Diagnosis by molecular methods

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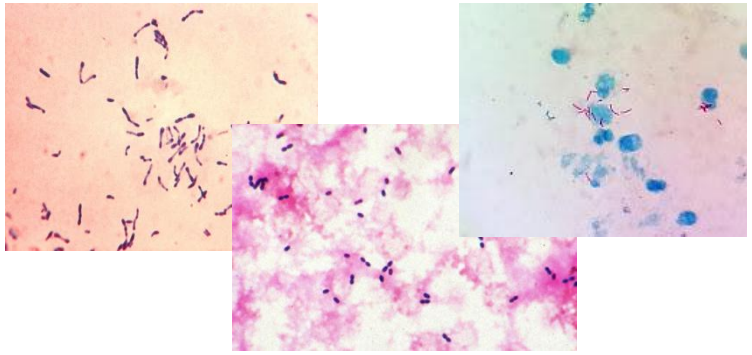
National Organization for Medicines, 14660/25-02-2011 and 47558/4-7-2012

Introduction

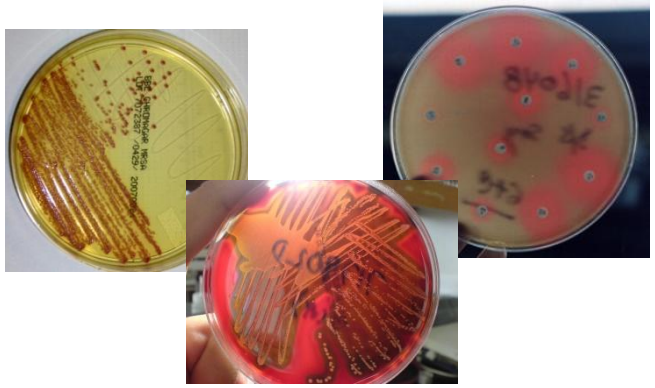
- Procedures and technical difficulties of molecular microbiological diagnosis of bone and joint infections
- State of the art techniques

Microbiological diagnosis

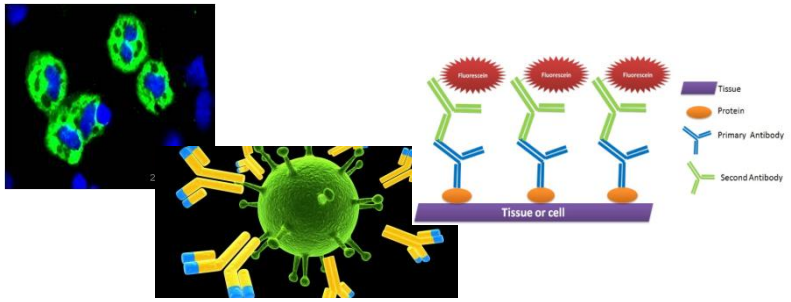
Microscopy



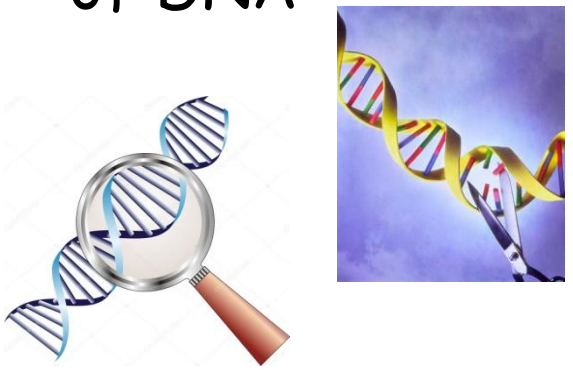
Culture



Serological detection of antibodies and antigens



Molecular detection of DNA



European Journal of Nuclear Medicine and Molecular Imaging (2019) 46:957–970
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GUIDELINES

Consensus document for the diagnosis of peripheral bone infection in adults: a joint paper by the EANM, EBJIS, and ESR (with ESCMID endorsement)

Andor W. J. M. Glaudemans[1] · Paul C. Jutte[2] · Maria Adriana Cataldo[3] · Victor Cassar-Pullicino[4] · Olivier Gheysens[5] · Olivier Borens[6] · Andrej Trampuz[7] · Klaus Wörtler[8] · Nicola Petrosillo[3] · Heinz Winkler[9] · Alberto Signore[10] · Luca Maria Sconfienza[11,12]

- √ European Bone and Joint Infection Society
- √ European Society of Clinical Microbiology and Infectious Diseases
- √ European Society of Radiology
- √ European Association of Nuclear Medicine

Glaudemans et al, 2019

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- √ **The gold standard for the correct identification of the causative microorganism of PBI is represented by culture of infected bone.**
- √ Bone biopsy samples should always be collected from a zone in which the bone structure is visibly inflamed.
- √ A minimum of three tissue samples should be collected. The more samples that are withdrawn, the less chance of an incorrect assessment due to contamination is reported.
- √ Collected pieces should be divided for bacteriology and histology.
- √ The samples should be sent for aerobic and anaerobic cultures; cultures for mycobacteria and fungi should be performed in patients with clinical and epidemiological features supporting a suspicion for these etiologies.
- √ Samples collected directly from the skin should be avoided since these biopsies are often contaminated with skin microbes, leading to false-positive results.

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infected bone. Prior to collecting microbiological samples, any antibiotic regimen should be discontinued for 2 weeks, provided the progression of the disease allows this.

Biopsies should be taken under image guidance to provide representative samples. Bone is easily visualized with conventional X-ray and fluoroscopy. However, bone biopsies are generally conducted using CT guidance, which has the advantage of providing higher contrast resolution and better visualization of surrounding soft tissues, thus allowing for better evaluation of the exact location of the lesion and position of the needle. MRI guidance is rarely used for obtaining a bone biopsy. Because of the electromagnetic radiation, MRI-guided bone biopsy requires a special needle made of non-ferromagnetic stainless steel. Other disadvantages of MRI are longer procedural time and higher costs. MRI guidance should only be used in very selected cases like pediatric ones [14].

Bone biopsy samples should always be collected from a zone in which the bone structure is visibly inflamed. Tissue near visible bone or sequestra is informative. Collected pieces should be divided into two pieces for bacteriology and histology.

A minimum of three tissue samples should be collected. The more samples that are withdrawn, the less chance of an incorrect assessment due to contamination is reported. Whenever bone biopsies are done, the samples should be sent for aerobic and anaerobic cultures, cultures for mycobacteria and fungi should be performed in patients with clinical and epidemiological features supporting a suspicion for these etiologies. Samples collected directly from the skin should be avoided since these biopsies are often contaminated with skin microbes, leading to false-positive results. Histopathological analysis is essential for confirming or excluding the diagnosis of infection. Visualization of granulatomatous lesions with positive Ziehl–Neelsen staining may allow the diagnosis of mycobacterial infection (e.g., *Mycobacterium tuberculosis*).

Because bone biopsy is an invasive diagnostic method, several studies examined the diagnostic values of sinus tract cultures. However, these tract cultures are often contaminated with skin microbes, leading to a higher number of false-positive results. Superficial swab cultures showed inferior diagnostic values to sinus tract cultures and bone biopsy, and should not be used. New molecular methods can further improve the microbiological diagnosis [15].

Radiological and nuclear medicine imaging methods and limitations

Several commonly used radiological and nuclear-medicine imaging methods are available (see Tables 1 and 2). An extensive description on the correct use of these techniques is provided in Appendix 2 [16–24]. The concerns on the use of ionizing radiation is described in Appendix 3 (https://ec.europa.eu/energy/sites/ener/files/documents/CELEX-32013L0059-EN-TXT.pdf, [25]).

Consensus statements

All performed PICOs for the statements and the papers finally included for the level of evidence are mentioned in Appendix 4.

1. **Patients presenting with clinical and radiological signs of peripheral bone infection or a positive probe-to-bone test may require further diagnostic procedures.**

   Level of evidence: 5

   In case of clinical and radiological suspicion of peripheral bone infection, further diagnostic testing can be indicated to reveal severity and extent of the infection. Patients with acute peripheral bone infection can present with local pain, swelling, erythema, and warmth at the site of infection, and systemic symptoms such as fever and general illness. If a fistula is present, a probe to the bone test can be performed. In diabetic foot, this is indicative of bone infection, however, there is no literature supporting that statement in PBI. In general, in the acute phase with clear clinical signs, advanced imaging is often not necessary.

2. **Fistula direct to the bone and purulent discharge are evidence of bone infection.**

   Level of evidence: 5

   There are no articles that provide evidence for this statement. It is based on common medical reasoning; bacteria that normally are present as part of skin flora superficially spread and colonize the exposed bone thereby causing local infection.

3. **CRP, ESR, and WBC counts should always be performed in patients suspected of having peripheral bone infection for diagnostic purposes.**

   Level of evidence: 4

   In patients with PBI, raised ESR and CRP can be present, even if inconsistently, and can orientate versus a diagnosis of infection. White blood cell counts are more rarely increased. In patients with contiguous pedal osteomyelitis, the positive predictive value of ESR in diagnosing osteomyelitis in patients without diabetes was 78%, and in those with diabetes was 81%, with a negative predictive value 58 and 31%,

New molecular methods can further improve the microbiological diagnosis [15].

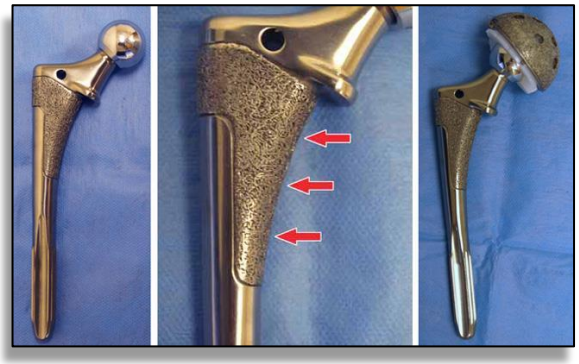
In a consensus guidelines paper of 14 pages and 7,362 words, signed by four international societies, the phrase "molecular methods" is only stated once and in a very ambiguous way

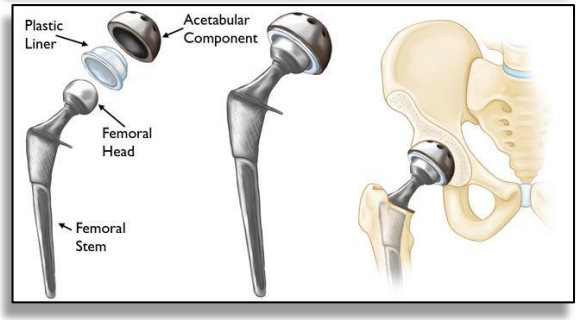
Glaudemans et al, 2019

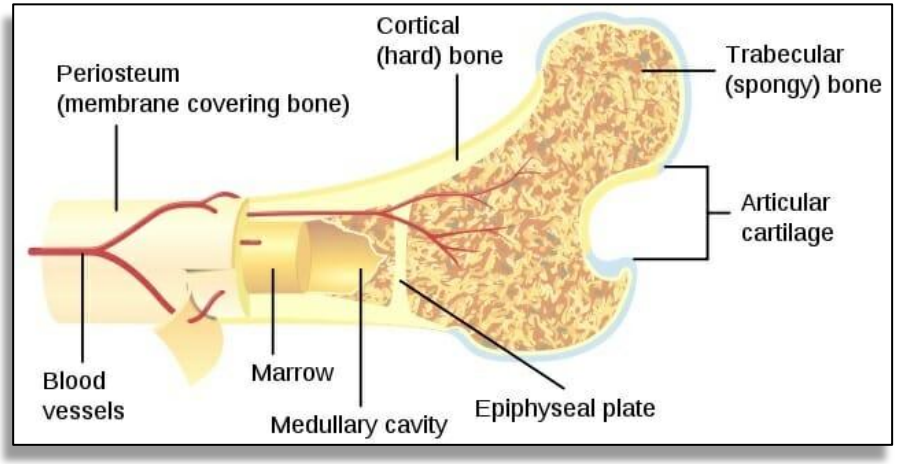
Issues with molecular microbiological diagnosis of bone infections

- The clinical specimen - the difficulty issue
- The most common pathogens - the easiness issue
- The PCR target(s) – the multiplex issue

- Issue No 1: the clinical specimen
  - √ Bone tissue → DNA extraction is extremely difficult and it requires harsh treatment of the bone with metallic beads, which can also easily destroy bacterial cells
  - √ Preferable specimen the surrounding soft tissues, but not always infected
  - √ Prosthetic devises (metal) → no DNA extraction is possible at all, only sampling of the biofilm surrounding the metallic devise

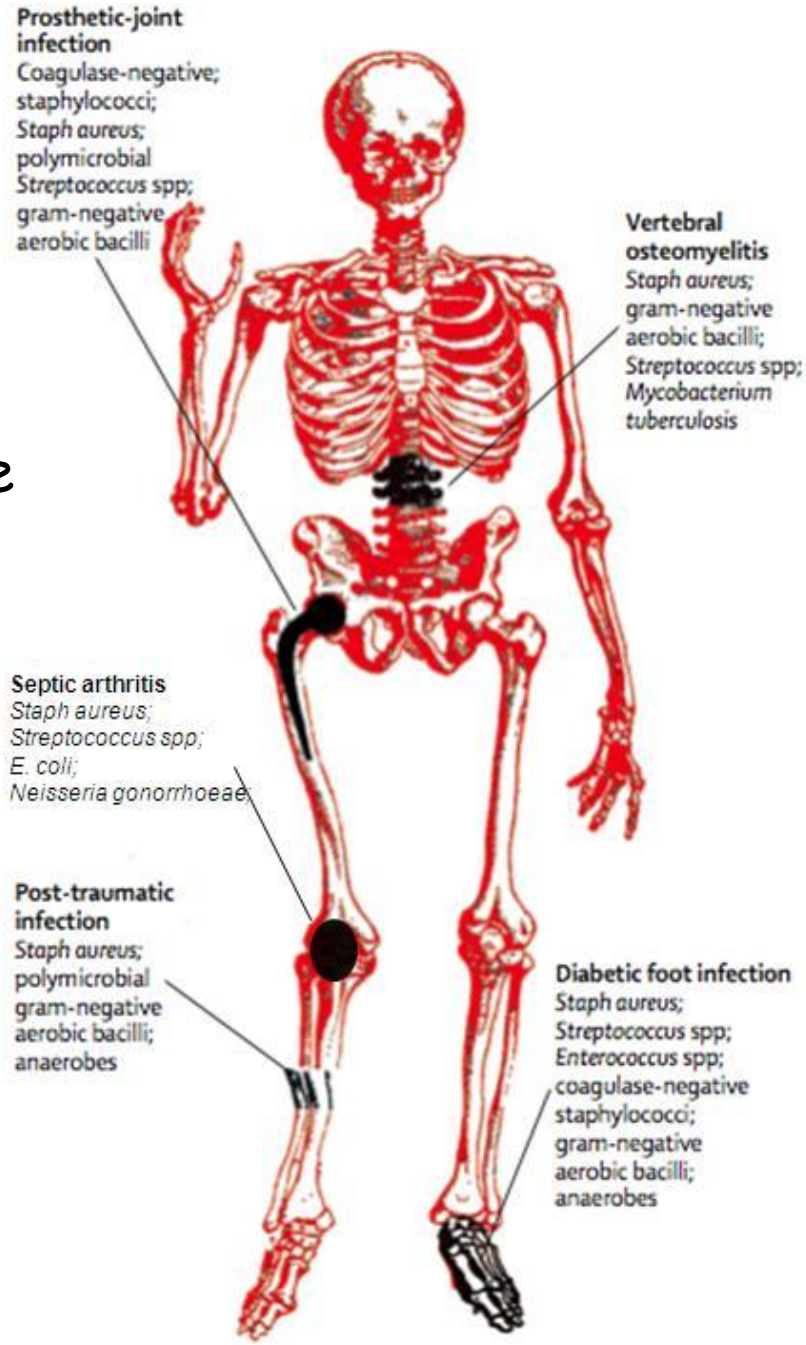




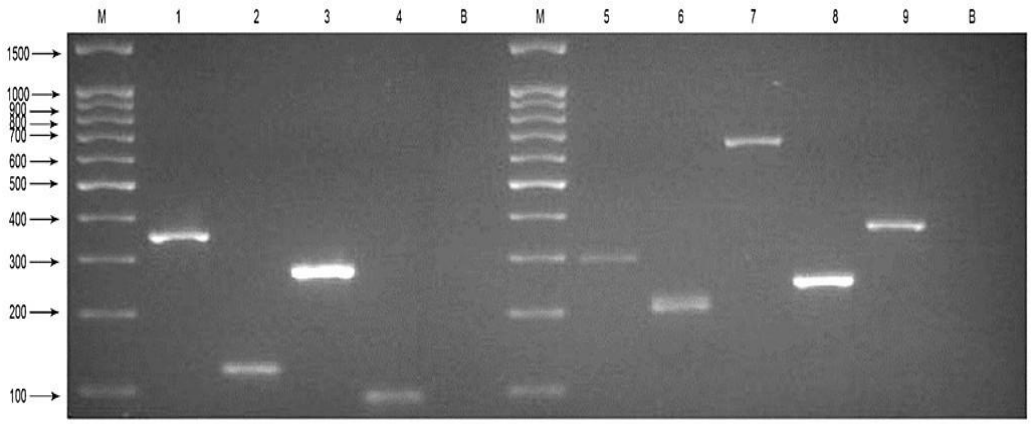


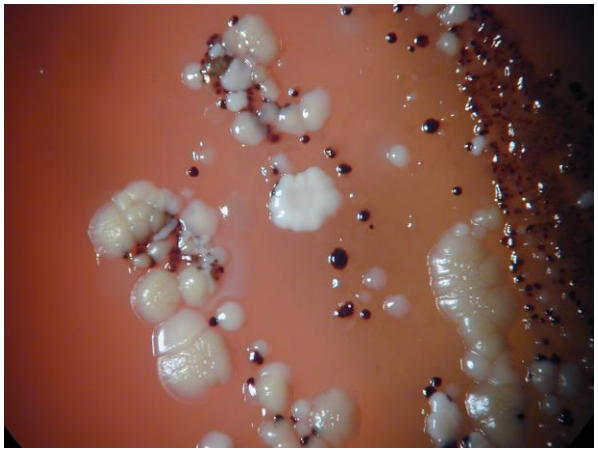
Florencio-Silva et al, 2015

Issue No 2: The most common pathogens are easy to culture



- Issue No 3: the number of the PCR targets
  - √ Until recently, PCR was performed for a single pathogens at any one time (single-plex), or, in the best case scenario, for a limited number of pathogens/targets (multi-plex) at any one time
  - √ The number of targets detected at the same time depends on the nucleotide sequence of each target
  - √ Thus, molecular diagnosis is species-specific, whereas conventional diagnosis is syndrome-specific





Common pathogens and DNA targets

Streptococcus pyogenes	*spy1*258 (transcription regulator) , *spe*B (toxin)
Staphylococcus aureus	*nuc* (nuclease), *mecA* (MRSA resistance)
Klebsiella pneumoniae	*rmpA* (regulator of polysaccharide synthesis)
Pseudomonas aeruginosa	*relBE, higBA, parDE (toxin-antitoxin system*)
Mycobacterium tuberculosis	IS610 (repeated insertion sequence)

AMERICAN Clinical Microbiology
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REVIEW

Check for updates

Syndromic Panel-Based Testing in Clinical Microbiology

Poornima Ramanan,[a] Alexandra L. Bryson,[a] Matthew J. Binnicker,[a] Bobbi S. Pritt,[a,b] Robin Patel[a,b]

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Ramanan et al, 2018

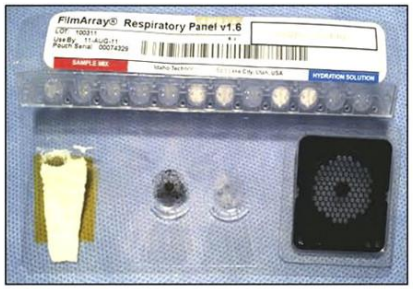
Curetis
Unyvero

bioMerieux
FilmArray

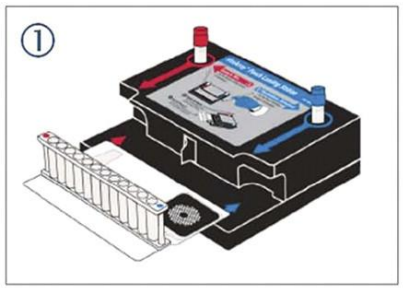
Roche
LightCycler

Luminex
Verigene

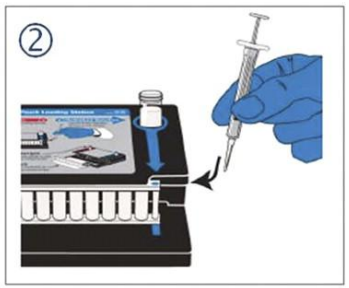
GenMark
ePlex



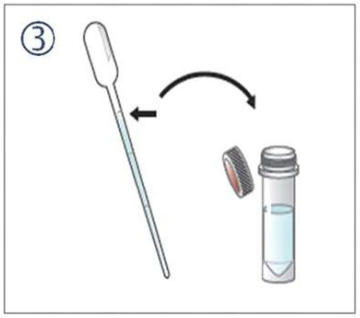
FilmArray RP Pouch



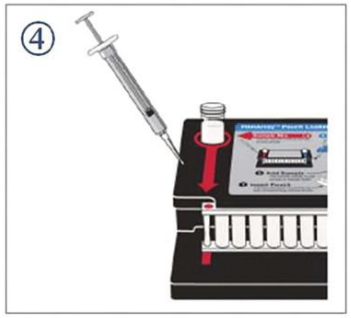
Load Pouch



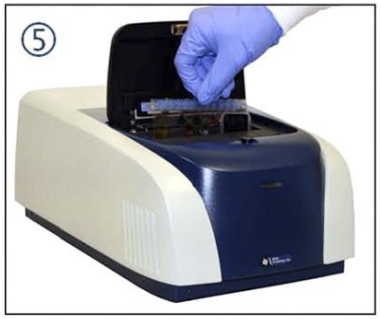
Inject Hydration Solution



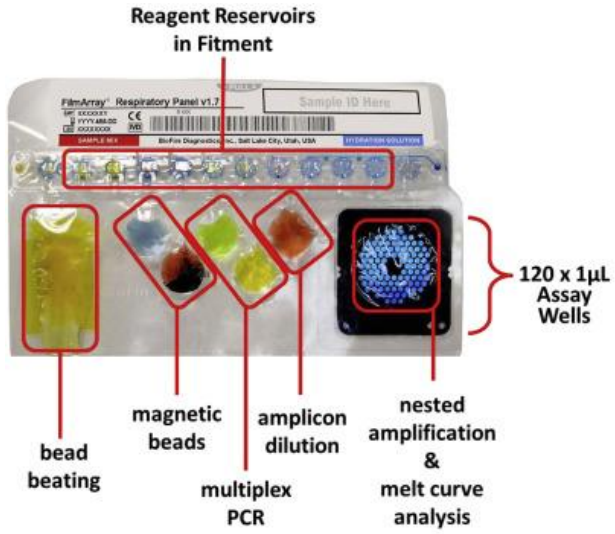
Add Sample to Buffer



Inject Sample



Load Pouch in FilmArray



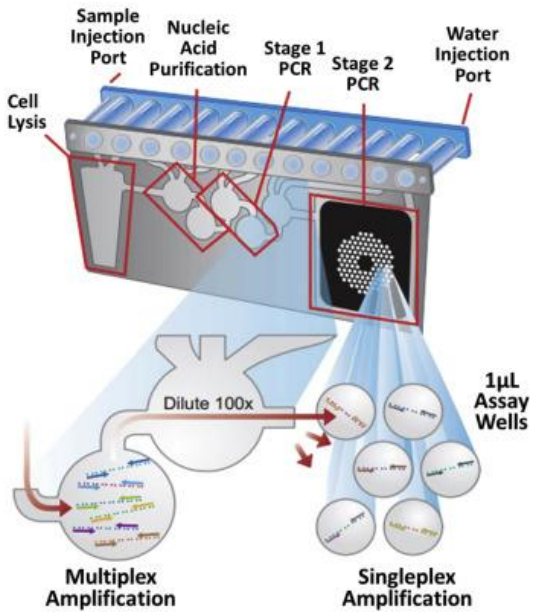


TABLE 1 FDA-approved/cleared panel-based molecular assays for detection of select microorganisms and select resistance genes in positive blood culture bottles

Parameter	FilmArray BCID	Verigene	
		Gram-positive blood culture	Gram-negative blood culture
Total no. of targets	27	15	14
Ability to detect pathogen			
Gram-positive bacteria			
Staphylococcus species			
Staphylococcus aureus			
Staphylococcus epidermidis			
Staphylococcus lugdunensis			
Streptococcus species			
Streptococcus agalactiae			
Streptococcus pyogenes			
Streptococcus pneumoniae			
Streptococcus anginosus group			
Enterococcus species			
Enterococcus faecalis			
Enterococcus faecium			
Listeria species			
Listeria monocytogenes			
Gram-negative bacteria			
Klebsiella oxytoca			
Klebsiella pneumoniae			
Serratia marcescens			
Proteus species			
Acinetobacter species			
Acinetobacter baumannii			
Haemophilus influenzae			
Neisseria meningitis			
Pseudomonas aeruginosa			
Enterobacteriaceae			
Escherichia coli			
Enterobacter species			
Enterobacter cloacae complex			
Citrobacter species			
Yeasts			
Candida albicans			
Candida glabrata			
Candida krusei			
Candida parapsilosis			
Candida tropicalis			
Ability to detect presence of resistance gene			
mecA	✓	✓	
vanA	✓	✓	
vanB	✓	✓	
bla_{KPC}	✓		✓
bla_{NDM}			✓
bla_{OXA}			✓
bla_{VIM}			✓
bla_{IMP}			✓
bla_{CTX-M}			✓
Time to result (h)	~1	~2.5	~2

TABLE 3 FDA-approved/cleared multiplex respiratory panels[a]

Parameter	FilmArray	Verigene	x-TAG RVP	x-TAG RVP Fast	NxTAG-RPP	eSensor RVP	ePlex
Analysis platform	FilmArray system or FilmArray Torch	Verigene system	Luminex 100/200	Luminex 100/200	Luminex Magpix	eSensor	ePlex system
No. of targets	20	16	12	8	20	14	17
Ability to detect pathogen							
Viruses							
Adenovirus	✓	✓					
Coronavirus							
Coronavirus HKU1	✓						
Coronavirus NL63	✓						
Coronavirus 229E	✓						
Coronavirus OC43	✓						
Human bocavirus							
Human metapneumovirus	✓	✓					
Influenza A virus	✓	✓					
Subtype H1	✓	✓					
Subtype H3	✓	✓					
Subtype 2009 H1N1	✓						
Influenza B virus	✓	✓					
Parainfluenza virus 1	✓	✓					
Parainfluenza virus 2	✓	✓					
Parainfluenza virus 3	✓	✓					
Parainfluenza virus 4	✓	✓					
Respiratory syncytial virus	✓						
Respiratory syncytial virus A		✓					
Respiratory syncytial virus B		✓					
Rhinovirus/enterovirus	✓	✓					
Bacteria							
Chlamydophila pneumoniae	✓						
Mycoplasma pneumoniae	✓						
Bordetella pertussis	✓	✓					
Bordetella parapertussis-Bordetella bronchiseptica		✓					
Bordetella holmesii		✓					
Time to result (h)	~1	~2–3					

[a]The acceptable specimen type for all panels is a nasopharyngeal swab. RVP, respir

TABLE 4 FDA-approved/cleared multiplex gastrointestinal panels[a]

Parameter	Verigene EP	Luminex GPP	BioFire GIP
Analysis platform	Verigene system	Magpix or Luminex 100/200 system	FilmArray system or FilmArray Torch
Acceptable specimen type	Stool in Cary-Blair medium	Fresh stool or stool in Cary-Blair medium	Stool in Cary-Blair medium
No. of targets	9	14	22
Ability to detect pathogen			
Bacteria			
Campylobacter species	✓	✓	✓
Salmonella species	✓	✓	✓
Shigella species/enteroinvasive *E. coli*[b]	✓	✓	✓
Vibrio species	✓		✓
Vibrio cholerae		✓	✓
Yersinia enterocolitica	✓		✓
Escherichia coli O157		✓	✓
Enterotoxigenic *E. coli*		✓	✓
Enteropathogenic *E. coli*			✓
Enteroaggregative *E. coli*			✓
Plesiomonas shigelloides			✓
Shiga toxin-producing *E. coli* (stx_1-stx_2)	✓[c]	✓	✓
Clostridium difficile (toxin A/B)		✓	✓
Viruses			
Norovirus GI/GII	✓	✓	✓
Rotavirus A	✓	✓	✓
Astrovirus			✓
Adenovirus 40/41		✓	✓
Sapovirus			
Parasites			
Cryptosporidium species			
Entamoeba histolytica			
Giardia lamblia			
Cyclospora cayetanensis			
No. of samples (throughput)			
Time to result (h)			

[a]EP, enteric pathogens; GPP, gastrointestinal pathogen
[b]The Verigene EP and Luminex GPP do not specifically
[c]The Verigene EP has separate targets for stx_1 and stx_2

TABLE 6 Organisms targeted by the FilmArray Meningitis/Encephalitis panel

Parameter	FilmArray Meningitis/Encephalitis panel
Pathogen detected	
Viruses	Cytomegalovirus, enterovirus, herpes simplex virus 1, herpes simplex virus 2, human herpesvirus 6, human parechovirus, varicella-zoster virus
Bacteria	*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*
Fungi	*Cryptococcus neoformans-C. gattii*
Analysis platform	FilmArray system or FilmArray Torch
Acceptable specimen type	CSF
Time to results (h)	~1

Ramanan et al, 2018

Future MICROBIOLOGY

Research Article

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Performance of the automated multiplex PCR Unyvero implant and tissue infections system in the management of diabetic foot osteomyelitis

Catherine Dunyach-Remy[‡,1], Charlotte Carrere[‡,1], Hélène Marchandin[2], Sophie Schuldiner[3], Anne-Marie Guedj[4], Nicolas Cellier[5], Axelle Cadière[6], Catherine Lechiche[7], Albert Sotto[8] & Jean-Philippe Lavigne*[,1]

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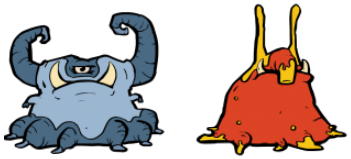
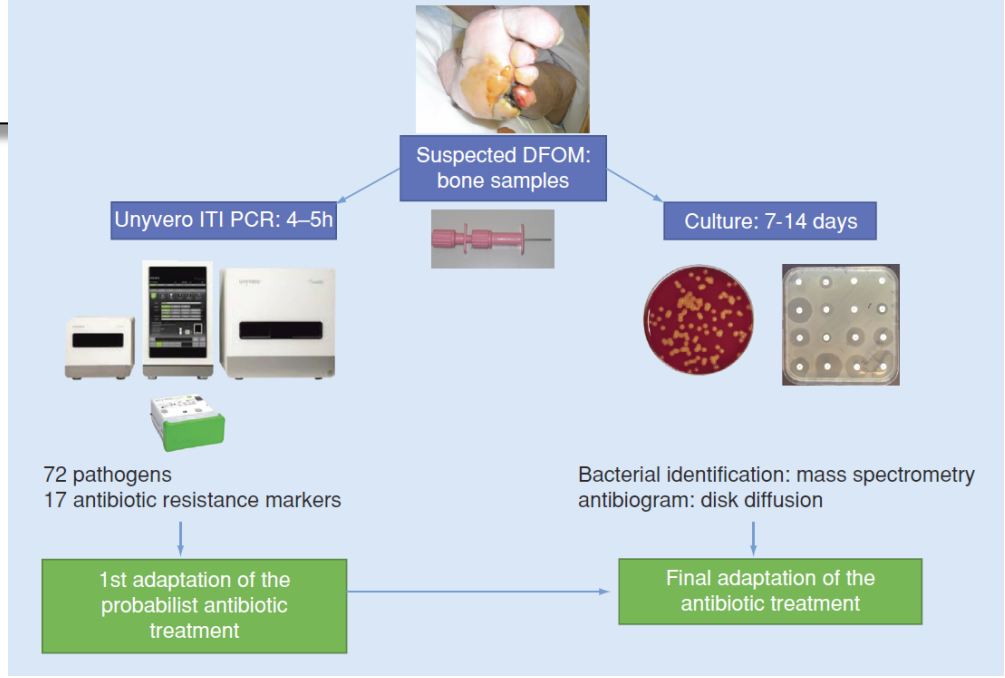
‡Authors contributed equally





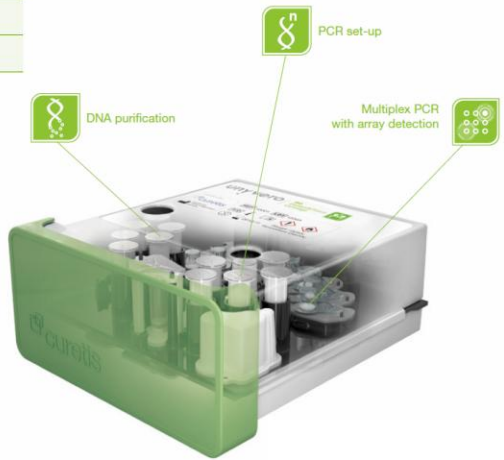
Dunyach-Remy et al, 2018

GROUP	PATHOGEN
Gram-positive bacteria	*Staphylococcus aureus*
	Coagulase negative staphylococci [1]
	Streptococcus agalactiae
	Streptococcus pyogenes [2]
	Enterococcus faecalis
	Enterococcus spp. [3]
Nutritionally variant Streptococci	*Granulicatella adiacens*
	Abiotrophia defectiva
Corynebacteriaceae	*Corynebacterium* spp. [4]

RESISTANCES	RESISTANCE AGAINST
mecA	Oxacillin/ Methicillin
mecC (LGA251)	Oxacillin/ Methicillin
aac(6')aph(2")	Carbapenem
ermA	Macrolide
ermC	Macrolide
van A	Vancomycin
vanB	Vancomycin
rpoB	Rifampin *(S.aureus)*

GROUP	PATHOGEN
Enterobacteriaceae	*Escherichia coli*
	Enterobacter cloacae complex
	Enterobacter aerogenes
	Proteus spp.[5]
	Klebsiella oxytoca
	Klebsiella pneumoniae [6]
Non-fermenting bacteria	*Acinetobacter baumannii* complex
	Pseudomonas aeruginosa
Anaerobic bacteria	*Propionibacterium acnes*
	Propionibacterium avidum/granulosum
	Finegoldia magna
	Bacteroides fragilis group [7]
Fungi	*Candida parapsilosis*
	Candida albicans

RESISTANCES	RESISTANCE AGAINST
ctx-M	3rd generation Cephalosporins
vim	Carbapenem
imp	Carbapenem
kpc	Carbapenem
ndm	Carbapenem
aacA4	Aminoglycoside
gyrA	Quinolones
oxa-23	Carbapenem
oxa-24	Carbapenem
oxa-48	Carbapenem
oxa-58	Carbapenem



Analyte	Detection Limit (Pathogens/mL)	Positivity Rate at 1/10 of the Detection Limit
Universal Bacteria	10^5	85%
Staphylococcus aureus	10^5	58%
Coagulase negative *Staphylococci*	10^4	Not tested
Streptococcus spp.	10^5	52%
Streptococcus pneumoniae	10^4	Not tested
Streptococcus agalactiae	10^4	Not tested
Streptococcus pyogenes/dysgalactiae	10^4	Not tested
Granulicatella adiacens	10^5	0%

Analyte	Detection Limit (Pathogens/mL)	Positivity Rate at 1/10 of the Detection Limit
Abiotrophia defectiva	10^5	50%
Enterococcus spp.	10^5	20%
Enterococcus faecalis	10^6	63%
Corynebacterium spp.	10^5	38%
Escherichia coli	10^4	Not tested
Enterobacter cloacae complex	10^5	25%
Enterobacter aerogenes	10^5	0%
Proteus spp.	10^4	Not tested
Klebsiella pneumoniae	10^5	Not tested
Klebsiella oxytoca	10^4	Not tested
Klebsiella variicola	10^4	Not tested
Citrobacter freundii/koseri	10^5	50%
Pseudomonas aeruginosa	10^4	Not tested
Acinetobacter baumannii complex	10^4	Not tested
Propionibacterium acnes	10^5	33%
Finegoldia magna	10^6	22%
Bacteroides fragilis group	10^4	Not tested
Candida spp.	10^5	68%
Candida albicans	10^5	47%
Candida tropicalis	10^8	0%
Candida glabrata	10^5	25%
Issatchenkia orientalis (C. krusei)	10^6	0%

Evaluation

- Sensitivity: 50.1% up to 100.0%
- Specificity: 91.7% up to 100.0%

- The sensitivity differences (and hence the negative predictive value differences) are due to:
  - √ The bacterial load at the site of the infection
  - √ The clinical specimen selection
  - √ The DNA target copy number per bacterial cell
- The specificity differences are due to the different CoNS targets

Ramanan et al, 2018

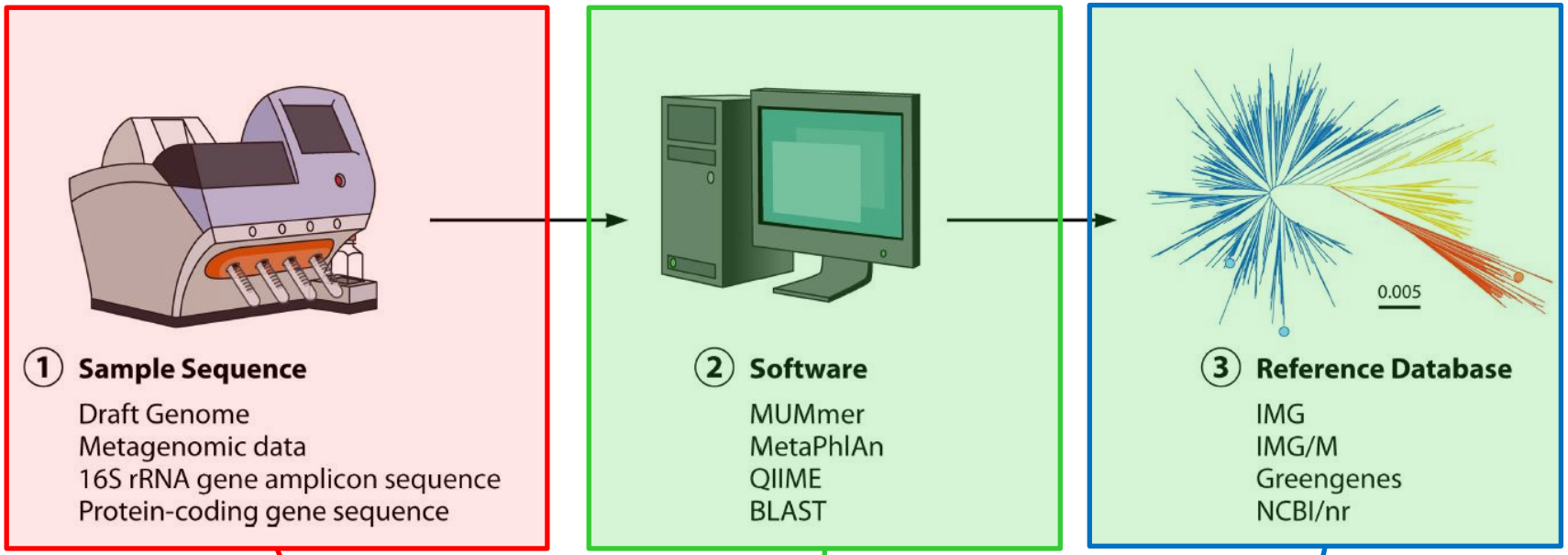
Strategies for Next Generation Sequencing

1. **Whole Genome Sequencing (WGS)**
   100+ Gb sequencing
2. **Whole exome sequencing (WES)**
   Capture all (200,00) exons & sequence them
   Less sequencing & analysis (38 Mbs)
3. **Target sequencing**
   Capture regions of interest & sequence them
4. **Transcriptone sequencing (RNAseq)**
   What is being actively transcribed in the cell of interest
   20,000 protein coding genes

Targeted NGS

1. Selection of DNA targets of clinical interest
2. Sequencing of the DNA sections with or without prior amplification
3. Evaluation of the obtained information and completion of the diagnostic process

NGS information workflow



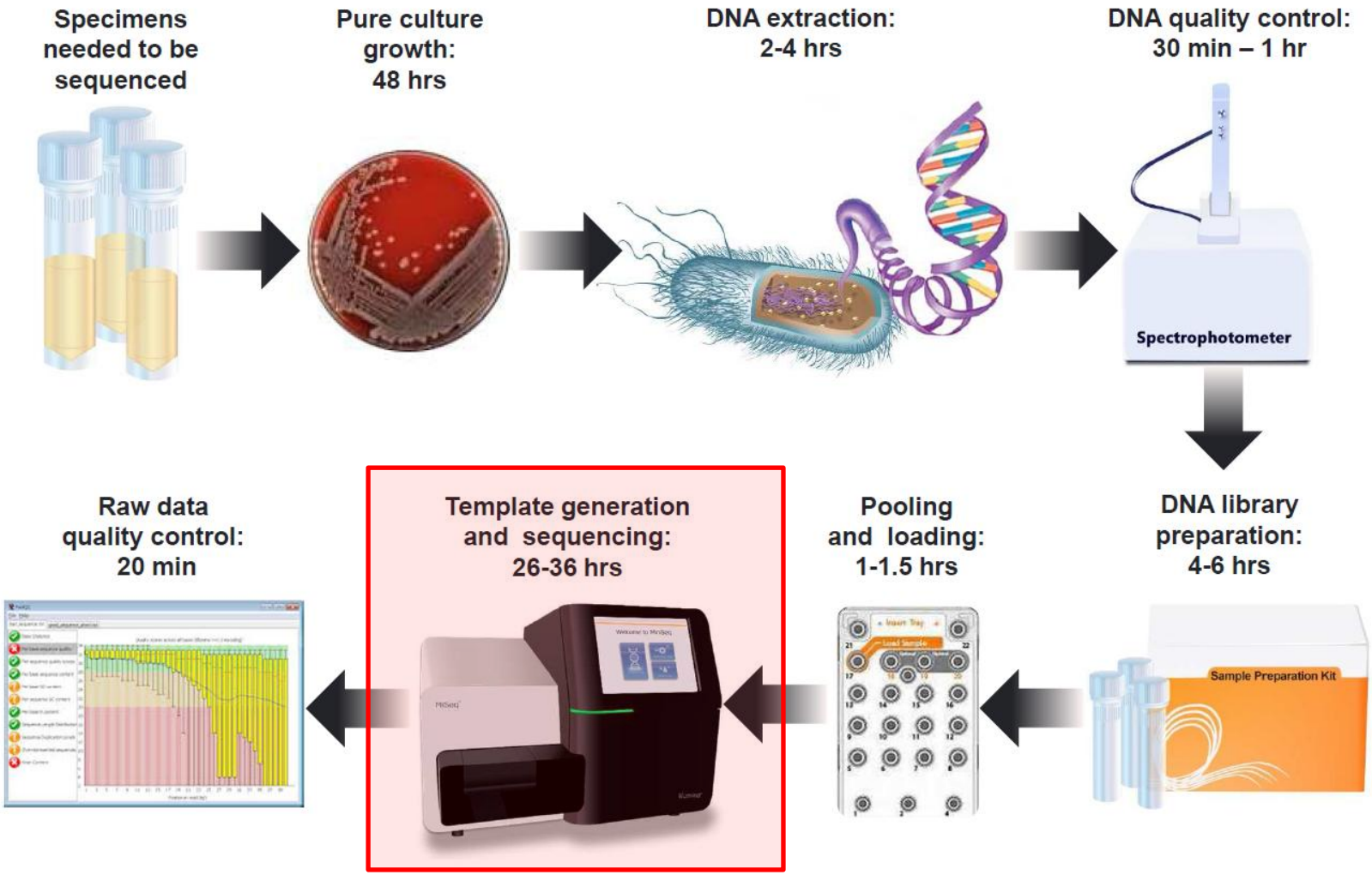
Production…

Processing…

Translation…

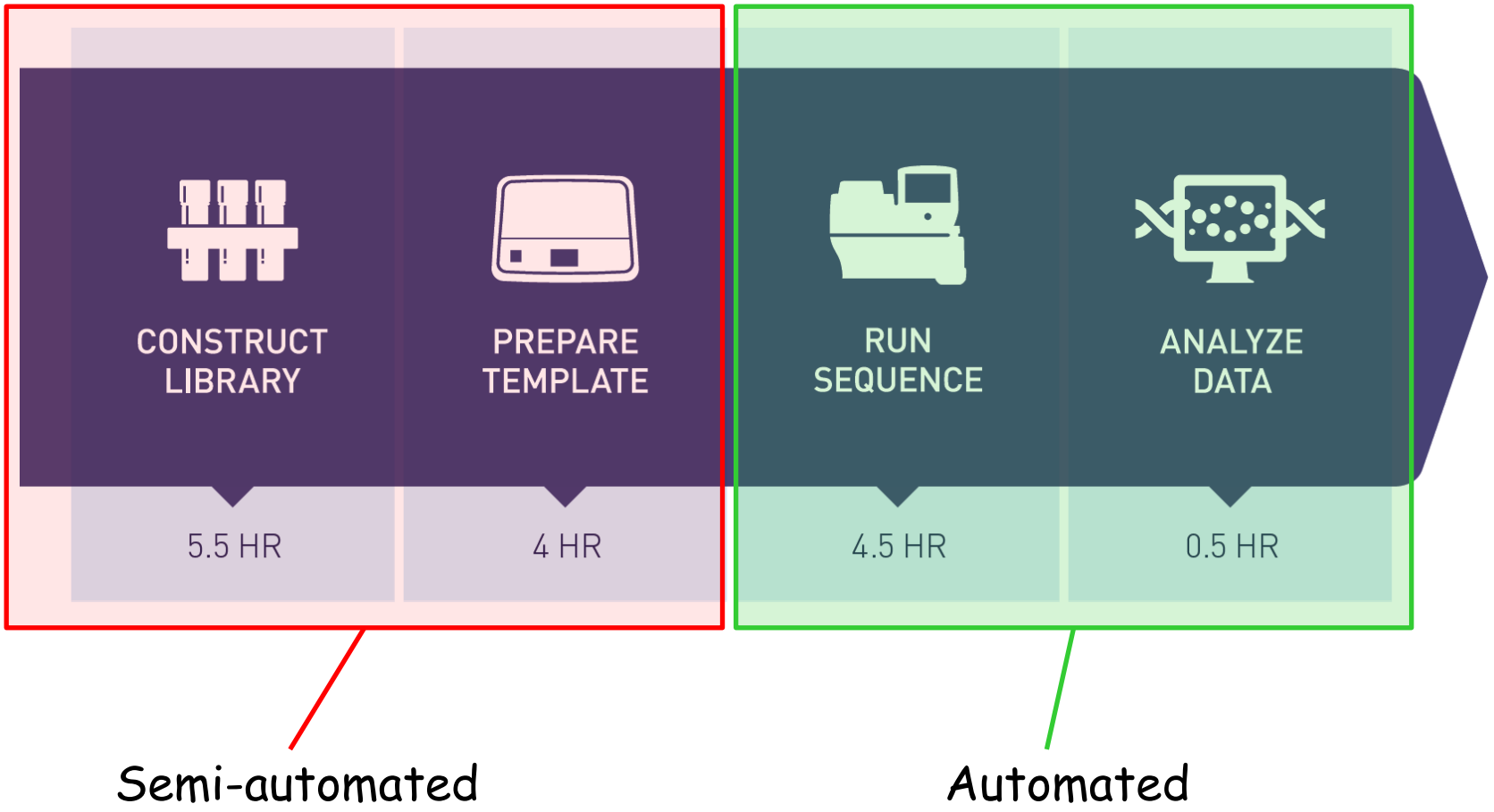
…of the information

NGS workflow



Besser et al, CMI 2017

NGS workflow



http://www.thermofisher.com

Advantages and disadvantages

Advantages

√ Targeted sequencing of DNA regions of clinical importance

√ Time management → results within the working hours

√ Information size → 10-50 Mbp vs >400 Mbp

√ Low detection limit (up to 1 copy per ml)

Disadvantages

√ Losing of the whole image (sequencing of the whole genome)

√ No identification of new DNA regions of potential clinical significance

Main companies and platforms

Table 1
Properties of current NGS platforms.

Company	Equipment	Output/run (Gb)	Maximum read length (bp)	Reads (x10^6)	Running time
Illumina	MiniSeq	0.6–7.5	2 × 150	25	4–24 h
Illumina	Miseq	0.3–15	2 × 300	25	5–55 h
Illumina	NextSeq	20–120	2 × 150	130/400	12–30 h
Illumina	HiSeq 3000	125–700	2 × 150	2500	<1–3.5 days
ThermoFisher	Ion PGM™	0.03–2	200–400	0.4–5.5	2–7 h
ThermoFisher	Ion 5S™	0.6–15	200–400	3–80	2.5–4 h
ThermoFisher	Ion 5S™ XL	0.6–15	200–400	3–80	<24 h
Oxford Nanopore	MinION	21–42	230,000–300,000	2.2–4.4	1 min–48 h
Pacific Biosciences[a]	Sequel	0.75–1.25	>20,000	370,000	30 min–6 h
Pacific Biosciences[a]	RSII	0.5–1	>20,000	55,000	30 min–4 h

[a] The Pacific Biosciences data are per smart cell; both the Sequel and the RSII can run 1–16 smart cells in one run.

- √ Platforms for smaller fragment sequencing
- √ Faster turn-around time

Deurenberget al, JB 2017

Table 2
Software packages frequently used for NGS data analyses in our laboratory.

Application	Software	Link	Note
Annotation	Prokka	www.vicbioinformatics.com	
	RAST	http://rast.nmpdr.org	
Assembly	BioNumerics	www.applied-maths.com	Commercial software
	CLC Genomic Workbench	www.clcbio.com	Commercial software
	SeqSphere	www.ridom.de	Commercial software
	SPAdes	http://bioinf.spbau.ru/spades	Unix-based
	Velvet	www.ebi.ac.uk/~zerbino/velvet	Unix-based
Data quality check	BaseSpace	https://basespace.illumina.com	Commercial software
	BioNumerics	www.applied-maths.com	Commercial software
	CLC Genomic Workbench	www.clcbio.com	Commercial software
	FastQC	www.bioinformatics.babraham.ac.uk	
Identification	K-merFinder	www.genomicepidemiology.org	
	NCBI BLAST	www.ncbi.nlm.nih.gov/blast	
Metagenomics	MEGAN	http://ab.inf.uni-tuebingen.de/software/malt	
Phylogeny	FastTree	www.microbesonline.org/fasttree	
	RAxML	http://sco.h-its.org/exelixis/software.html	
	SeqSphere	www.ridom.de	Commercial software
	SNPTree	www.genomicepidemiology.org	
Resistance	ARDB	https://ardb.cbcb.umd.edu	
	CARD	https://card.mcmaster.ca	
	ResFinder	www.genomicepidemiology.org	
SNP calling	BioNumerics	www.applied-maths.com	Commercial software
	CLC Genomic Workbench	www.clcbio.com	Commercial software
	Samtools	www.htslib.org	
	SeqSphere	www.ridom.de	Commercial software
Typing (wgMLST)	BIGSdb	http://bigsdb.readthedocs.io	
	BioNumerics	www.applied-maths.com	Commercial software
	CLC Genomic Workbench	www.clcbio.com	Commercial software
	EnteroBase	https://enterobase.warwick.ac.uk	
	SeqSpere	www.ridom.de	Commercial software
Virulence	VFDB	www.mgc.ac.cn/VFs	
	VirulenceFinder	www.genomicepidemiology.org	
Visualisation & comparative study	ACT	www.sanger.ac.uk/science/tools	
	Artemis	www.sanger.ac.uk/science/tools	
	BRIG	https://sourceforge.net/projects/brig/	
	ClustalW	www.genome.jp/tools/clustalw	
	DNA plotter	www.sanger.ac.uk/science/tools	
	WebACT	www.webact.org	

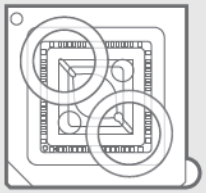
Deurenberget al, JB 2017

Main workflow steps

- √ Primer design and protocol optimization
- √ Up to 6.144 primer pairs can be used at the same time

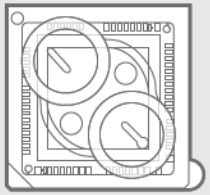
- √ Clinical specimen → DNA extraction → amplification → sequencing
- √ Evaluation and translation of the information in large data bases (cloud computing)

- √ Major advantages (1) Low turnaround time, (2) fast information processing, (3) low detection limit



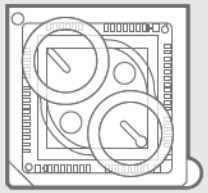
314

Ion 314™ Chip
1 million wells
400–550 thousand reads
for 200-base sequencing



316

Ion 316™ Chip
6 million wells
2–3 million reads
for 200-base sequencing



318

Ion 318™ Chip
11 million wells
4–5.5 million reads
for 200-base sequencing

Sequencing is performed in small semiconductors

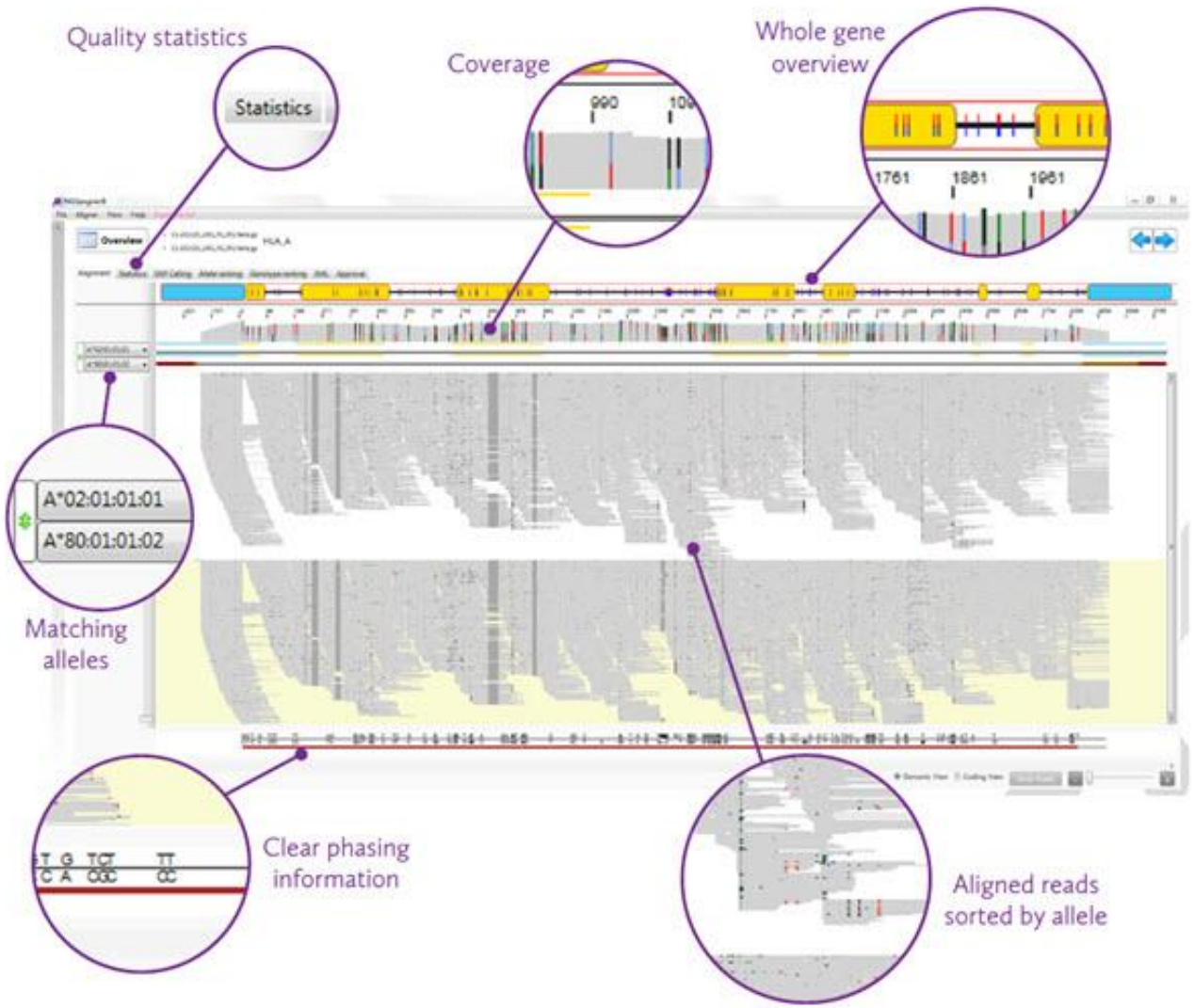
Up to 1,2 billion microwells allow sequencing of up to 496.000.000 DNA fragments

Semiconductor Sequencing Chips

Chip Types[1]	314	316	318	IP1/IP2/IP3*
# Wells per Chip	1,262,528	6,348,216	11,302,473	165 M/660M/1.2B
Volume, µL	7	30	30	55
# of Reads[1]	295,736	1,592,020	4,580,123	124-496,000,000
Yield/Q20, bases	24.6/**21.9** Mb	146.7/**122.5** Mb	600/**500** Mb	**10** / **60** / **480** Gb
Mean Read[1], bp	83	92	129	Up to 300
Longest Reads[1]	396	307	386	640
Run Time[1], Hrs	2.4	3.1	4.5	~4
Processing, Hrs[1]	0.3	2.0	4.5	Up to 8 hrs
Analysis[2], Hrs	12	18	30	Up to 1 day
Template Molecules	2.5×10^7	5×10^7	5×10^7	2.5×10^7
Cost per Run	$400	$500	$800	$1,000

http://www.thermofisher.com

Analysis and translation



http://www.thermofisher.com

Diagnosis of Periprosthetic Joint Infection: The Potential of Next-Generation Sequencing

Majd Tarabichi, MD, Noam Shohat, MD, Karan Goswami, MD, Abtin Alvand, MD, PhD, FRCS, Randi Silibovsky, MD, Katherine Belden, MD, and Javad Parvizi, MD, FRCS

Investigation performed at The Rothman Institute at Thomas Jefferson University, Philadelphia, Pennsylvania

www.nature.com/scientificreports

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Targeted next-generation sequencing of the 16S-23S rRNA region for culture-independent bacterial identification - increased discrimination of closely related species

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Artur J. Sabat[1,2], Evert van Zanten[2], Viktoria Akkerboom[1], Guido Wisselink[2], Kees van Slochteren[2], Richard F. de Boer[2], Ron Hendrix[2], Alexander W. Friedrich[1], John W. A. Rossen[1] & Anna M. D. (Mirjam) Kooistra-Smid[1,2]

J. Bone Joint Infect. 2019, Vol. 4 50

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Journal of Bone and Joint Infection

2019; 4(1): 50-55. doi: 10.7150/jbji.30615

Case Report

Metagenomic next-generation sequencing contribution in identifying prosthetic joint infection due to Parvimonas micra: a case report

Zida Huang[1*], Chongjing Zhang[1*], Wenbo Li[1], Xinyu Fang[1], Qijin Wang[1], Li Xing[3], Yingzhen Li[3], Xifang Nie[3], Bin Yang[2], Wenming Zhang[1]

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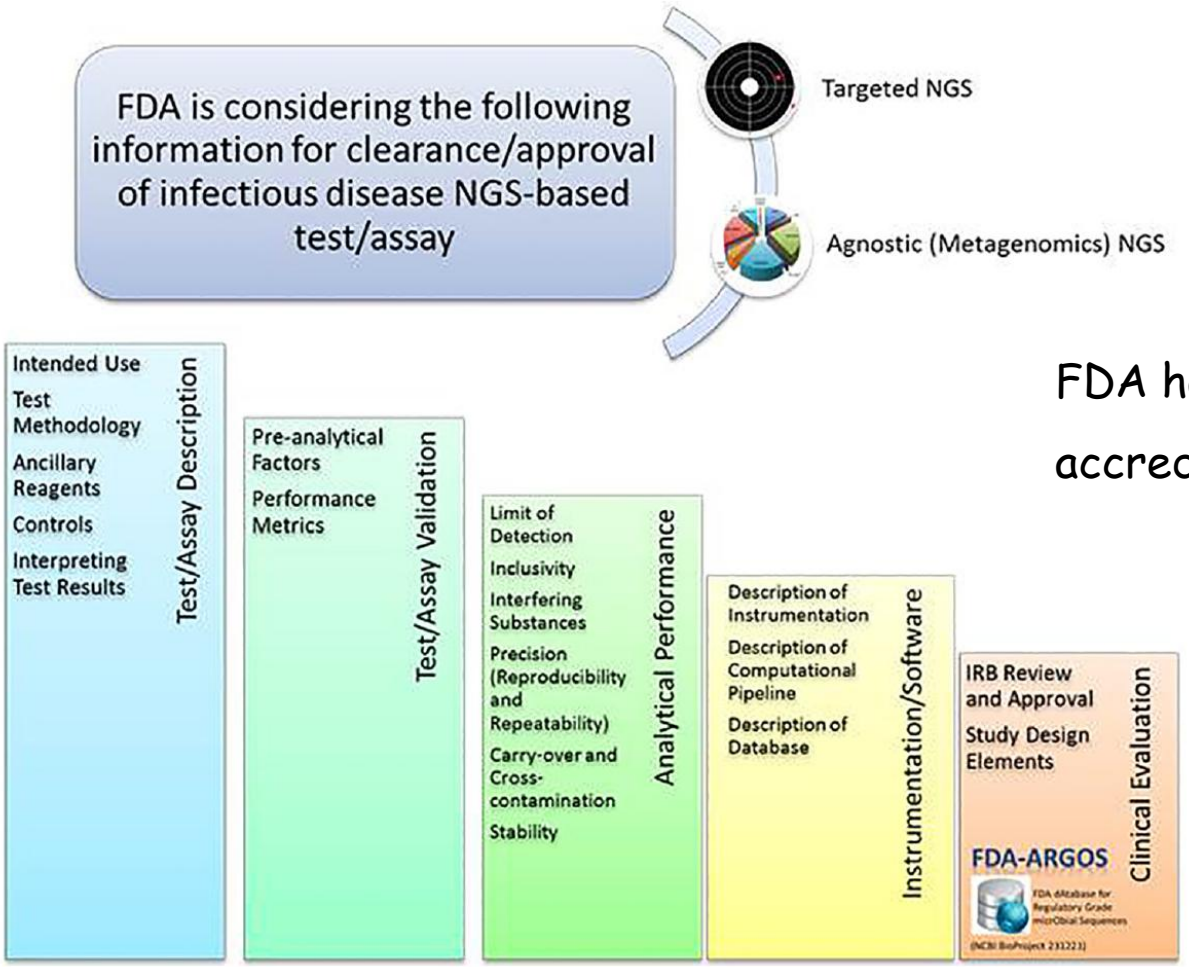
Direct Detection and Identification of Prosthetic Joint Infection Pathogens in Synovial Fluid by Metagenomic Shotgun Sequencing

Morgan I. Ivy,[a] Matthew J. Thoendel,[b] Patricio R. Jeraldo,[c] Kerryl E. Greenwood-Quaintance,[a] Arlen D. Hanssen,[d] Matthew P. Abdel,[d] Nicholas Chia,[c] Janet Z. Yao,[c] Aaron J. Tande,[b] Jayawant N. Mandrekar,[e] Robin Patel[a,b]

Applications of NGS in bone and implant infections

Next milestone: incorporation of the technique in the Microbiology Lab routine

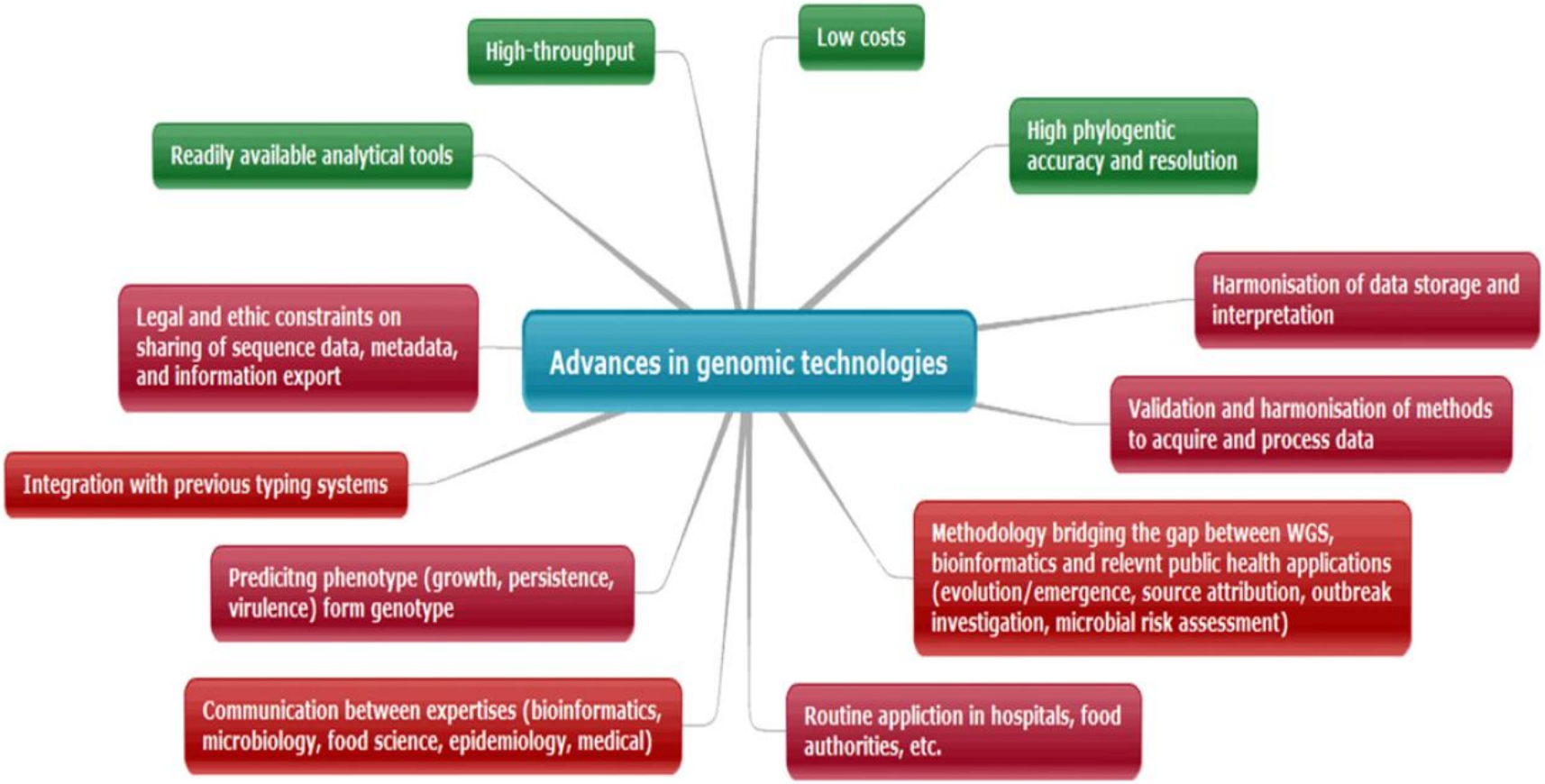


FDA has already proposed an accreditation procedure

Goldberg et al, mBio 2015

Next milestone: incorporation of the technique in the Microbiology Lab routine

But the chapters still open to discussion are more than the ones that are closed



Franz et al, IJFM 2014

Conclusions

- √ Well into the 21st century, the Gold Standard for diagnosis of bone and joint infections is still based on a technique established during the late 19th century (with major optimizations of course)
- √ Nevertheless, new techniques, already used in other disciplines, "slowly" find their way to orthopedic infection diagnosis
- √ Syndromic molecular diagnostic approach seems to be the most promising tool for the time being
- √ NGS will require additional time, but eventually will replace all other molecular techniques


Thank you for
your attention