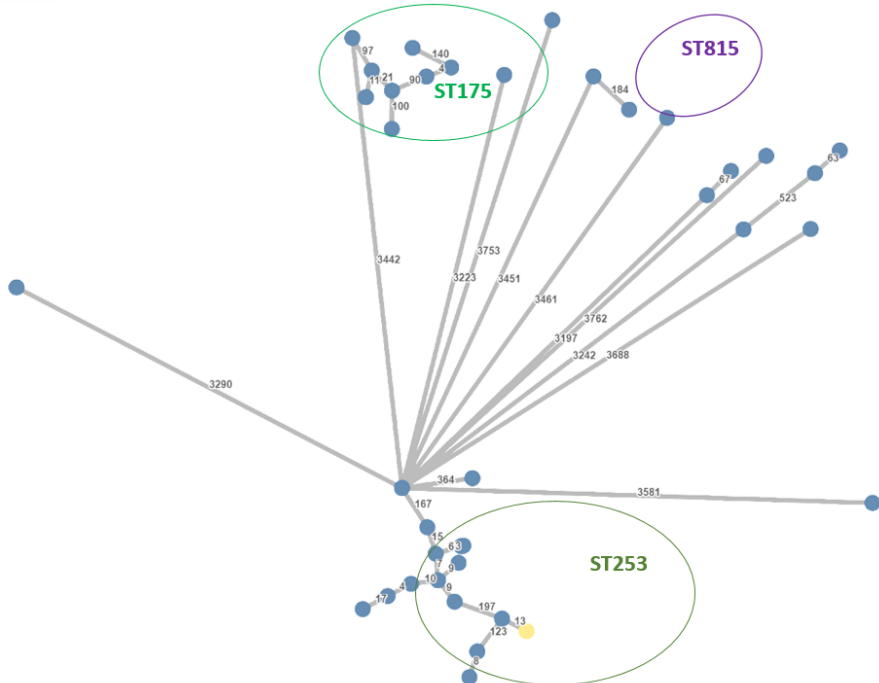
Whole Genome Sequencing



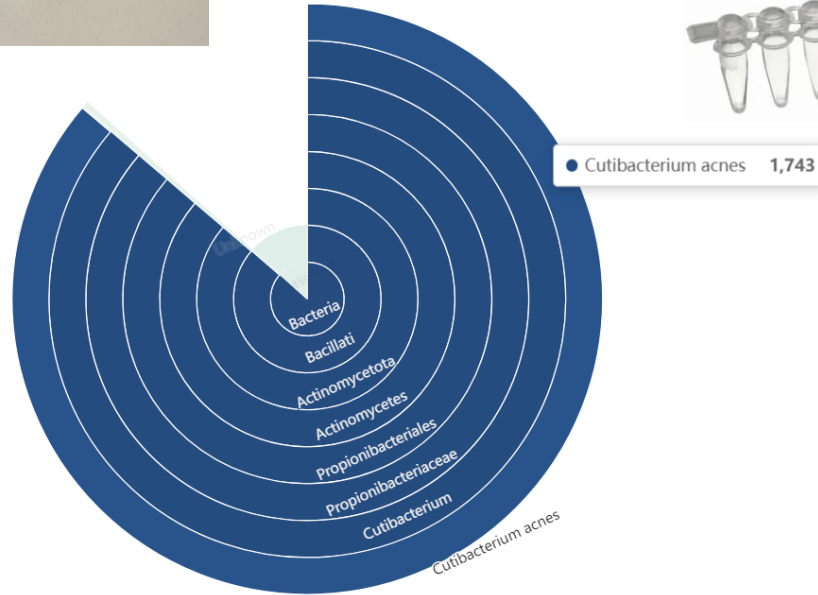
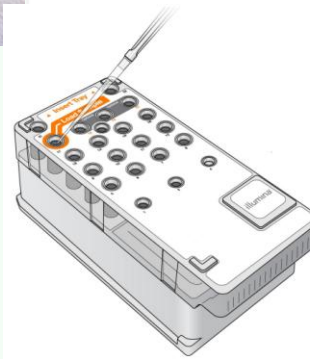
Resistome

Allele search

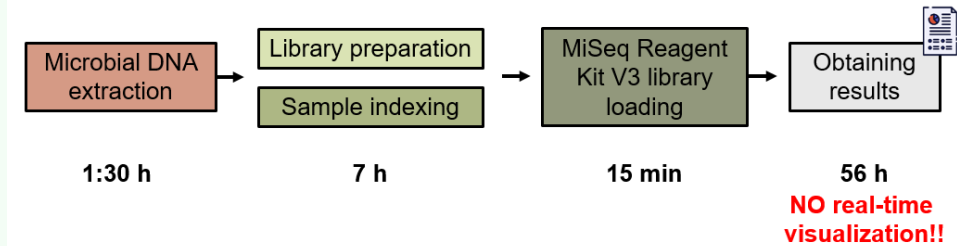
Locus	May confer resistance to drugs	May confer resistance to drug families
aac(6')-Ib	Amikacin, Arbekacin, Dibekacin, Gentamicin, Gentamicin b, Gentamicin c, Isepamicin, Kanamycin, Kanamycin a, Neomycin, Netilmicin, Plazomicin, Sisomicin, Tobramycin	Aminoglycosides
aadA6	Spectinomycin, Streptomycin	Aminoglycosides
aph(3')-IIb	Butirosin, G418, Gentamicin b, Kanamycin, Kanamycin a, Neomycin, Paromomycin, Ribostamycin	Aminoglycosides
arnA	Polymyxin	Peptides, Polypeptides
basS	Polymyxin	Polypeptides
bcr-1	Acridine dye, Benzalkonium chloride, Bicyclomycin, Cephalosporins, Diaminopyrimidine, Fosfomicin, Glycylcycline, Isoniazid, Lincosamides, Macrolides, Penam, Rhodamine, Tetracycline	Acridine, Antibacterial free fatty acids, Beta-lactams, Fosfomicins, Isonicotinic acids, Macrolides/lincosamides/streptogramins, Nitroimidazole, Nucleoside, Oxazolidinone, Peptides, Phenicols, Pyrimidine analogs, Quinolones, Rifamycins, Special, Tetracyclines
catB7	Azidamfenicol, Chloramphenicol, Thiamphenicol	Phenicols
crpP		Quinolones
emrE	Erythromycin, Gentamicin c, Macrolides, Tetracycline	Aminocoumarin, Aminoglycosides, Macrolides/lincosamides/streptogramins, Phenicols, Polyketides, Tetracyclines
fosA	Fosfomicin	Fosfomicins
OXA-15	Amoxicillin, Ampicillin, Aztreonam, Ceftazidime, Cephalosporins, Penam, Piperacillin	Beta-lactams



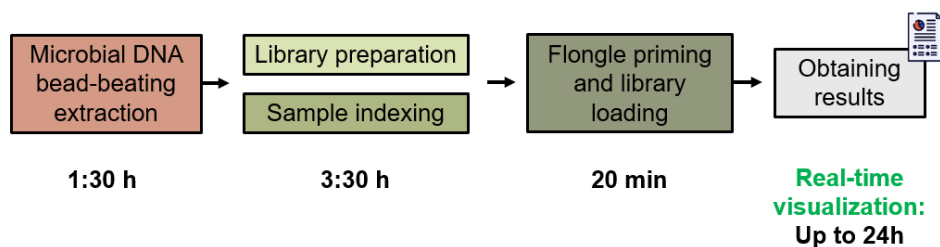
Targeted NGS



Illumina 16S sequencing flowchart:



ONT 16S Barcoding flowchart:



50422323-1
 HC+
 8 Sep 2025 09:50 AM
 Bacteremia
 Upload batch 20250908094208

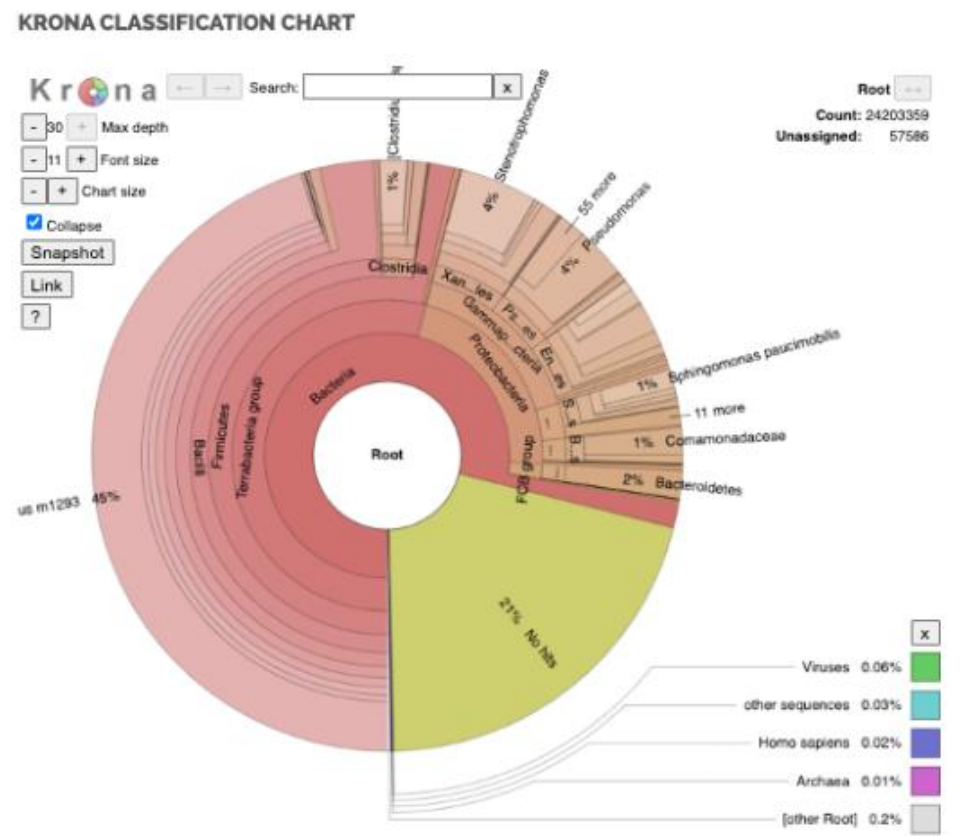
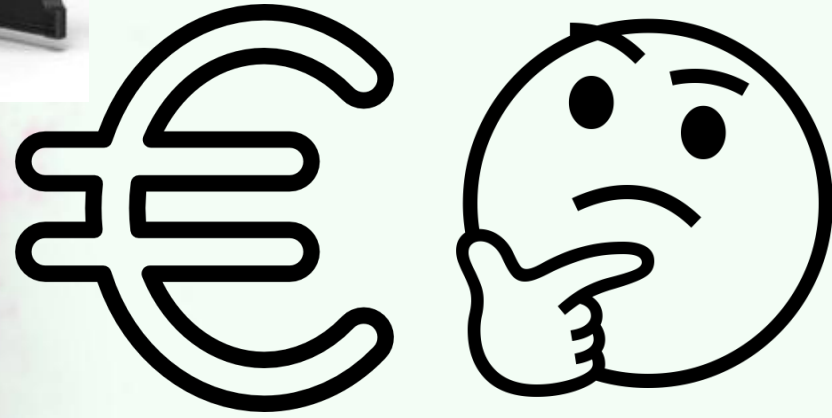
Sample data quality: ●
 Number of reads: Total reads: 773 reads
 Passing filters: 740 reads (95.73%)
 Read length: ●
 Sequencing quality: ●
 Targeted regions: ● Bacterial: Full-length 16S (740 reads, 95.73%)
 ● Fungal: Not selected

Read length distribution
 Read count vs Read length (0 to >2000)

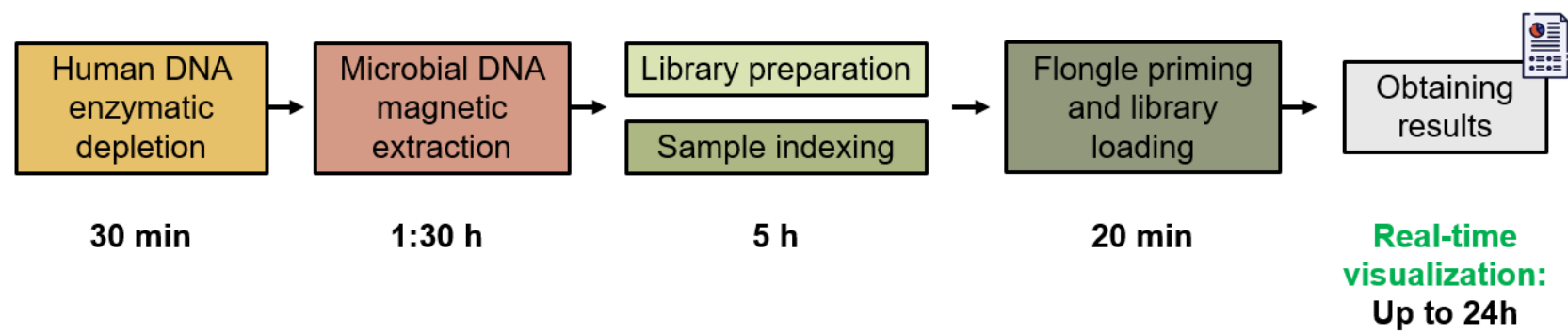
Approve	Reject	Contaminant	Organism identification	Approval status	Identification level	Confidence score	Number of associated reads ↓	Closest species	Closest confidence score
<input type="radio"/>	<input type="radio"/>	<input type="checkbox"/>	Streptococcus anginosus group		Indistinguishable species	99.54 %	365 (49.32 % of bacterial reads)	NA	NA
<input type="radio"/>	<input type="radio"/>	<input type="checkbox"/>	Clostridium cadaveris		Species	99.45 %	307 (41.62 % of bacterial reads)	NA	NA

2 bacteria / 2 organisms
 Display results below the identification cutoff (text in grey)
 ADD TAG CONTROL REVIEW

Shotgun NGS



ONT Shotgun flowchart:





Contents lists available at ScienceDirect

Diagnostic Microbiology & Infectious Disease

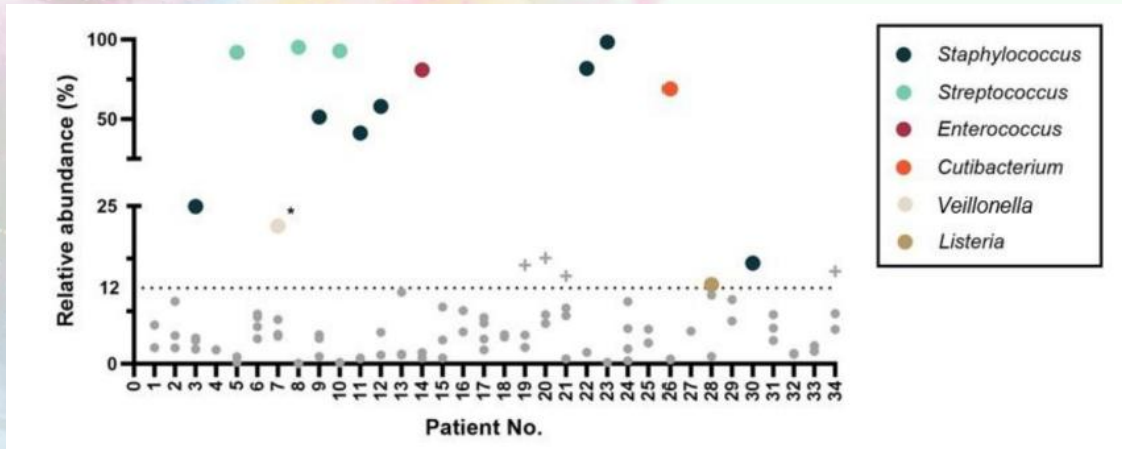
journal homepage: www.elsevier.com/locate/diagmicrob



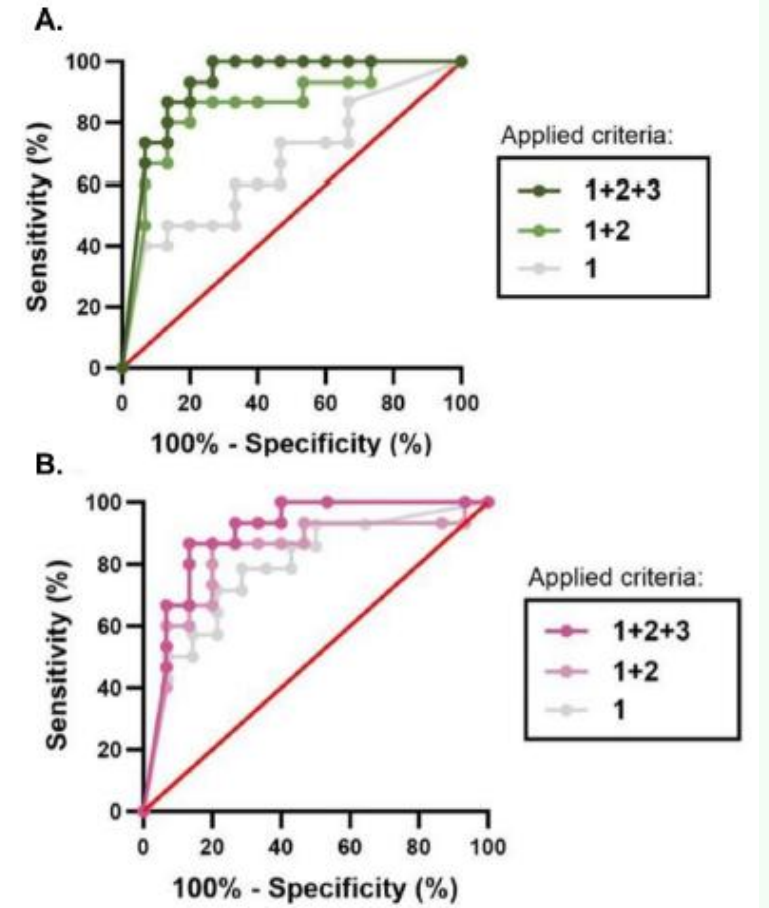
Original Article

Toward a clinical algorithm for the detection of periprosthetic joint infections using targeted NGS

Ander Uribarri^{a,b,*}, Lucia Henriquez^{a,b}, Iñaki Beguiristain^{a,b}, Ivan Rodriguez^{a,b}, Manuel Alfaro^a, Ignacio Sancho^{a,b}, Maria Eugenia Portillo^{a,b,c}



	Applied criteria:		
	1	1 + 2	1 + 2 + 3
AUC (SE)	0.671 (0.099)	0.856 (0.073)	0.924 (0.052)
95 % CI	0.476 – 0.867	0.712 - 0.999	0.823 - 1.000
p-value	0.110	0.001	<0.0001
Choosing >12 % classifiable reads as our cut off:			
Sensitivity	100 %	100 %	100 %
Specificity	13.04 %	71.43 %	90.50 %
PPV	35.48 %	68.42 %	86.67 %
NPV	100 %	100 %	100 %



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Version Examination	2011 MSIS Criteria ¹⁰²	2013 ICM Criteria ¹⁰³	2018 ICM Criteria ¹⁰⁴	2021 EBJIS Criteria ¹⁰⁵
Clinical indicators	Sinus tract (major); Puruce (minor).	Sinus tract(major); Purulence(removed).	Sinus tract (major); Purulence (minor,3 points).	Sinus tract (infection confirmed); Purulence (infection likely); Periprosthetic fracture nonunion (infection likely); Recent history of fever (infection likely); Secretions around the prosthesis (infection likely).
Peripheral blood serological	CRP and ESR : ↑ (minor)	CRP : > 10mg/L (chronic) ESR : > 30mm/h (chronic) (minor)	CRP>10 mg/L (preoperative,minor,2 points); D-dimer>860ng/mL(preoperative,minor,2points); ESR > 30 mm/h (preoperative,minor,1 point).	CRP > 10 mg/L (infection likely)
Synovial fluid cytological	WBC: ↑ (minor); PMN%: ↑(minor).	WBC>3000/μL (chronic) (minor); PMN%>80% (chronic) (minor).	WBC>3000/μL (preoperative,minor,3 points); PMN%>90% (preoperative,minor,2 points).	WBC>1500/μL (infection likely); >3000/μL (infection confirmed), PMN%>65% (infection likely); >80% (infection confirmed).
Synovial fluid serological	/	LE: (++) (minor)	α-defensin:positive(preoperative,minor,3points); LE: (++) (preoperative,minor,3 points).	α-defensin: positive (infection confirmed)
Pathogen detection	Periprosthetic tissue or synovial fluid culture: ≥ 2 positive results (major); 1 positive result (minor).	Periprosthetic tissue or synovial fluid culture: ≥ 2 positive results (major); 1 positive result (minor).	Periprosthetic tissue or synovial fluid culture: ≥2 positive results (postoperative,major); 1 positive result (postoperative,minor,2 points).	Periprosthetic tissue or synovial fluid culture: ≥ 2 positive results (infection confirmed); 1 positive result (infection likely. If the preoperative puncture and intraoperative tissue culture yield the same microorganism, it can be regarded as two positive confirmatory samples), sonication fluid>1CFU/mL(infection likely); > 50CFU/mL(infection confirmed).
Histopathological	> 5 neutrophils in each of 5 high-power fields (minor)	> 5 neutrophils in each of 5 high-power fields (minor)	>5 neutrophils in each of 5 high-power fields (postoperative, minor, 3 points)	> 5 neutrophils in each of 5 high-power fields(infection confirmed); > 5 neutrophils in 1 high-power field (infection likely), visible microorganisms (infection confirmed).
Nuclear imaging	/	/	/	Radiological signs of loosening within 5 years after implantation (infection likely), positive WBC scintigraphy (infection likely).



Unified Criteria for Periprosthetic Joint Infections (PJI)

Standalone criteria

Clinical features

- A sinus tract communicating from the joint to the outside environment that develops or persists after the incision has or should have healed

Microbiology

- Two positive cultures with a phenotypically indistinguishable organism from periprosthetic tissue
- One positive culture from synovial fluid or sonicate fluid PLUS one positive culture from periprosthetic tissue with a phenotypically indistinguishable organism

Inflammatory markers and histology

- Synovial leucocyte count >3000 cells/ μ L
- Synovial polymorphonuclear cells >75%
- Positive histology: 5 or more neutrophils in each of 5 or more high power fields (400x)

All without any alternative explanation¹

Specificity >95%

Supportive criteria

Microbiology

- A single positive synovial fluid, sonicate fluid or periprosthetic tissue culture
- A positive molecular test of any organism in synovial fluid, tissue or sonication fluid

Imaging

- A positive WBC-scintigraphy³
- A positive [¹⁸F]-FDG-PET/CT when performed more than 6 months after the index arthroplasty⁴

Inflammatory markers

- Synovial leucocyte count 1500 - 2999 cells/ μ L
- Synovial polymorphonuclear cells 65 - 74%
- Any alternative positive synovial fluid biomarker⁵

All without any alternative explanation¹

Specificity >80%

Confirmed PJI

One standalone criterion in any category

Probable PJI

One supportive microbiology criterion PLUS one supportive inflammatory criterion or imaging criterion

Microbial aetiology work-up across different continents.

Characteristics	Europe N = 55	Asia N = 24	Oceania N = 10	North America N = 3	South America N = 7	Africa N = 8	p value	Overall
Average number of samples: n (%)								
<3	0	10 (41.7)	1 (10)	0	1 (14.3)	0	<0.001	12 (11.2)
3-4	12 (21.8)	8 (33.3)	3 (30)	2 (66.7)	0	4 (50)		29 (27.1)
5-6	36 (65.5)	4 (16.7)	5 (50)	1 (33.3)	6 (85.7)	3 (37.5)		55 (51.4)
>6	7 (12.7)	2 (8.3)	1 (10)	0	0	1 (12.5)		11 (10.3)
Sonication performed	30 (54.5)	8 (33.3)	3 (30)	0	5 (62.5)	0	0.08	46 (43)
MALDI-TOF	49 (89.1)	12 (50)	10 (100)	1 (33.3)	5 (71.4)	3 (37.5)	0.001	80 (74.8)
Vitek/Vitek 2	2 (3.6)	9 (37.5)	1 (10)	0	2 (28.6)	1 (12.5)		15 (14)
>1 method	7 (12.7)	4 (16.7)	1 (10)	0	1 (14.3)	0		13 (12.1)
Don't know	0	0	0	2 (66.7)	0	4 (50)		6 (5.6)
Susceptibility method: n (%)								
Vitek/Vitek 2	30 (54.5)	16 (66.7)	10 (100)	1 (33.3)	4 (57.1)	3 (37.5)		64 (59.8)
Disc Diffusion	26 (47.2)	10 (41.7)	3 (30)	0	5 (71.4)	2 (25)		46 (43)
E-tests	9 (16.4)	0	1 (10)	0	3 (42.9)	0		13 (12.1)
MicroScan	9 (16.4)	0	0	0	0	0		9 (8.4)
>1 method	23 (41.8)	5 (20.8)	3 (30)	0	6 (75)	2 (25)		39 (36.4)
Don't know	0	0	0	2 (66.7)	0	4 (50)		6 (5.6)
Standard of susceptibility interpretation: n (%)								
EUCAST	52 (94.5)	1 (4.2)	9 (90)	1 (33.3)	1 (14.3)	5 (62.5)	<0.001	69 (64.5)
CLSI	0	22 (91.7)	1 (10)	2 (66.7)	1 (14.3)	2 (25)	<0.001	28 (26.2)
Both	2 (3.6)	0	0	0	0	0		2 (1.9)
Other	1 (1.8)	1 (4.2)			5 (71.4)	1 (12.5)		8 (7.5)
Media: n(%)								
Blood	51 (92.7)	21 (87.5)	9 (90)	3 (100)	6 (75)	7 (87.5)		97 (90.7)
Chocolate	35 (63.6)	13 (54.2)	10 (100)	3 (100)	5 (85.7)	5 (62.5)		71 (63.3)
MacConkey	24 (43.6)	14 (58.3)	5 (50)	2 (66.7)	5 (71.4)	4 (50)		54 (50.5)
Nutrient broth	35 (63.6)	11 (45.8)	8 (80)	1 (33.3)	4 (57.1)	4 (50)		63 (58.9)
Anaerobic	43 (78.2)	12 (50)	5 (50)	2 (66.7)	3 (42.8)	3 (37.5)		68 (63.6)
Other	10 (18.2)	8 (33.3)	1 (10)	0 (0)	3 (37.5)	1 (12.5)		23 (21.5)
>/=3 media	41 (74.5)	15 (62.5)	8 (80)	2 (66.7)	5 (62.5)	5 (62.5)		76 (71)
Culture duration and extension								
Median duration of incubationdays (IQR)	6 (5-9.5)	4 (3-5)	5 (5-6)	14 (5-21)	11 (12.25-14)	6.5 (2.25-11)		5 (3-7)
</=5 days (%)	12 (22.2)	16 (76.2)	8 (80)	1 (33.3)	3 (42.9)	3 (37.5)	<0.001	43 (41.7)
7 days (%)	20 (38.5)	4 (19)	1 (10)	0	3 (42.8)	0		28 (27.2)
>7 days (%)	20 (38.5)	1 (4.8)	1 (10)	2 (66.7)	1 (14.3)	5 (62.5)		30 (29.4)
Culture extension: n (%)	38 (73.1)	14 (66.7)	9 (90)	3 (100)	4 (57.1)	7 (87.5)		75 (72.8)
Median extension of cultures, days (range)	14 (7-30)	14 (7-28)	12 (10-14)	21 (10-28)	14 (14-14)	21 (7-42)		14 (7-30)
PCR Sequencing (16 s rDNA or metagenomics)	30 (54.5)	7 (29.2)	2 (20)	2 (66.7)	1 (14.3)	4 (50)	0.061	46 (43)
	23 (41.8)	5 (20.8)	10 (100)	1 (33.3)	1 (14.3)	4 (50)	<0.001	44



A global survey of diagnostic practices in prosthetic joint infections

Shradha Subedi ^{a,b,*}, Patrick NA Harris ^{a,c}, Paul Chapman ^{d,e}, Antonia F Chen ^f, Joshua S Davis ^g, Po-Yu Liu ^{h,i}, Leonard C Marais ^j, Mauro J Salles ^k, Jason A Roberts ^{a,e,l,m}, Jesus Rodriguez-Bano ^{n,q}, Marjan Wouthuyzen-Bakker ^o, David L Paterson ^{a,p}, Natividad Benito ^{a,q,r,**}



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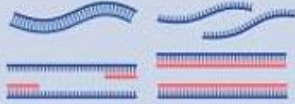
Conclusiones

- 1- Técnicas moleculares como **complemento** a los métodos convencionales.
- 2- Especialmente útiles en casos:
 - con **cultivos negativos**
 - infecciones **polimicrobianas**
 - cuando se requiere una **identificación rápida**
 - **patógenos infrecuentes** o de difícil crecimiento.
- 3- Gran **heterogeneidad: interpretación cuidadosa** de los hallazgos.
- 4- La validación sistemática, el análisis económico y la optimización de las vías regulatorias serán fundamentales para **convertir** estos avances en herramientas de **uso cotidiano**

Microbial Tests



Culture - aerobic and anaerobic



Multiplex nucleic acid amplification

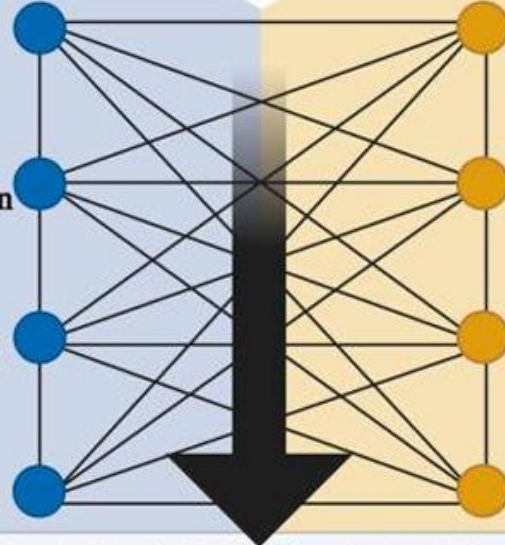


Shotgun metagenomic sequencing



16S rRNA PCR/sequencing
Microbial transcriptomic analysis

Integrated Multi-omic Analysis



Host Response Tests



Histopathology



Human cellularity profiling



Human proteomic analysis



Human transcriptomic analysis

Integrated Multi-omic Analysis - Arthroplasty Failure

Patient name: John Doe

Patient ID: 123456789

Specimen type(s): synovial fluid, periprosthetic tissue, sonicate fluid

Tests run: Microbial Tests

- Culture - aerobic and anerobic
- Multiplex nucleic acid amplification
- Shotgun metagenomic sequencing
- 16S rRNA PCR/sequencing
- Microbial transcriptomic analysis

Host Response Tests

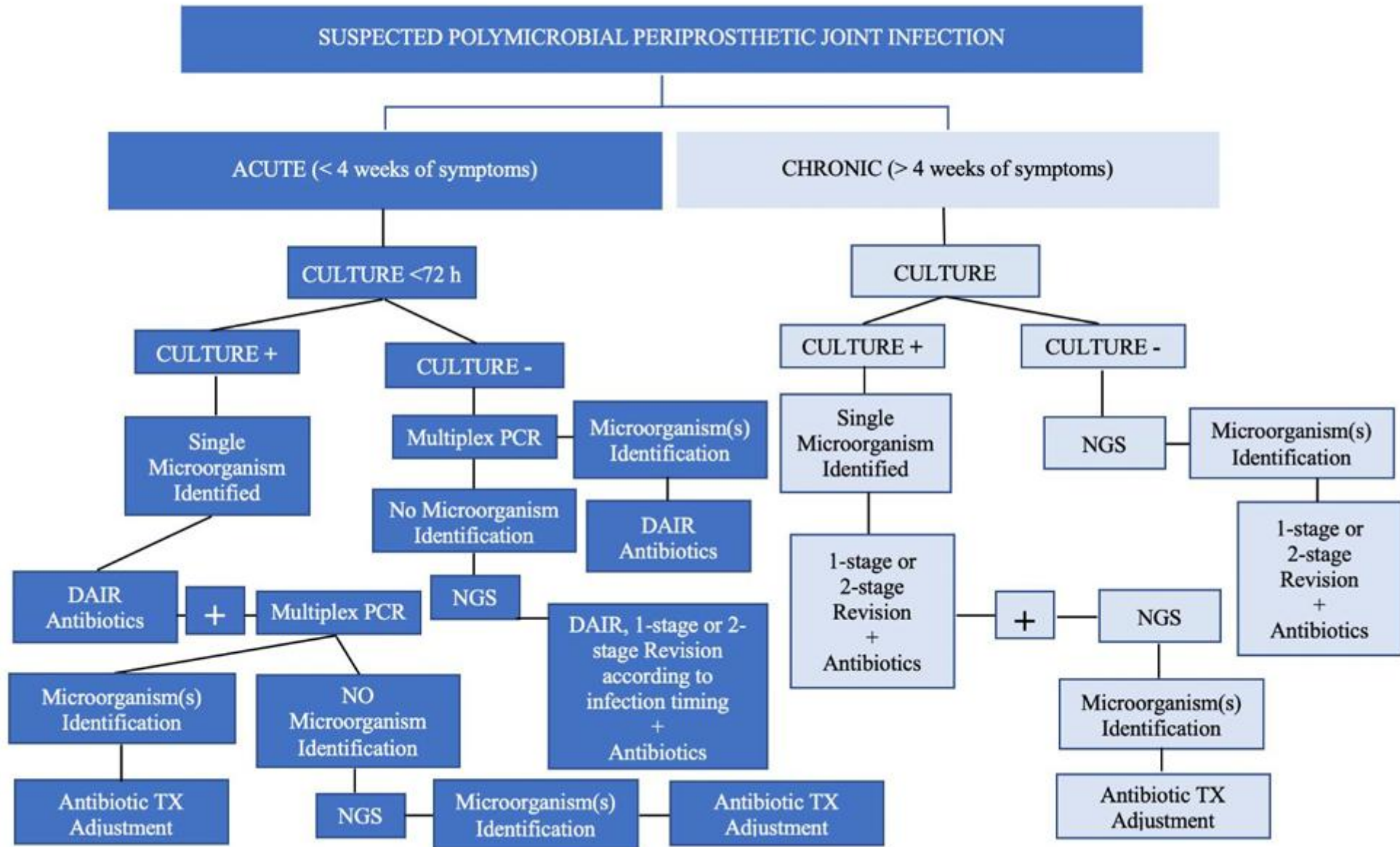
- Histopathology
- Human cellularity profiling
- Human proteomic analysis
- Human transcriptomic analysis

Results

- Likelihood of infection:95%
- Predicted cause:*Staphylococcus aureus*
- Resistance genes and mutations detected:*mecA*

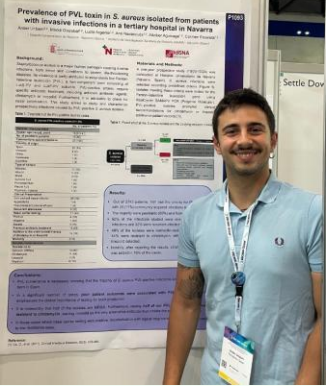
Suggested treatment regimen:





HK36: What is the diagnostic relevance of molecular techniques for polymicrobial PJI?





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