Place des nouvelles techniques de diagnostic microbiologique dans les Infections Ostéo-Articulaires

Martin Rottman
CRIOA HUPIFO
GH Hôpitaux Universitaires Paris IdF Ouest
Université de Versailles St Quentin

Déclaration d’intérêts de 2012 à 2015

- **Intérêts financiers :** **NON**
- **Liens durables ou permanents :** **Diaxonhit**
- **Interventions ponctuelles :** **Cepheid**
- **Intérêts indirects :** **NON**

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Plan

Définition et impact du biofilm

Définitions de l'infection sur prothèse

Spécificité liées au biofilm

- Sonication
- Broyage
- Culture prolongée

Biologie moléculaire

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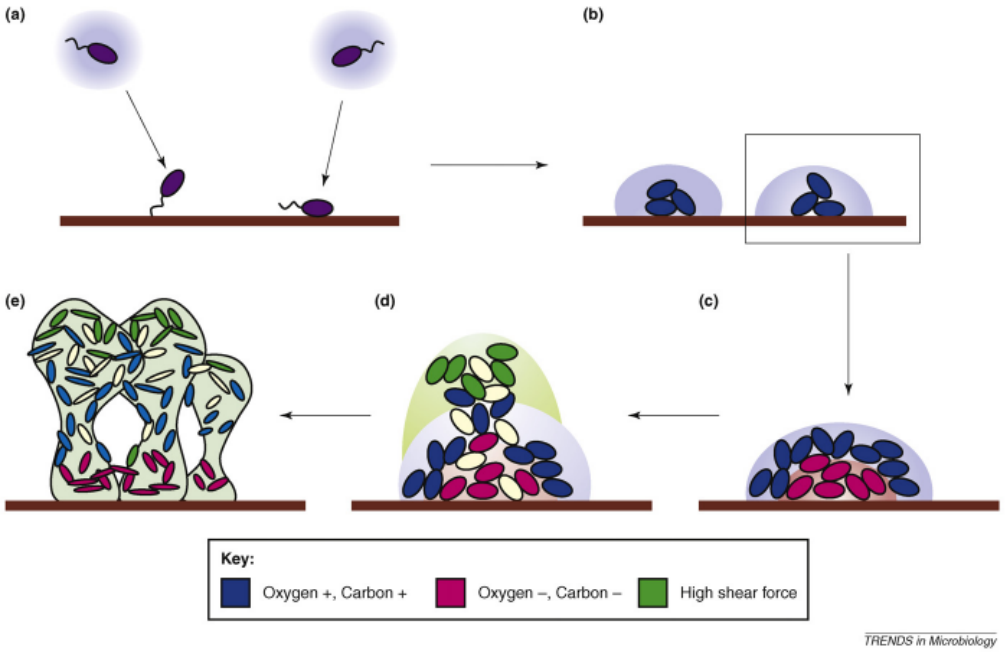
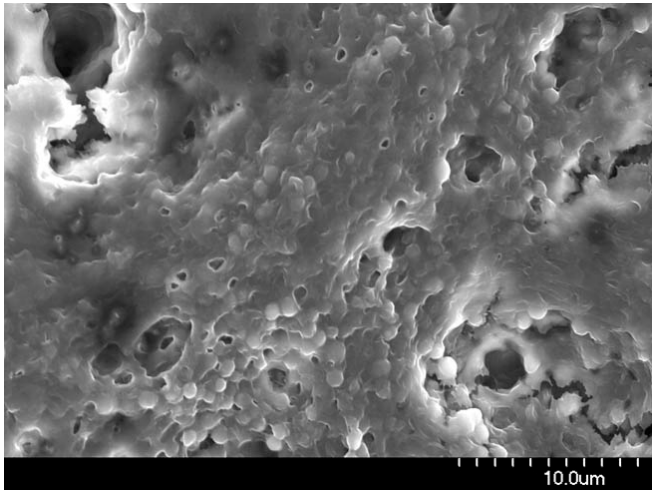
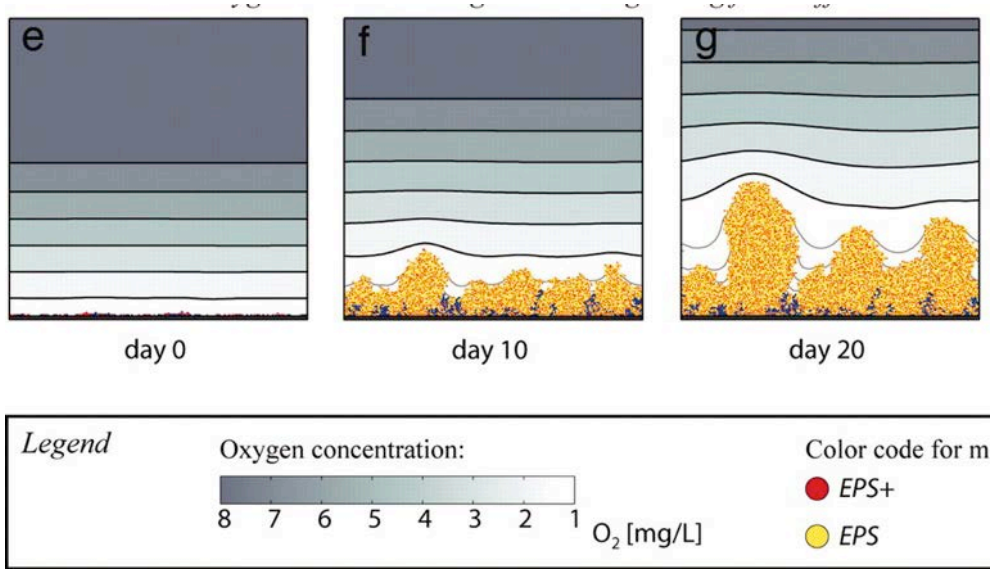
Infection sur matériel et biofilm

Biofilm bactérien : communauté bactérienne dans une matrice extracellulaire





Différenciation des bactéries au sein du biofilm



Xavier J B , Foster K R PNAS 2007;104:876-881

Modèlise les observations *in vitro: pas d'espace mort in vivo*

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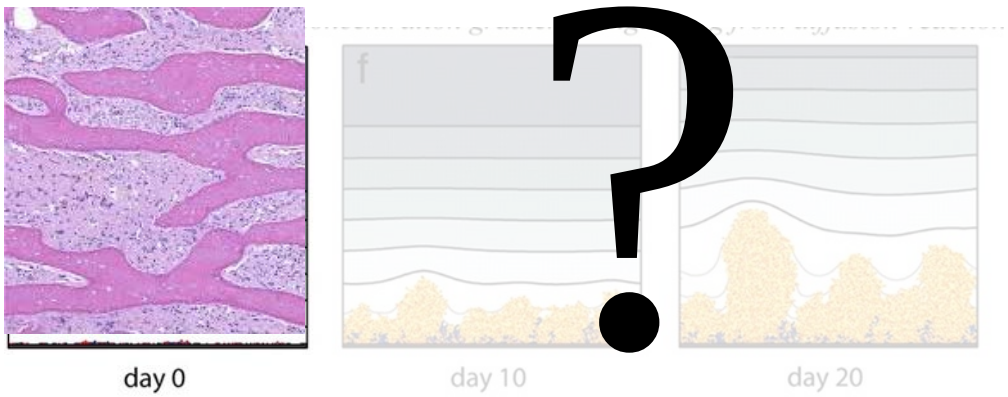
Invasion tissulaire?



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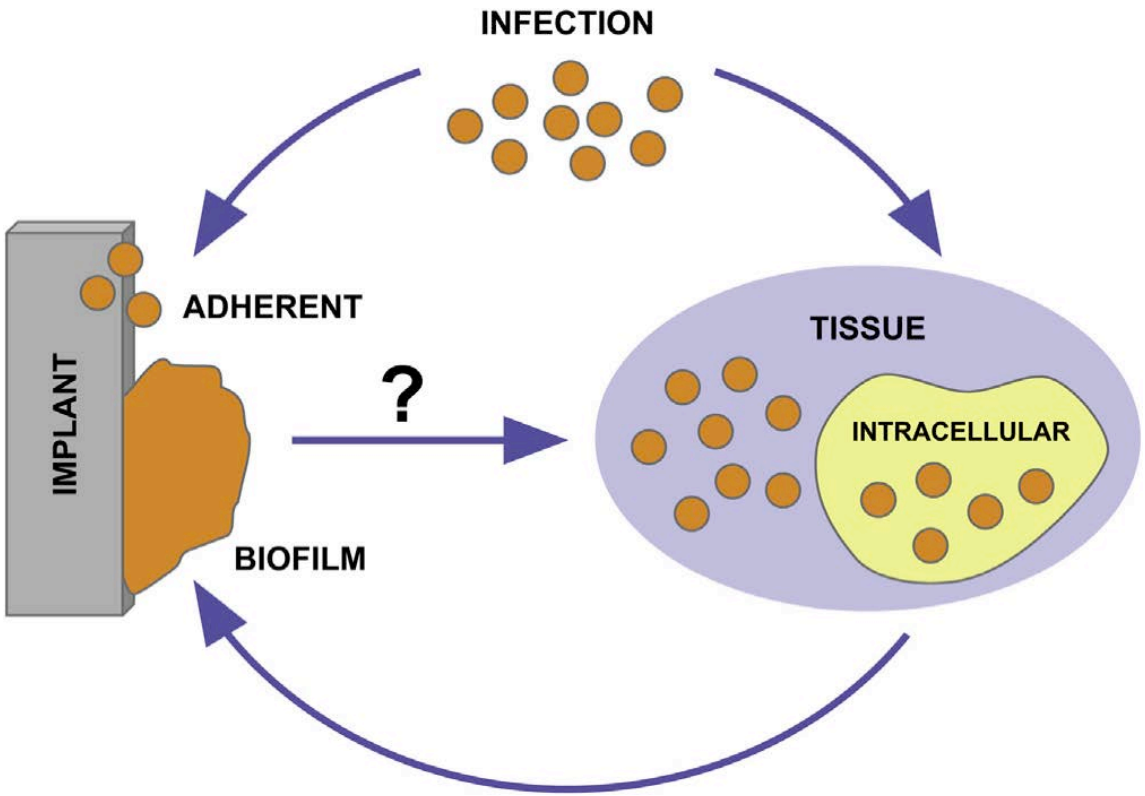
Implantation de titane « biofilmé »



M.Riooletal./ActaBiomaterialia10(2014)5202–5212

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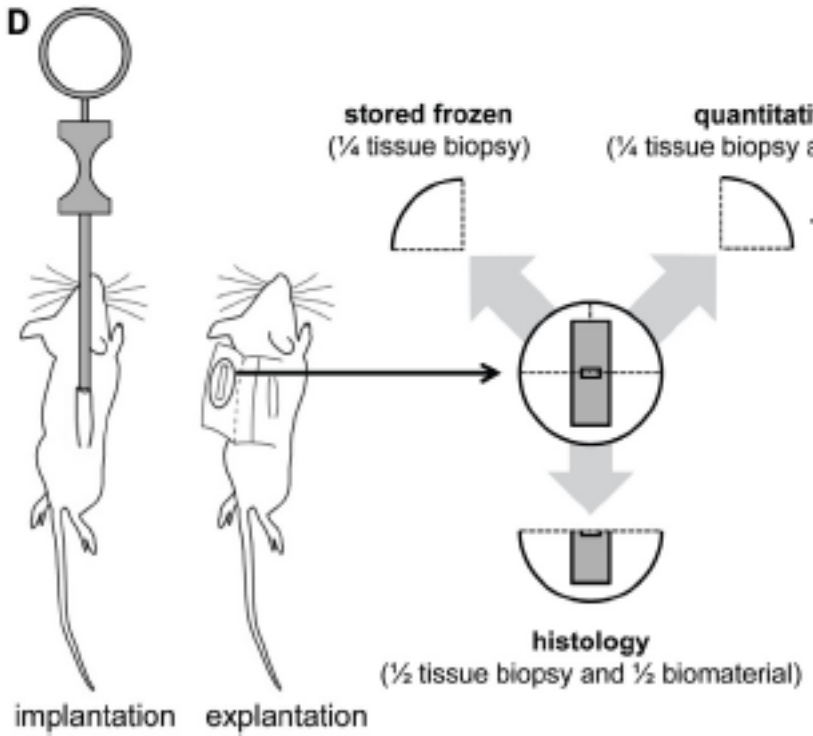
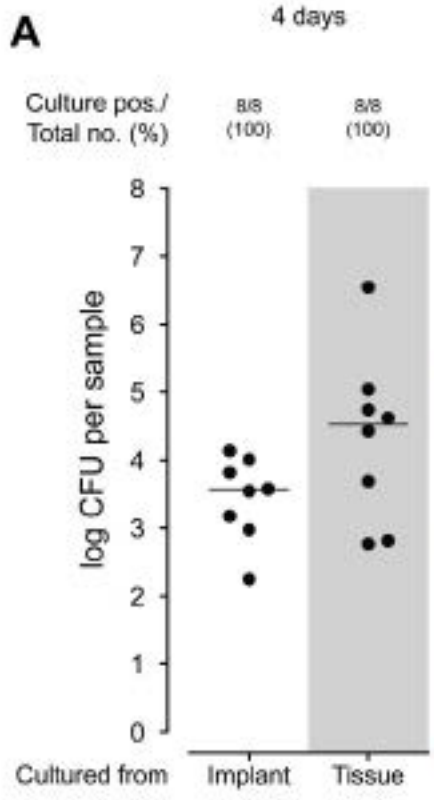
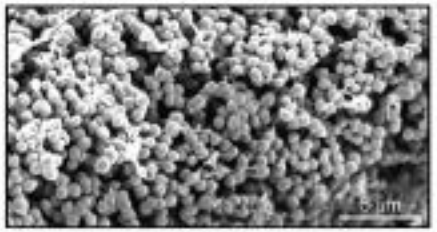
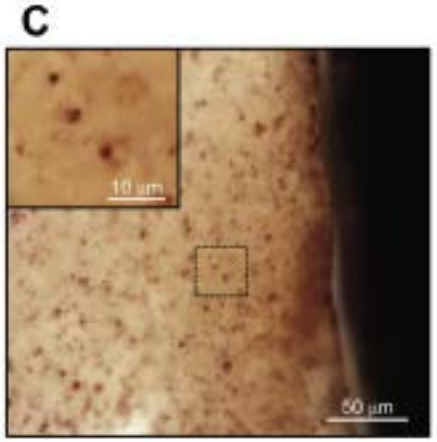
Implantation de titane « biofilmé »









M.Riooletal./ActaBiomaterialia10(2014)5202–5212

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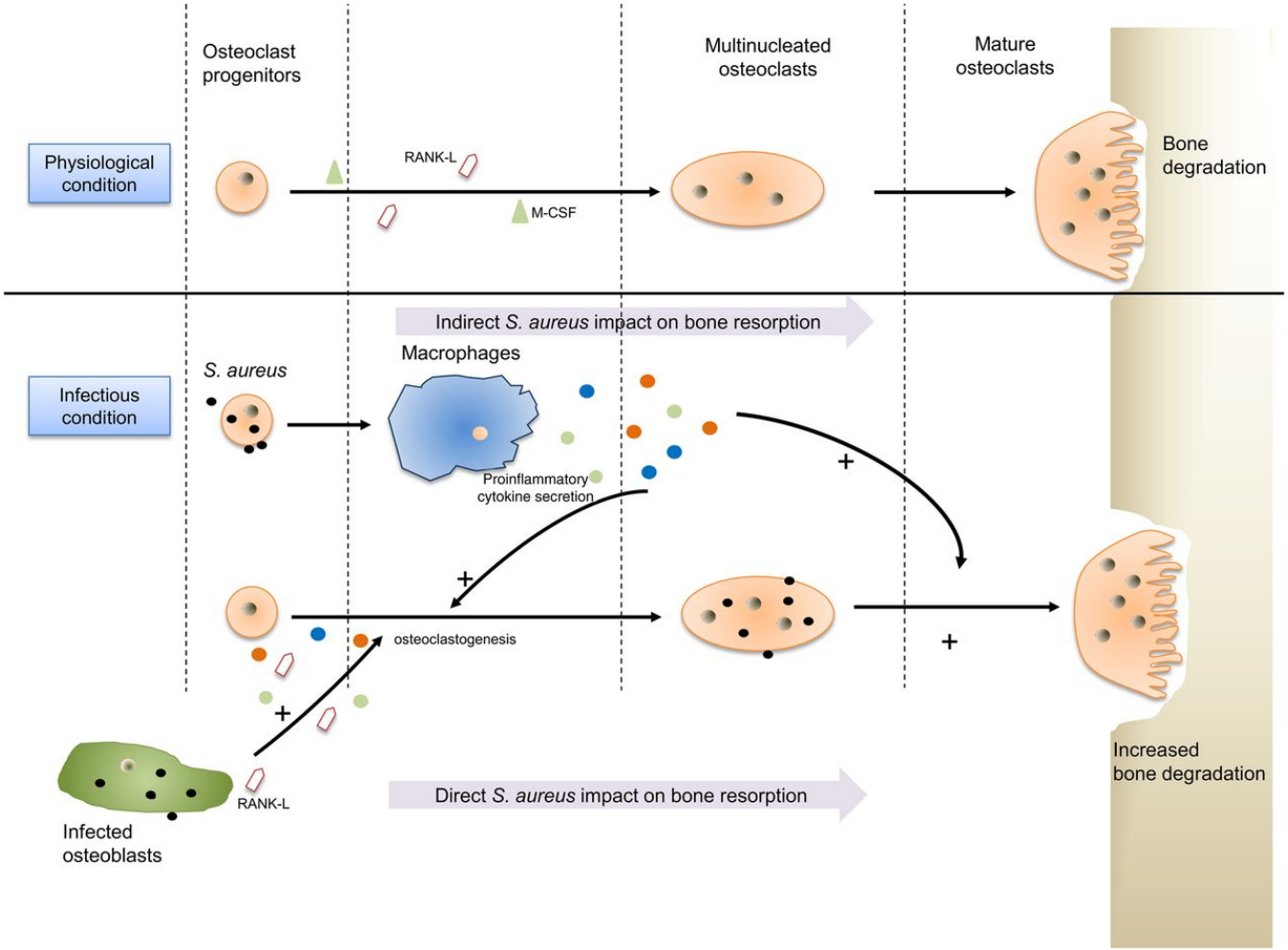
Schematic representation of the impact of *Staphylococcus aureus* on bone degradation.



Sophie Trouillet-Assant et al. J Infect Dis. 2015;211:571-581

The Journal of Infectious Diseases

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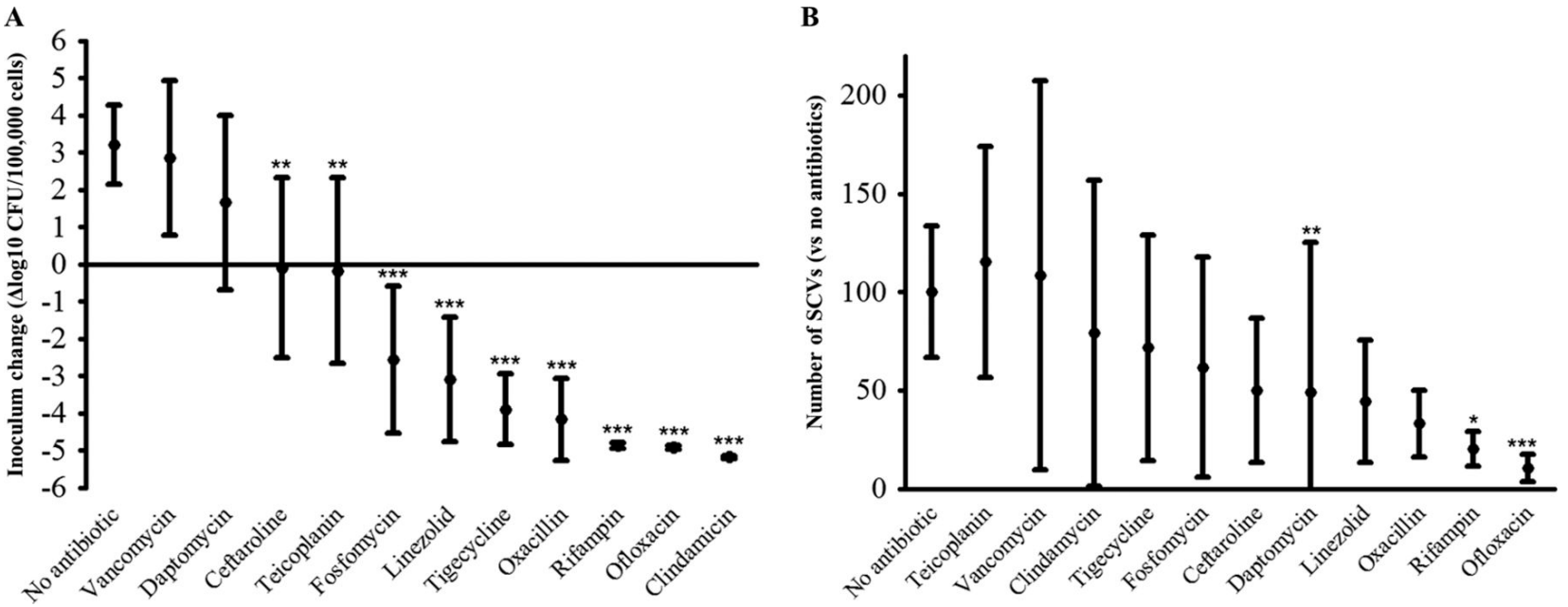
Intraosteoblastic inoculum change and intracellular proportion of small-colony variants in the presence of the main antistaphylococcal molecules at the usual bone concentration.



Florent Valour et al. Antimicrob. Agents Chemother. 2015;59:2029-2036

Antimicrobial Agents and Chemotherapy

Définitions de l'infection sur prothèse

MSIS – Consensus international 2013: PJI is defined as:

- Two positive periprosthetic cultures with phenotypically identical organisms, or
- A sinus tract communicating with the joint, or
- Having three of the following minor criteria:
  - Elevated serum C-reactive protein (CRP) AND erythrocyte sedimentation rate (ESR)
  - Elevated synovial fluid white blood cell (WBC) count OR ++change on leukocyte esterase test strip
  - Elevated synovial fluid polymorphonuclear neutrophil percentage (PMN%)
  - Positive histological analysis of periprosthetic tissue
  - A single positive culture

Delegate Vote: Agree: 85%, Disagree: 13%, Abstain: 2% (Strong Consensus)

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Anatomo-pathologie

APPEL A TEMOINS

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Spécificités liées au biofilm

Techniques culturales spécialisées

- Sonication des implants
- Broyage des prélèvements tissulaires
- Culture monitorée (sur milieux d'hémocultures)

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Gros matériels (Clou, prothèse…)

Sonication des implants:

Extraction du biofilm de la surface de l'implant

Images N. Desplaces GHDCSS

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Comparison

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Publication princeps

Table 1. Comparison of Microbiologic Tests for the Diagnosis of Prosthetic-Joint Infection.

Test	Patients with Prosthetic-Joint Infection (N=79)	Patients with Aseptic Failure (N=252)	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
	*no. of patients with positive specimens**		*% (95% confidence interval)*			
Synovial-fluid culture	18/32	2/108	56.3 (37.7–73.6)	98.1 (93.5–99.8)	90.0 (68.3–98.8)	88.3 (81.2–93.5)
Periprosthetic-tissue culture†						
≥1 positive culture	58	23	73.4 (62.3–82.7)	90.9 (86.6–94.1)	71.6 (60.5–81.1)	91.6 (87.4–94.7)
≥2 positive cultures	48	2	60.8 (49.1–71.6)	99.2 (97.2–99.9)	96.0 (86.3–99.5)	89.0 (84.7–92.4)
Sonicate-fluid culture†‡						
≥1 CFU	64	28	79.0 (68.5–87.3)	88.5 (83.9–92.2)	68.8 (58.4–78.0)	93.9 (88.9–95.8)
≥2 CFU	63	8	79.7 (69.2–88.0)	96.8 (93.8–98.6)	88.7 (79.0–95.0)	93.8 (90.2–96.4)
≥3 CFU	63	5	79.7 (69.2–88.0)	98.0 (95.4–99.4)	92.6 (83.7–97.6)	93.9 (90.3–96.5)
≥4 CFU	62	5	78.5 (67.8–86.9)	98.0 (95.4–99.4)	92.5 (83.4–97.5)	93.6 (89.9–96.2)
≥5 CFU	62	3	78.5 (67.8–86.9)	98.8 (96.6–99.8)	95.4 (87.1–99.0)	93.6 (90.0–96.2)
≥6 CFU	62	3	78.5 (67.8–86.9)	98.8 (96.6–99.8)	95.4 (87.1–99.0)	93.6 (90.0–96.2)
≥7 CFU	60	3	75.9 (65.0–84.9)	98.8 (96.6–99.8)	95.2 (86.7–99.0)	92.6 (89.2–95.7)
≥8 CFU	59	3	74.7 (63.6–83.8)	98.8 (96.6–99.8)	95.2 (86.5–99.0)	92.6 (88.8–95.4)
≥9 CFU	58	3	73.4 (62.3–82.7)	98.8 (96.6–99.8)	95.1 (86.3–99.0)	92.2 (88.4–95.1)
≥10 CFU	57	3	72.2 (60.9–81.7)	98.8 (96.6–99.8)	95.0 (86.1–99.0)	91.9 (88.0–94.8)
≥25 CFU	55	2	69.6 (58.2–79.5)	99.2 (97.2–99.9)	96.5 (87.9–99.6)	91.2 (87.2–94.3)
≥50 CFU	54	1	68.4 (56.9–78.4)	99.6 (97.8–100.0)	98.2 (90.3–100.0)	90.9 (86.9–94.1)
Gram's staining of sonicate fluid	34/76	0/250	44.7 (33.3–56.6)	100.0 (98.5–100.0)	100.0 (89.7–100.0)	85.6 (81.1–89.4)

* Where the denominator is shown, data were not available for all study patients.
† The number of cultures positive for the same microorganism is given.
‡ The number of colony-forming units (CFUs) per agar plate growing on either aerobic or anaerobic plates (whichever yielded higher counts) is given. According to the receiver-operating-characteristic analysis, the inflection point (i.e., the optimal cutoff) was 1 CFU or more. However, 5 CFU or more was selected as the ideal cutoff, because high specificity was considered more important than an optimal trade-off between sensitivity and specificity.

Trampuz A et al. N Engl J Med 2007;357:654-663.

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Comparison

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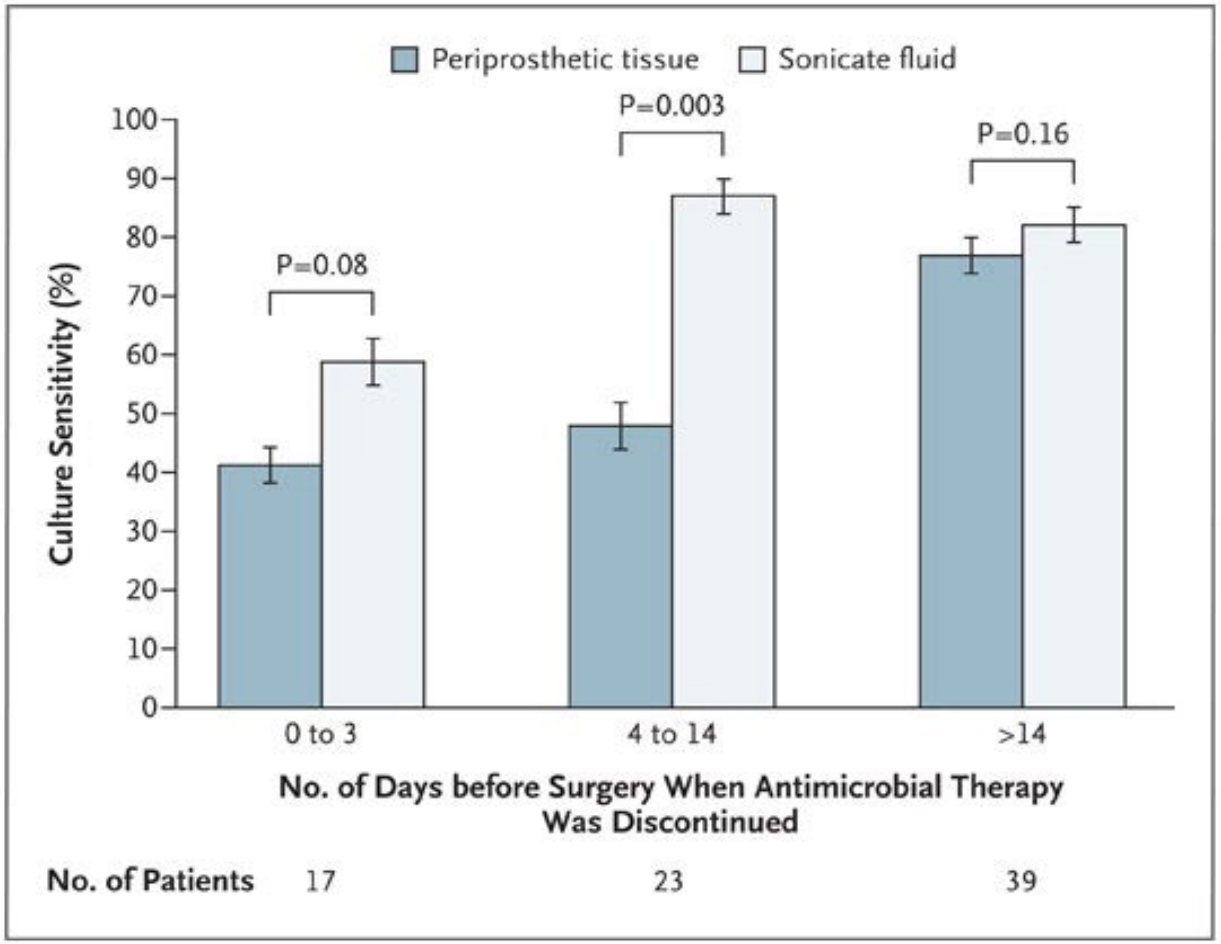
Trampuz A et al. N Engl J Med 2007;357:654-663.

Avantage pour l'ATBthérapie préalable



Trampuz A et al. N Engl J Med 2007;357:654-663.

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Effet centre?

TABLE 3. Comparison of periprosthetic tissue culture, sonication fluid culture, and multiplex PCR of sonication fluid in 37 cases of PJI

Infection type and microorganism	No. of episodes	No. of episodes with positive result by diagnostic test:		
		Periprosthetic tissue culture	Sonication fluid culture	Multiplex PCR of sonication fluid
Single microorganism	31	22	20	26
Staphylococcus aureus	9	5	5	9
Coagulase-negative staphylococci	11	9	8	11
Streptococcus mitis	1	1	1	1
Streptococcus agalactiae	1	1	1	1
Streptococcus dysgalactiae	1	1	0	1
Streptococcus gallolyticus	1	0	0	1
Streptococcus pneumoniae	1	0	0	1
Propionibacterium acnes	5	4	4	0
Candida albicans	1	1	1	1
Polymicrobial infection[a]	6	2	3	3
Total no. of episodes (%)	37 (100)	24 (65)	23 (62)	29 (78)

[a] Included coagulase-negative staphylococci, *Klebsiella pneumoniae/oxytoca* and *Enterococcus faecalis* ($n = 1$), *P. acnes* and coagulase-negative staphylococci ($n = 1$), *S. aureus* and coagulase-negative staphylococci ($n = 2$), *Corynebacterium* species and coagulase-negative staphylococci ($n = 1$), and *P. acnes* and *S. aureus* ($n = 1$).

Achermann Y et al. J. Clin. Microbiol. 2010;48:1208-1214

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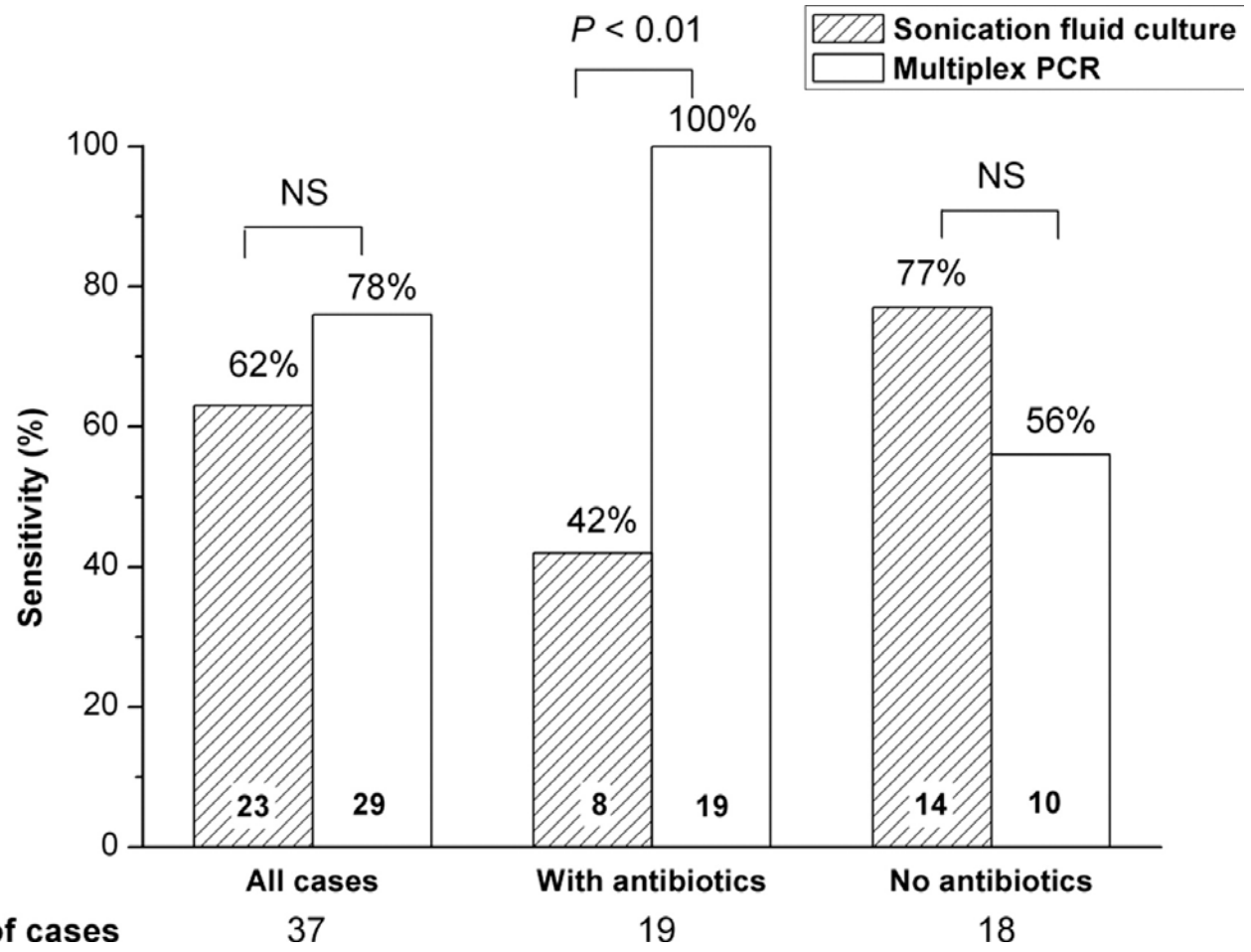
Sensitivity of culture and multiplex PCR of sonication fluid.



Achermann Y et al. J. Clin. Microbiol. 2010;48:1208-1214

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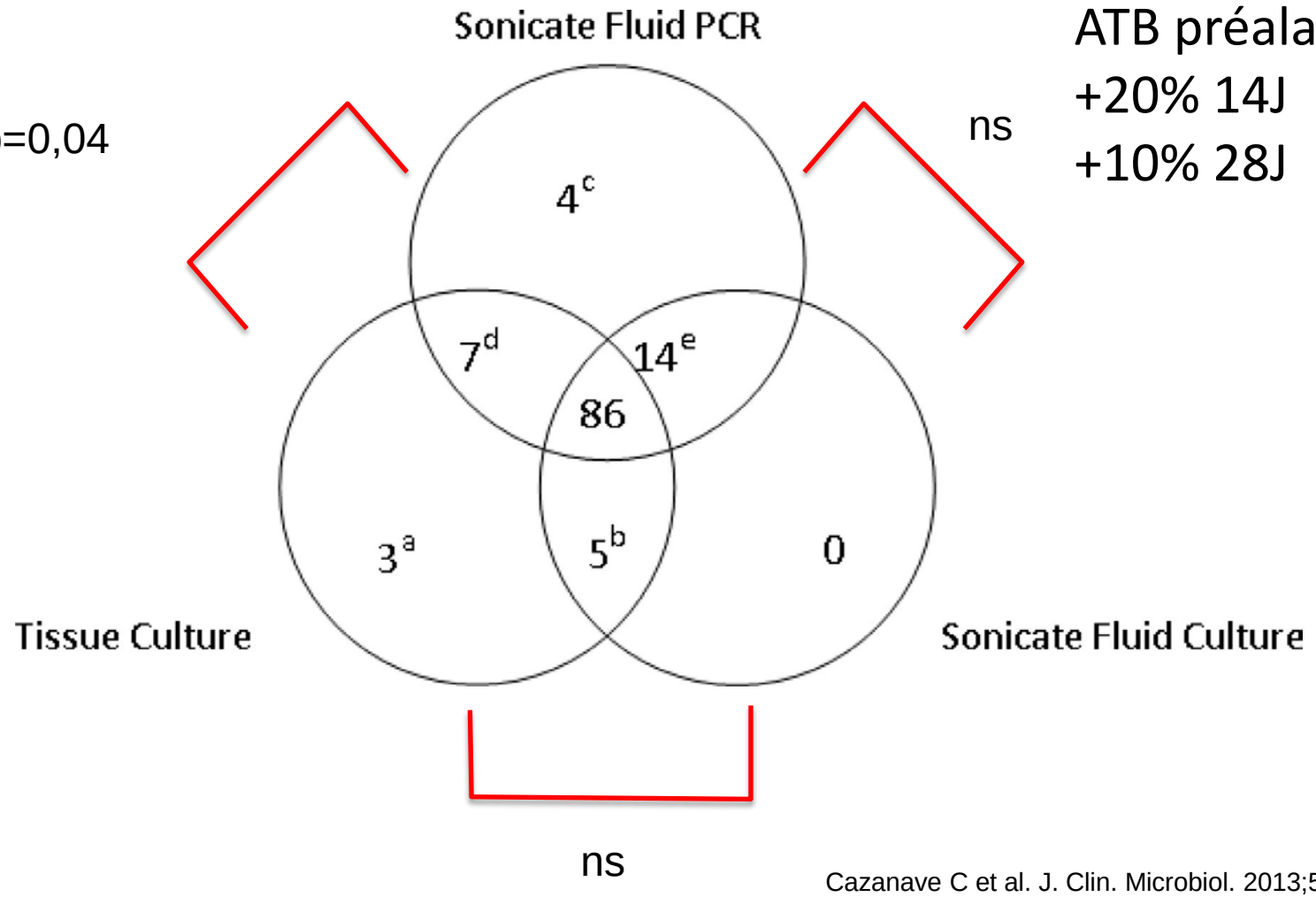
Performance des méthodes microbiologiques



ATB préalable+++
+20% 14J
+10% 28J

Cazanave C et al. J. Clin. Microbiol. 2013;51:2280-2287

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Apport de la sonication

EN CAS D'ANTIBIOTHERAPIE PREALABLE

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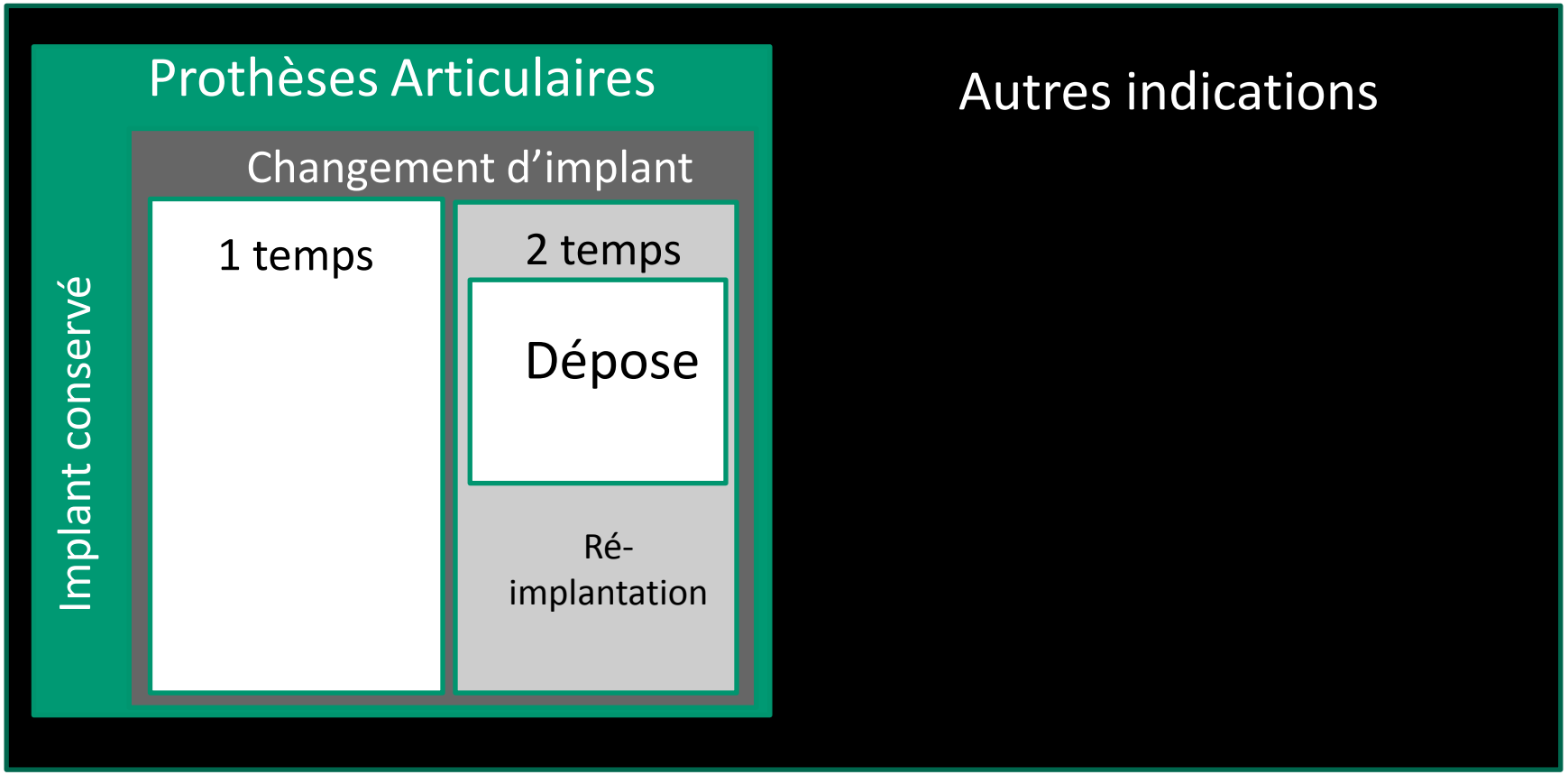
Limites de la sonication



Le traitement du matériel explanté concerne une fraction des infections ostéo-articulaires traitées par les laboratoires

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Spécificités liées au biofilm

Techniques culturales spécialisées

- Sonication des implants
- Broyage des prélèvements tissulaires
- Culture monitorée (sur milieux d'hémocultures)

Broyage mécanique des pièces opératoires

Diagnosis of prosthetic joint infection by beadmill processing of a periprosthetic specimen

A.-L. Roux[1,2], V. Sivadon-Tardy[1,3], T. Bauer[4,5], A. Lortat-Jacob[4,5], J.-L. Herrmann[1,2], J.-L. Gaillard[1,3] and M. Rottman[1,2]

Clin Microbiol Infect 2010

Applicable à tout prélèvement solide/petit matériel

Evolutions:

- Billes en inox 5mm
- Bouillons sur flacons d'hémoculture

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Broyeur à billes « Beadmil »

Patient characteristics		
Number of cases of PJI	92	
Patient age	64.2	
Location (hip/knee)	54/38	
Sex ratio (M/F)	0.7	(38/54)
Number of samples per patient	5.8	(±1.4)

Microbiological findings	**n**	**%**
Microbiological documentation	77	83.7%
Polymicrobial infections	16	20.8%
Negative culture	7	7.6%
Contaminant	8	8.7%

A.-L. Roux *et al.* Clin Microbiol Infect. 2011 Mar;17(3):447-50. Epub 2010 Nov 5

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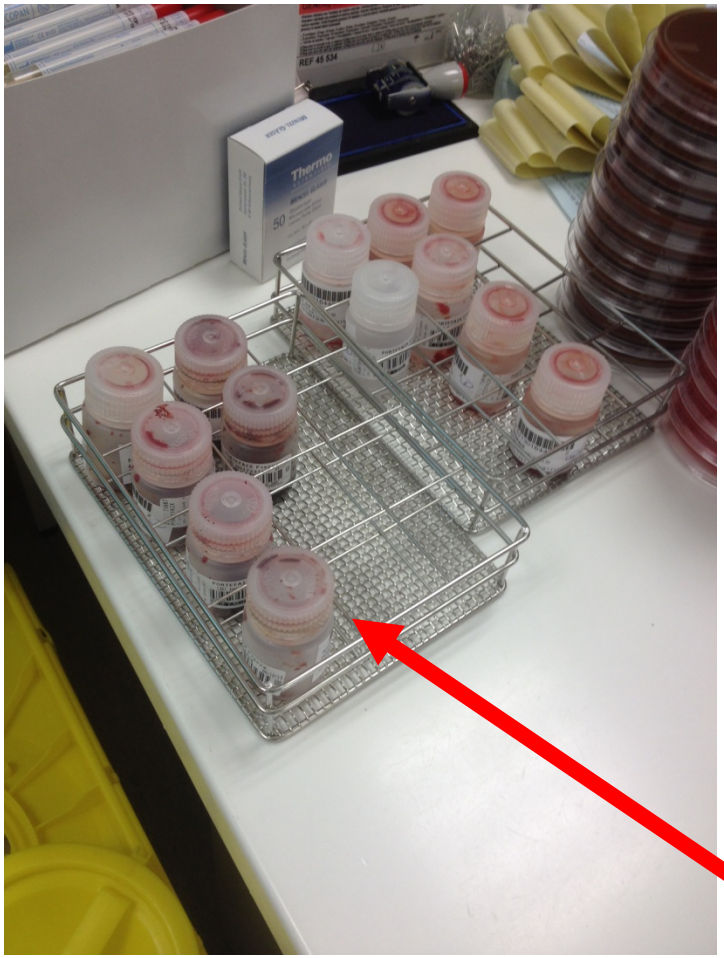
Processus de broyage



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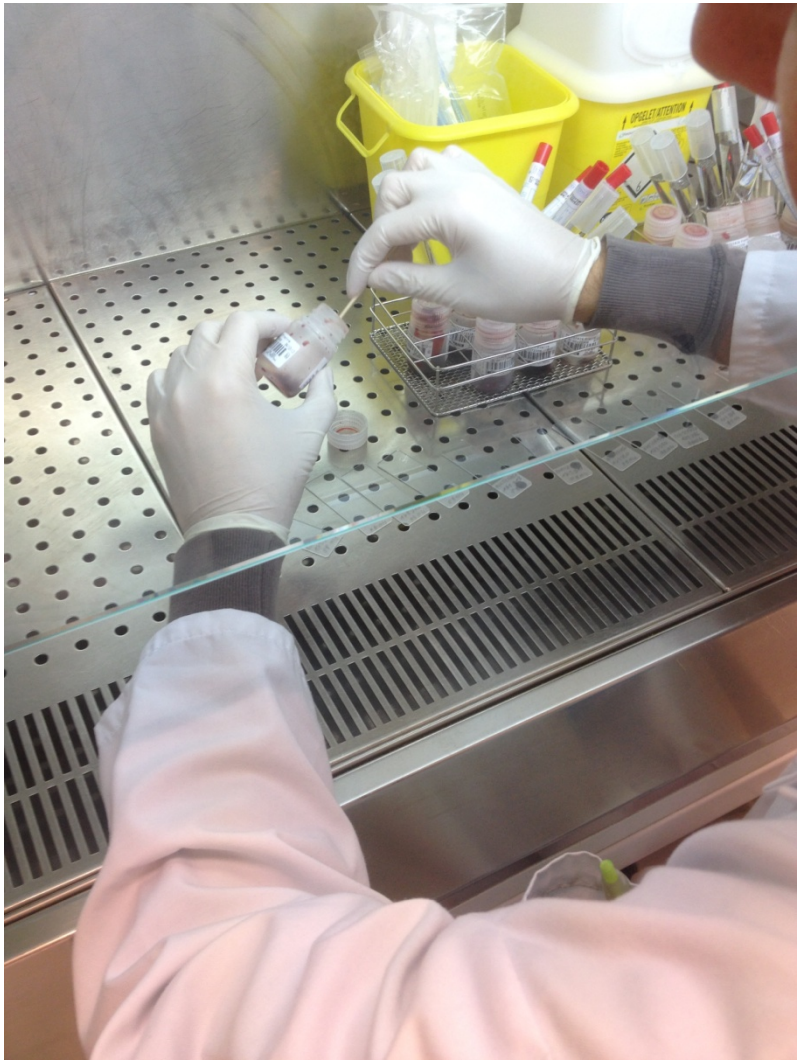
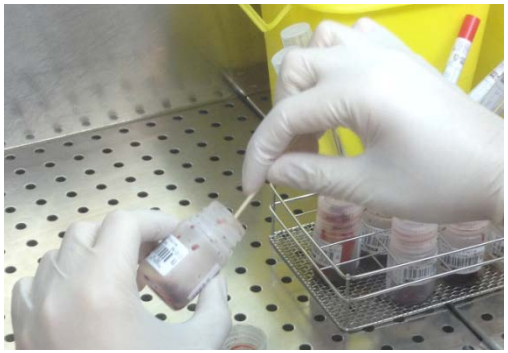
Examen direct





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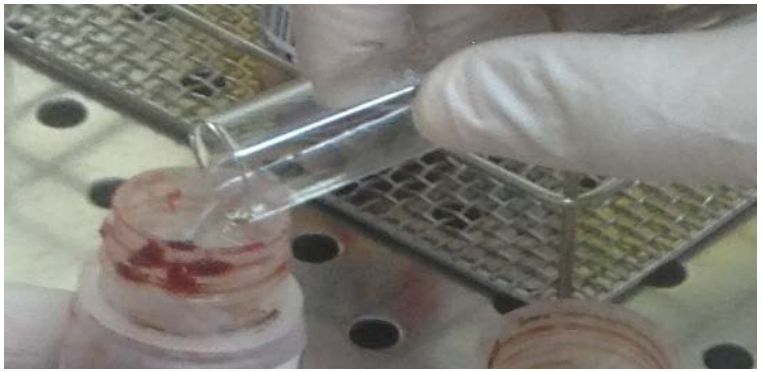
Ajouts ddH_2O / billes



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Broyage « Beadmill »

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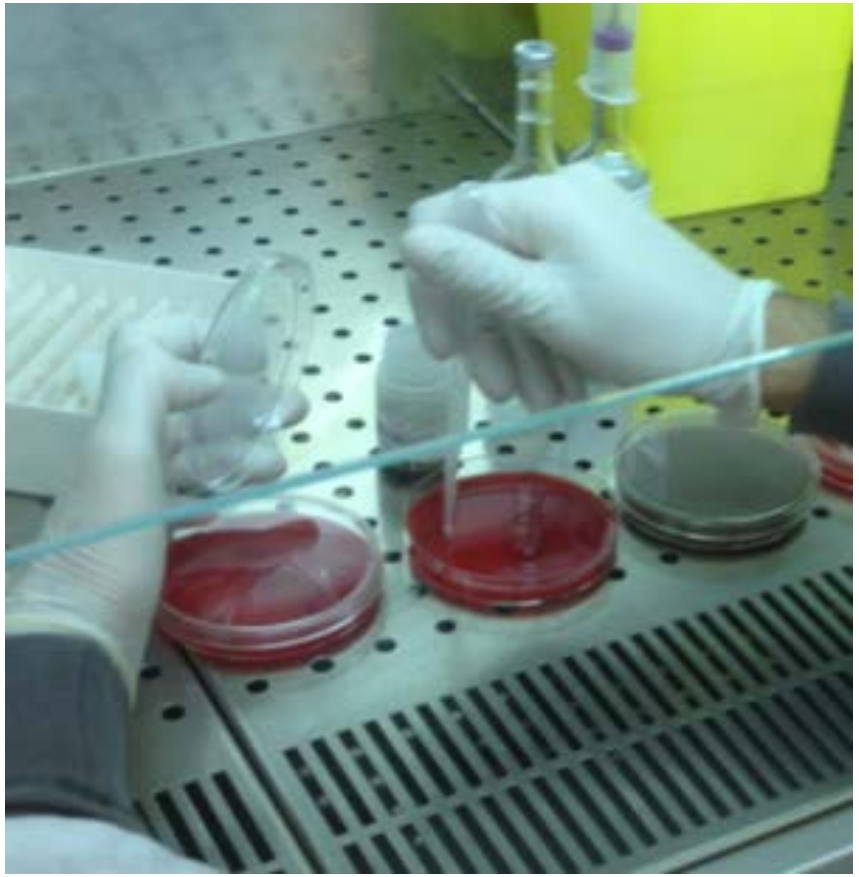
Ensemencement sur milieux solides



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Ensemencement sur milieux liquides

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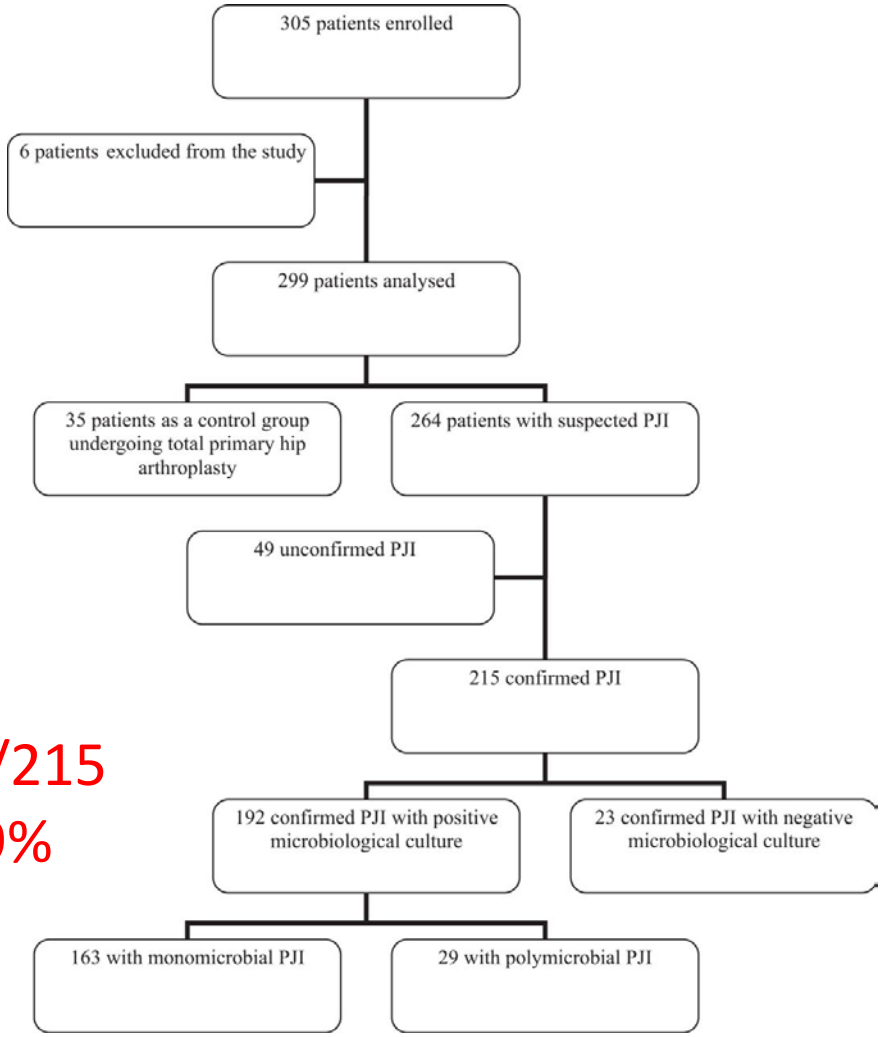
Broyage mécanisé des prélèvements



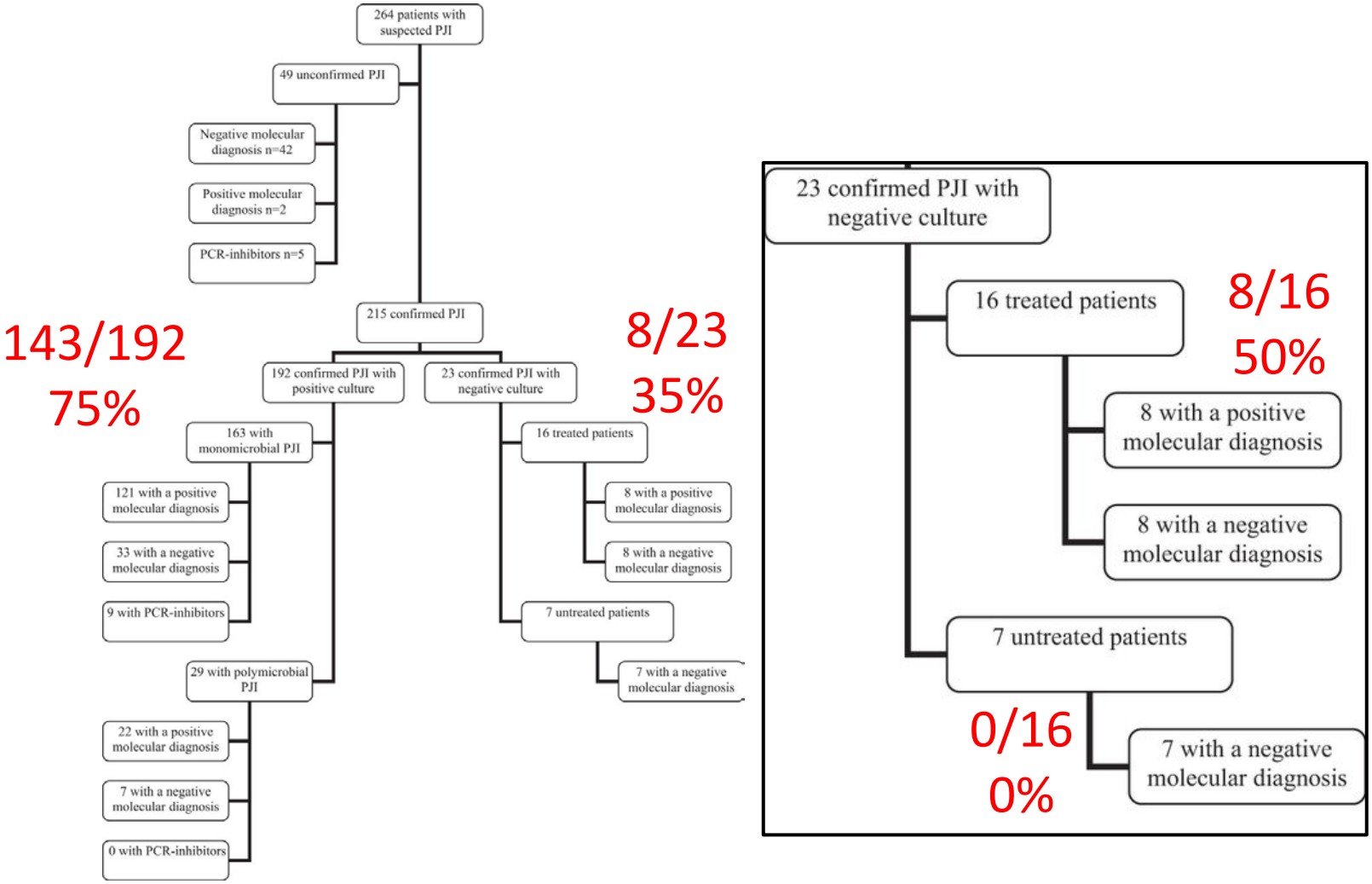
192/215

89%

Bémer P et al. J. Clin. Microbiol. 2014;52:3583-3589

Journal of Clinical Microbiology

Amplification génique sur broyat



Pascale Bémer et al. J. Clin. Microbiol. 2014;52:3583-3589

Journal of Clinical Microbiology

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UFR DES SCIENCES DE LA SANTÉ SIMONE VEIL

Spécificités liées au biofilm

Techniques culturales spécialisées

- Sonication des implants
- Broyage des prélèvements tissulaires
- Culture monitorée (sur milieux d'hémocultures)

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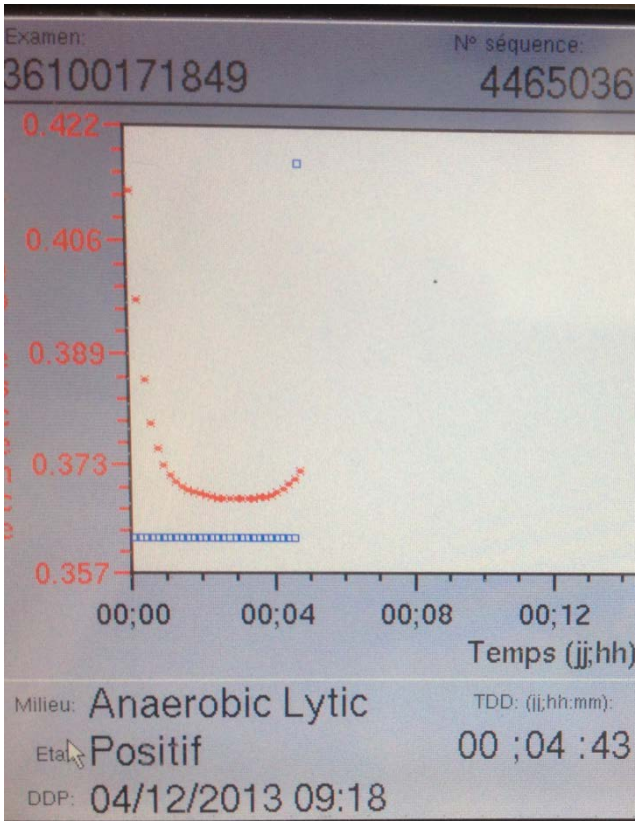
Incubation automatisée



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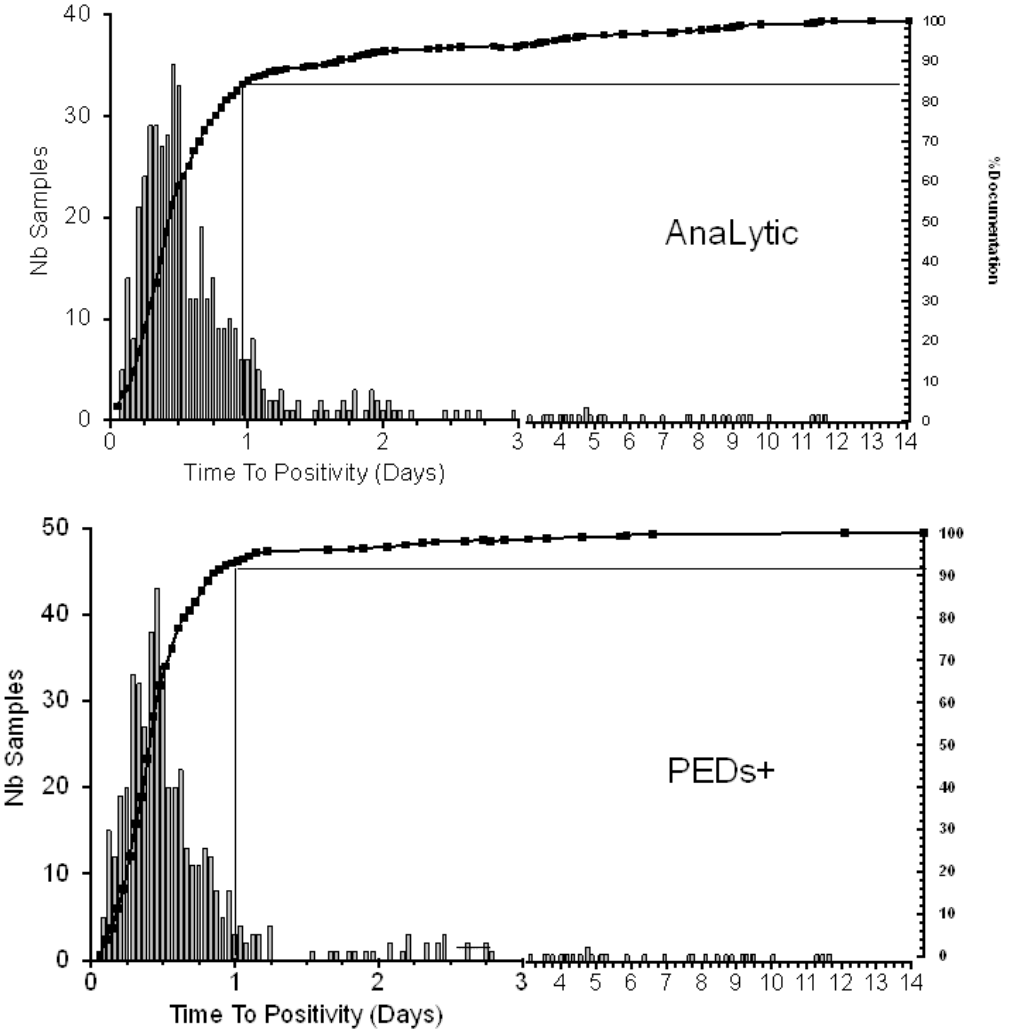
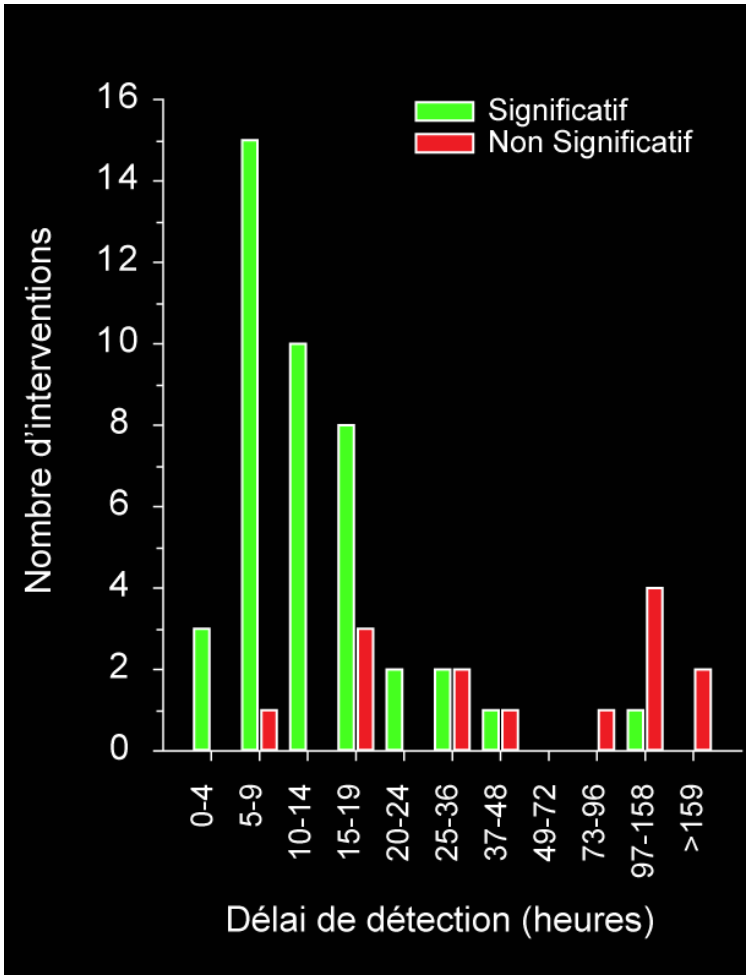
Apport du broyage/Flacons d'hémoculture





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Milieux de culture automatisés

Minassian *et al. BMC Infectious Diseases* 2014, **14**:233
http://www.biomedcentral.com/1471-2334/14/233

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Use of an automated blood culture system (BD BACTEC™) for diagnosis of prosthetic joint infections: easy and fast

Angela M Minassian[1,2*], Robert Newnham[1], Elizabeth Kalimeris[1], Philip Bejon[1,2], Bridget L Atkins[1,2] and Ian CJW Bowler[1]

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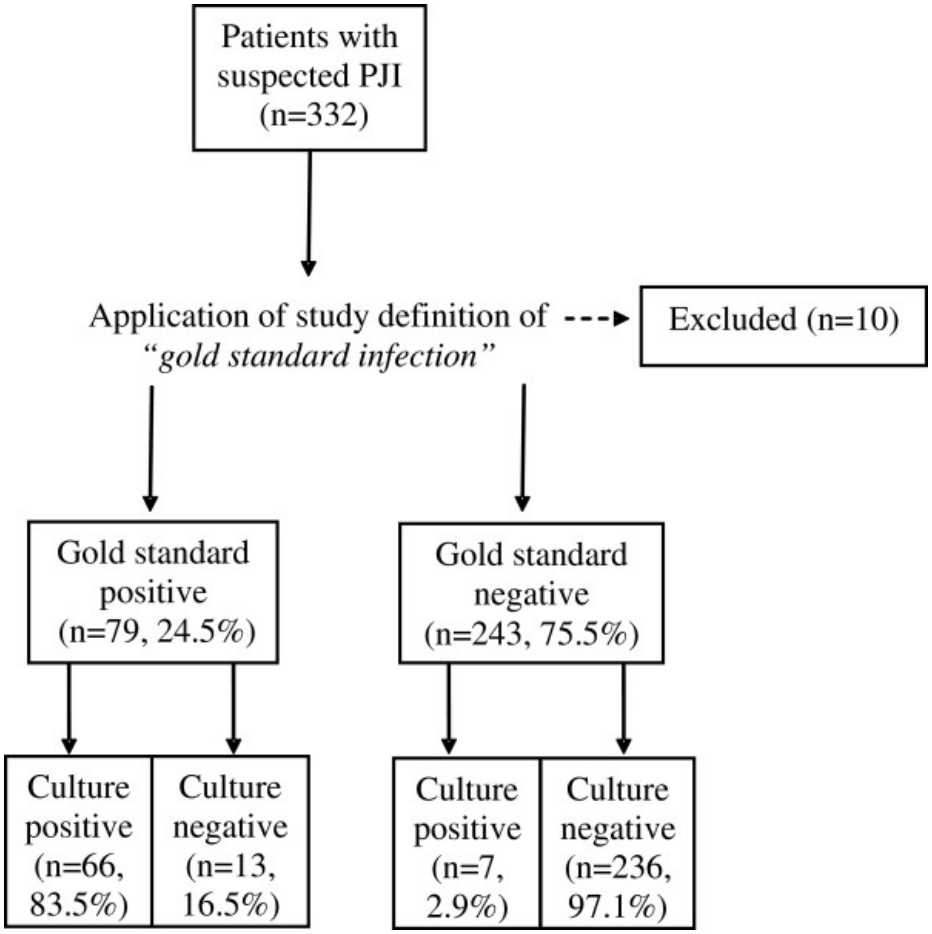
Milieux de culture automatisés



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Milieux aérobie et anaérobie

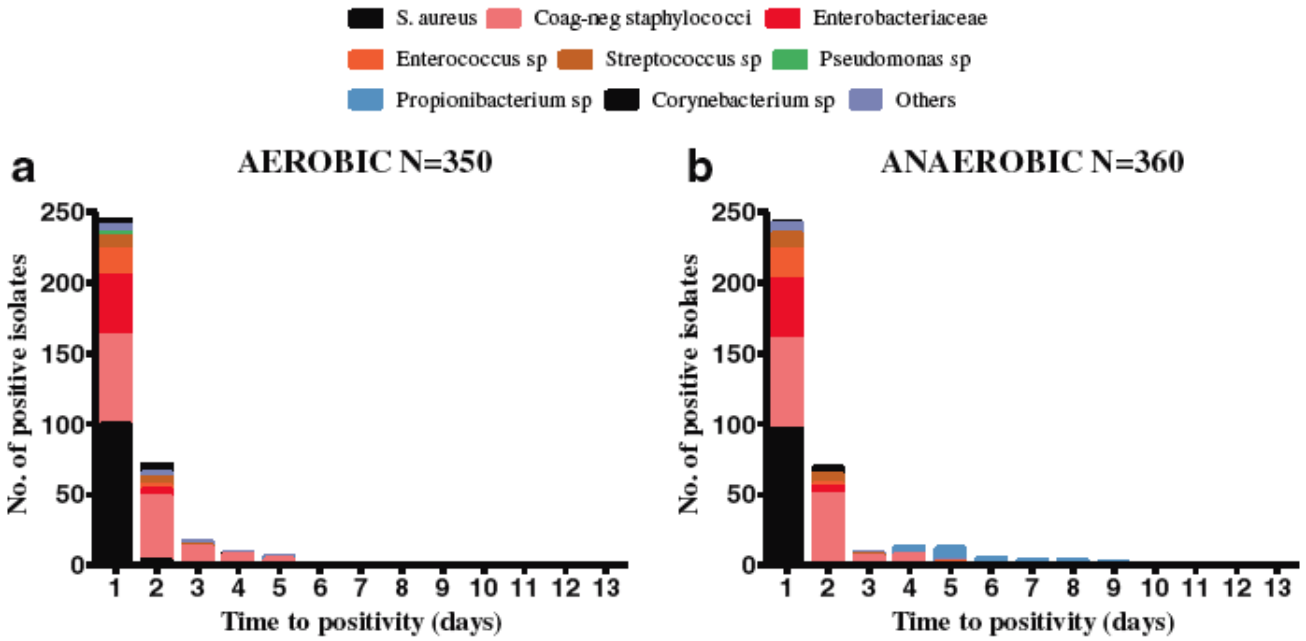

Figure 2 Time to positivity (TTP) (days) by organism: data for aerobic (a) and anaerobic (b) positive cultures shown separately, for ALL isolates grown from all 1328 periprosthetic samples. Data include no determination of whether or not *"gold standard infection"*. N = 360 means 360 of the 455 isolates flagged in the aerobic bottle; N = 350 means 350 of the 455 isolates flagged in the anaerobic bottle.

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Milieux aérobie et anaérobie

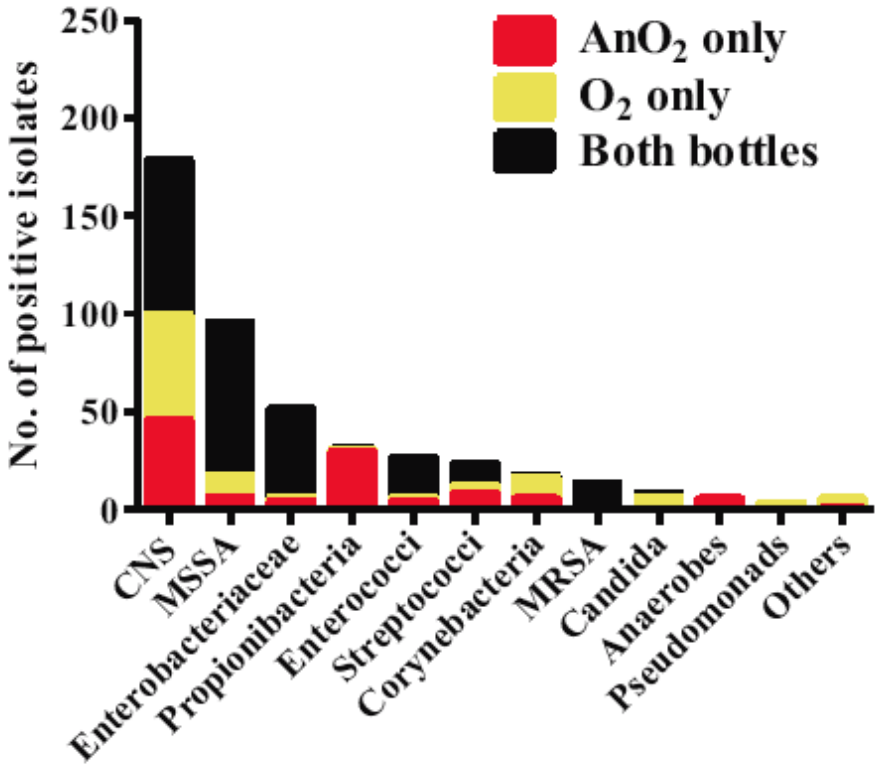

Figure 3 Number of isolates flagging in one or both BACTEC™ bottles (aerobic, O_2, and anaerobic, AnO_2), by organism type. Graph shows that a significant proportion of isolates would remain undetected without dual culture conditions in parallel.

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Incubation prolongée?

MAJOR ARTICLE

Prolonged Bacterial Culture to Identify Late Periprosthetic Joint Infection: A Promising Strategy

Peter Schäfer,[1] Bernd Fink,[2] Dieter Sandow,[1] Andreas Margull,[1] Irina Berger,[3] and Lars Frommelt[4]

[1]Ambulatory Healthcare Center, Labor Ludwigsburg, Ludwigsburg, [2]Clinic of Joint Replacement, General and Rheumatic Orthopaedics, Orthopaedic Clinic Markgröningen, Markgröningen, [3]Institute of Pathology, Klinikum Kassel, Kassel, and [4]ENDO-Klinik, Hamburg, Germany

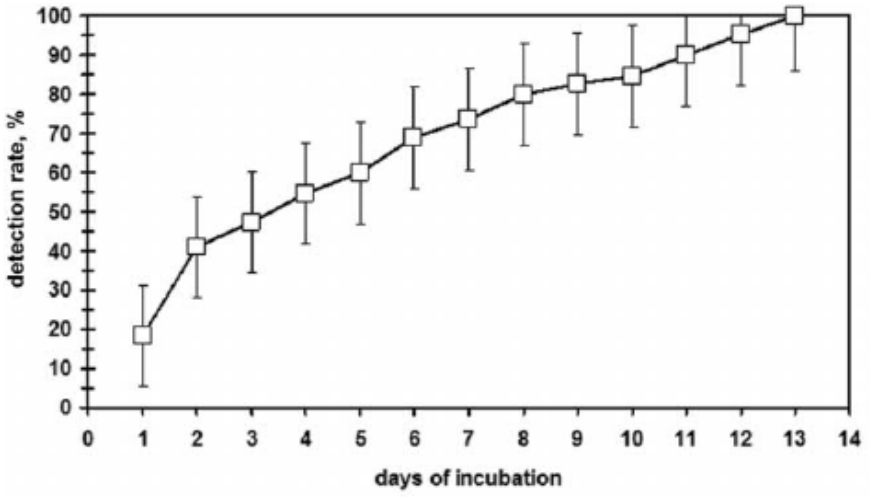

Figure 1. Time to diagnosis of infection by culture. Whisker lines span the 95% Hall-Wellner CI.

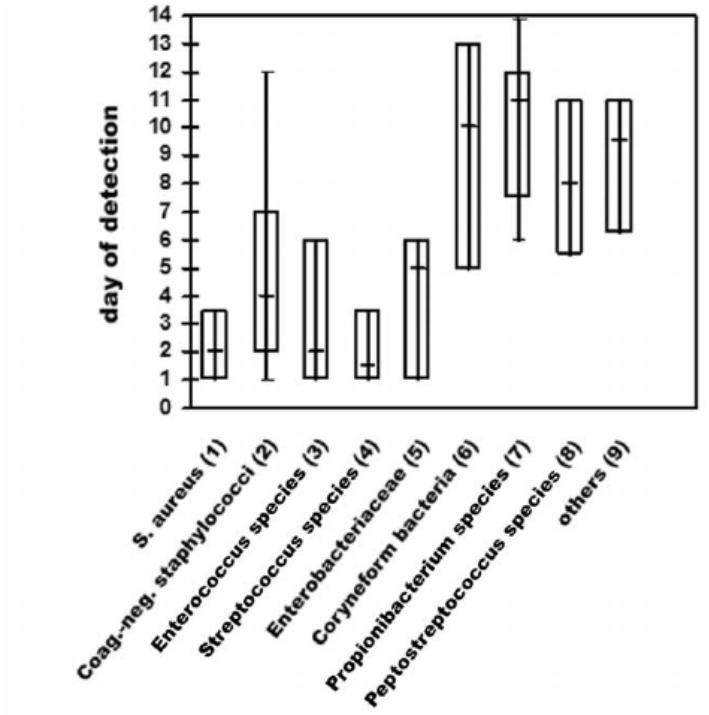

Figure 2. Time to detection of the different species in all patient samples (n = 284). Horizontal bars, boxes, and whisker lines indicate median values, 25%–75% percentiles, and 5%–95% percentiles, respectively. Early-detected species included *Staphylococcus aureus,* coagulase-negative (Coag.-neg.) staphylococci, *Enterococcus* species, *Streptococcus* species, and Enterobacteriaceae. Late-detected species included coryneform bacteria, *Propionibacterium* species, *Peptostreptococcus* species, and other species. There were no statistically significant differences among the late-detected species with regard to time to detection.

Impact de la culture prolongée

Effectif concerné:

- Bénéfice potentiel pour <7% des patients infectés
- 10-30% d'infection sur les révisions -> 70% à 90%

Conséquences:

- Antibiothérapie probabiliste injustifiée
- Durée d'hospitalisation

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Incubation prolongée?

Prolonged Bacterial Culture t
Periprosthetic Joint Infection:

Peter Schäfer,[1] Bernd Fink,[2] Dieter Sandow,[1] Andreas Margull,
[1]Ambulatory Healthcare Center, Labor Ludwigsburg, Ludwigsburg, [2]Clinic of Joi
Clinic Markgröningen, Markgröningen, [3]Institute of Pathology, Klinikum Kassel,

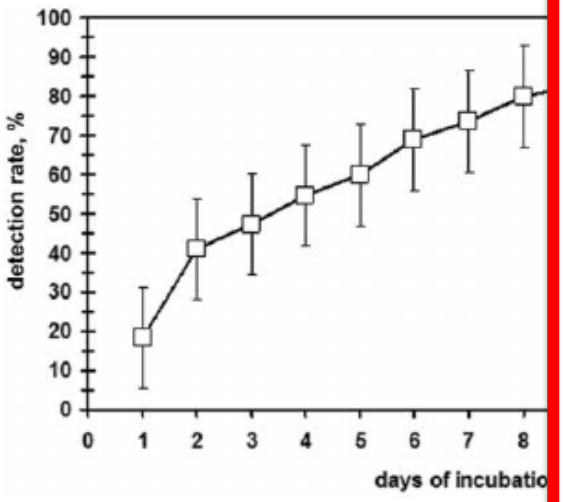


Figure 1. Time to diagnosis of infection by
the 95% Hall-Wellner CI.

Table 2. Overall association of culture with histologic findings.

Result of histologic analysis	Total no. of samples	No. of culture-positive tissue samples,[a] no. of samples		
		≥2[b]	1	0
Positive	104	80	18	6
Negative	180	12	47[c]	121
All	284	92	65	127

[a] For ≥2 positive tissue samples versus 1 positive tissue sample, $P < .001$. For 1 positive tissue sample versus no positive tissue sample, $P < .001$.
[b] Growth of indistinguishable organisms.
[c] Contaminating strains.

contaminés (=1): 22,9%
sensibilité (≥2): 76,9%
spécificité (≥2): 87,5%

Pb méthodologique:
P. acnes est responsable d'infections à histologie négative

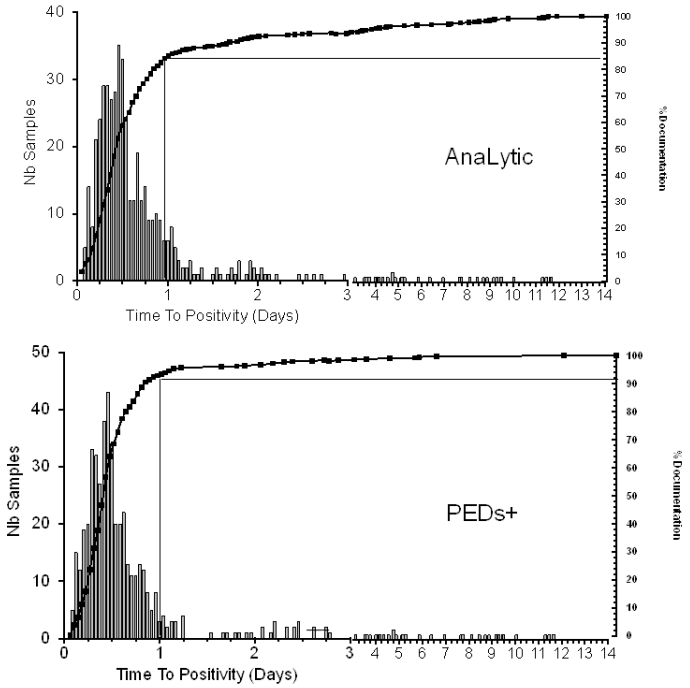
Apport incubation prolongée 2009-2015

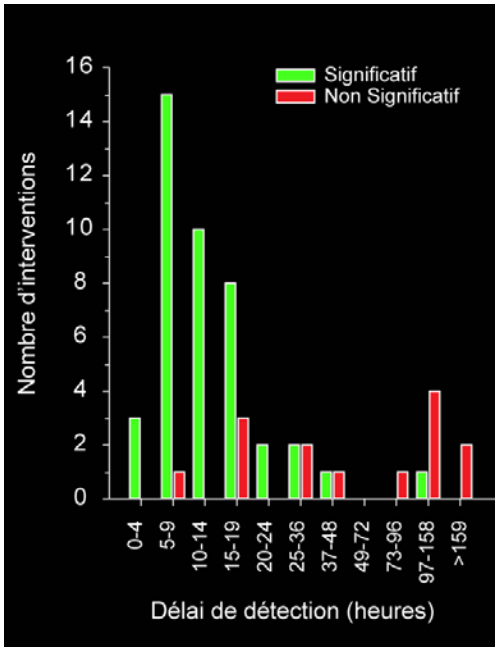
3 cas d'infections diagnostiquées à >J8

1 cas d'infection diagnostiquée à >J12

(Post ATB)

<1/1000…





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Remerciements

CRIOAs Ile de France

GH Diaconesses Croix-St-Simon

Simon Marmor

Nicole Desplaces

Ambroise Paré

Thomas Bauer

Beate Heym

Jean-Louis Gaillard

Raymond Poincaré

Benoit Combourieu

Anne-Laure Roux

JL Herrmann

Equipe Labo

Bloc opératoire

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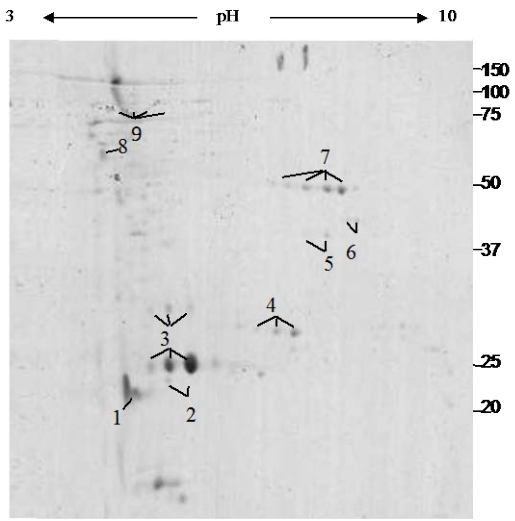
Identification immunoprotéomique des Ags



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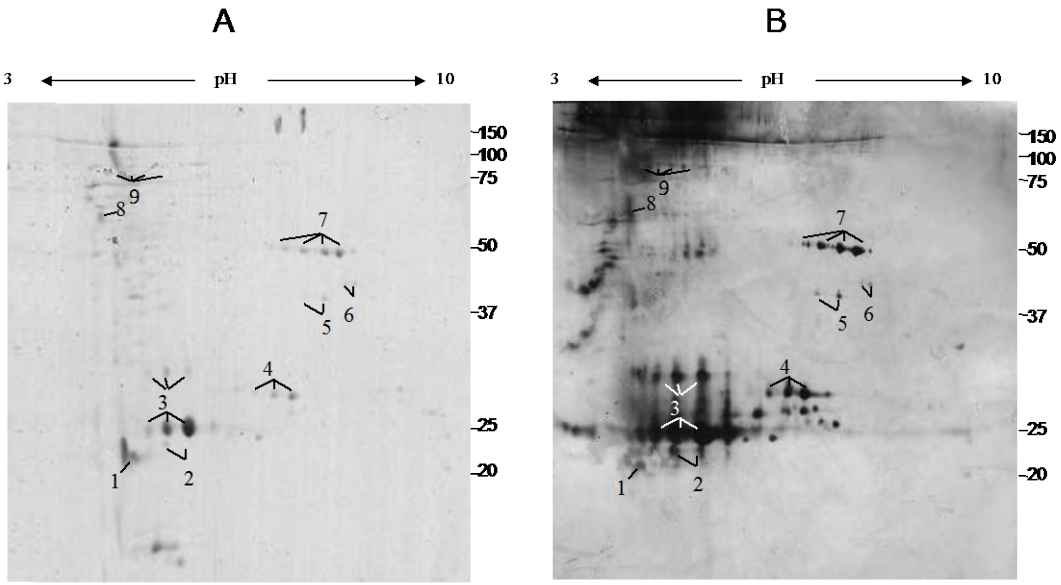

Extracellular proteins from exponential-phase *S. epidermidis* RP62A strain. Extracellular proteins from exponential-phase cells were separated on 2-DE gels (pH gradient for isoelectric focusing, pH 3 to 10).
(A) Proteins detected by Coomassie staining.
(B) Proteins detected by sera from PJI-patients following Western blotting. The numbers correspond to the protein spots listed in Table 2 and the text. The approximate positions of molecular mass markers (kDa) are indicated.

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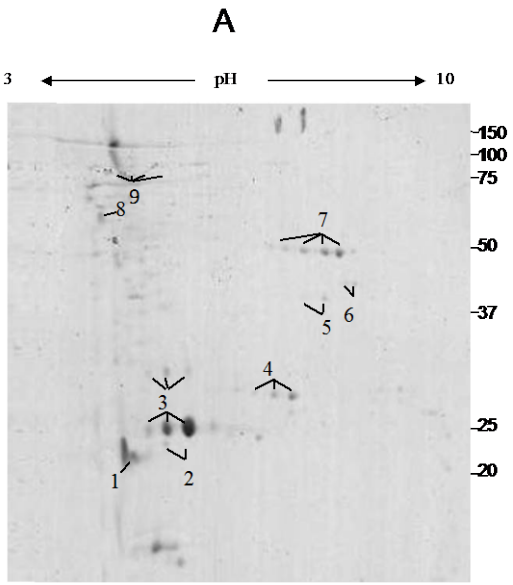

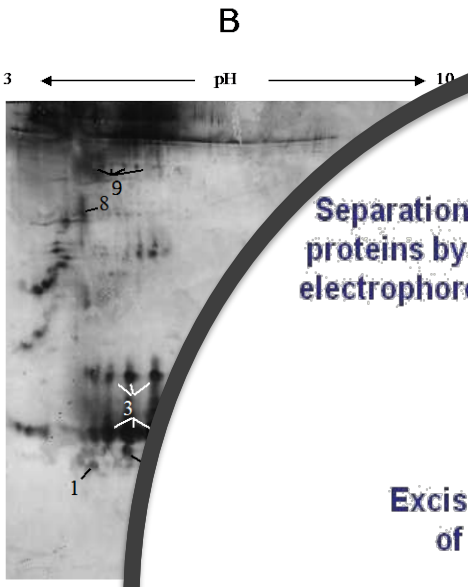

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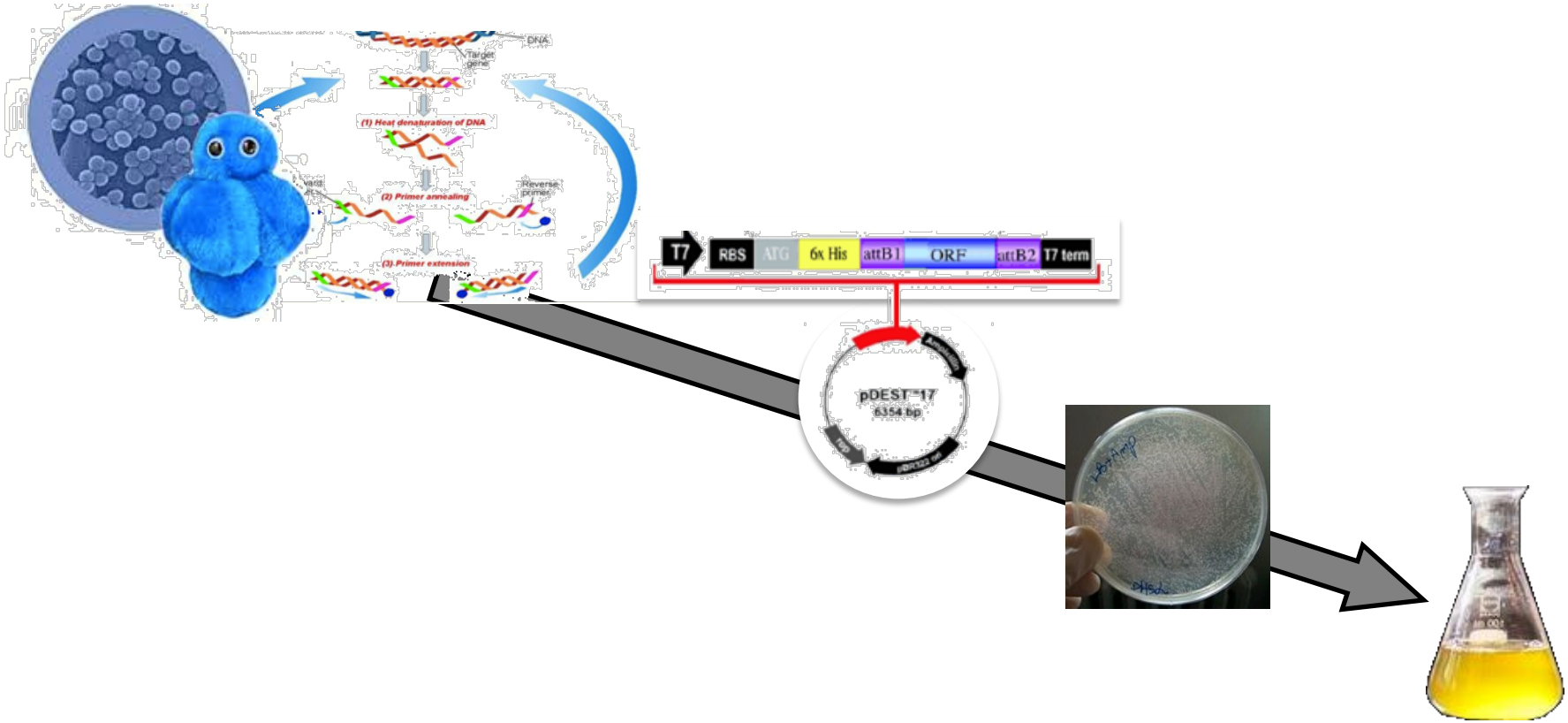
Production des Ags recombinants



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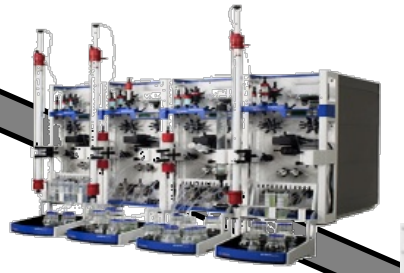
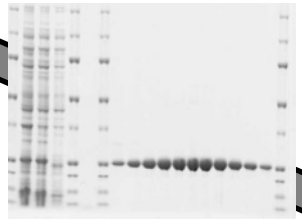
Production des Ags recombinants

Bacterial lysis



Purification by chromatography



Analysis, estimation of the purity with densitometry

ELISA
High throughput screening

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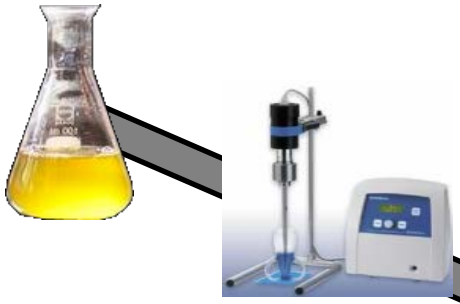
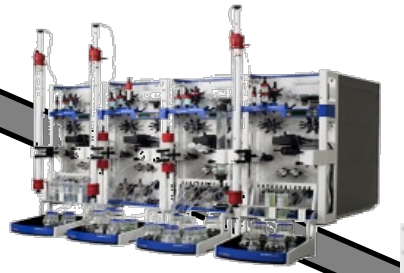
Production des Ags recombinants



Bacterial lysis



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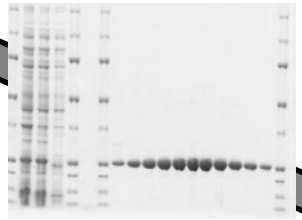

Analysis, estimation of the purity with densitometry

ELISA
High throughput screening

	Identified	Cloned produced	Screened on ELISA	Selected for dev on Luminex
Nb of Ag	450	300	300	50

Mise en œuvre du test BJI InoPlex ®

Staphylococcus: 8 Ags

S.aureus, S. epidermidis, S. lugdunensis

Streptococcus agalactiae (4 Ags)

Propionibacterium acnes (4 Ags)

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Test:

16 Antigènes testés en multiplex -> 16 résultats quantitatifs

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Streptococcus agalactiae (4 Ags)

Propionibacterium acnes (4 Ags)

Test:

16 Antigènes testés en multiplex -> 16 résultats quantitatifs

Algorithme BJI InoPlex

Synthèse en 3 résultats indépendants

- Staphylocoques: Positif/Négatif/Indéterminé
- Strepto B: Positif/Négatif/Indéterminé
- *P. acnes*: Positif/Négatif/Indéterminé

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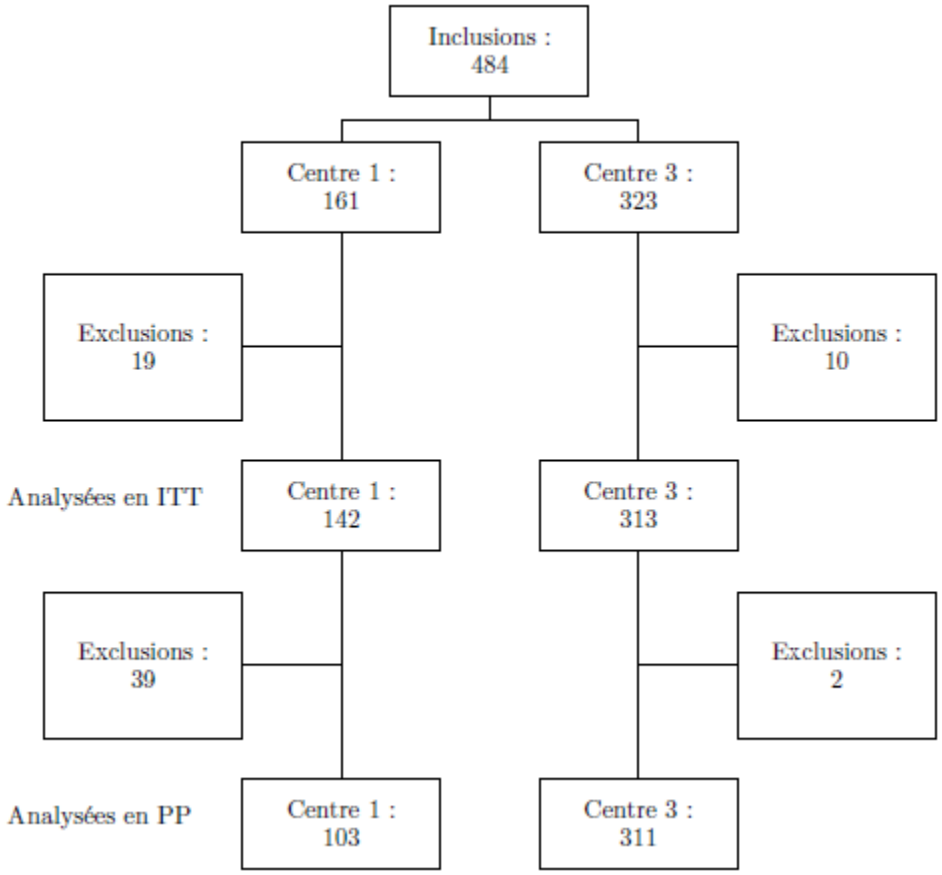
Population de l'étude



Etude prospective / Reprises de prothèse toutes indications

CRIOAs Ile de France : Ambroise Paré et GH Diaconesses Croix-Saint-Simon

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Population de l'étude

Données démographiques	
Age : moyenne (± sd)	71 (±24)
Sexe : Homme/Femme	216/237
Localisation : N (%) Hanche Genou Epaule	 301 (66,3%) 135 (29,7%) 18 (4%)
Age de la prothèse: Mediane (q1 ; q3)	4,8 (1,4 ; 10,8)
Nombre de reprises (%) 1ère 2ème 3ème 4ème	 69% 24% 7% 1%
Microbiologie (%) *Staphylococcus aureus* *Staphylococcus epidermidis* *Propionibacterium acnes* *Staphylococcus lugdunensis* *Streptococcus agalactiae*	 30% 26% 9% 6% 4%

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Résultats performances *Staphylocoques*

	S. aureus	***S. epidermidis***	***S. lugdunensis***	***Global Staph***
Sensibilité	73,8% (31/42)	74,3% (26/35)	100% (9/9)	75,9% (63/83)

Calcul sur échantillons positifs en culture peropératoire (≥2 prélèvements positifs)

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Résultats performances *Staphylocoques* Selon VS-CRP

	S. aureus	*S. epidermidis*	*S. lugdunensis*	*Global Staph*
Sensibilité	73,8% (31/42)	74,3% (26/35)	100% (9/9)	75,9% (63/83)
Sensibilité VS-CRP ↗	72,4% (21/29)	89,5% (17/19)	100% (5/5)	

Calcul sur échantillons positifs en culture peropératoire (≥2 prélèvements positifs)

Résultats performances *Propionibacterium*

	P. acnes	Tous *Propionibacterium*
Sensibilité	38,5% (5/13)	43,8% (7/16)
Spécificité	81,9% (190/232)	85,7% (114/133)

Valeur pronostique du test ? Distinction entre colonisation et infection ?

Calcul sur échantillons positifs en culture peropératoire (≥2 prélèvements positifs)

Résultats par famille

	Staphylocoques	*S. agalactiae*	*P. acnes*
Sensibilité	75,9% (63/83)	66,7% (4/6)	38,5% (5/13)
Spécificité	82,2% (180/219)	92,4% (208/225)	81,9% (190/232)

Calcul sur échantillons positifs en culture peropératoire (≥2 prélèvements positifs)

Espèces ciblées pour la famille « Staphylocoques » : *S. aureus, S.epidermidis, S. lugdunensis*

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Comparaison avec la ponction

Bactérie	Estimations	BJI	Préopératoire	P
Staph (1)	Concordance	205/254 80.7% [75.6, 85.4]	237/254 93.3% [90.2, 96.1]	0.001
	Sensibilité*	50/67 74.6% [64.2, 85.1]	57/67 85.1% [76.1, 92.5]	0.1
	Spécificité	155/187 82.9% [77.5, 88.2]	180/187 96.3% [93.6, 98.9]	0.001
S. aureus	Concordance	175/215 81.4% [76.3, 86.5]	206/215 95.8% [93, 98.1]	0.001
	Sensibilité	20/28 71.4% [53.6, 85.7]	22/28 78.6% [64.3, 92.9]	0.8
	Spécificité	155/187 82.9% [77.5, 88.2]	184/187 98.4% [96.3, 100]	0.001
S. epidermidis	Concordance	179/220 81.4% [75.9, 86.4]	210/220 95.5% [92.7, 98.2]	0.001
	Sensibilité	24/33 72.7% [57.6, 87.9]	27/33 81.8% [66.7, 93.9]	0.4
	Spécificité	155/187 82.9% [77.5, 88.2]	183/187 97.9% [95.7, 99.5]	0.001
S. lugdunensis	Concordance	163/195 83.6% [78.5, 88.7]	195/195 100% [100, 100]	0.001
	Sensibilité	8/8 100% [100, 100]	08/8 100% [100, 100]	NaN
	Spécificité	155/187 82.9% [77.5, 88.2]	187/187 100% [100, 100]	0.001
Strep	Concordance	181/198 91.4% [87.4, 94.9]	195/198 98.5% [96.5, 100]	0.001
	Sensibilité	4/5 80% [40, 100]	3/5 60% [20, 100]	1
	Spécificité	177/193 91.7% [87.6, 95.3]	192/193 99.5% [98.4, 100]	0.001
PACN	Concordance	169/212 79.7% [74.1, 84.9]	196/212 92.5% [88.7, 95.8]	0.001
	Sensibilité	5/12 41.7% [16.7, 66.7]	6/12 50% [25, 75]	1
	Spécificité	164/200 82% [76.5, 87]	190/200 95% [92, 98]	0.001

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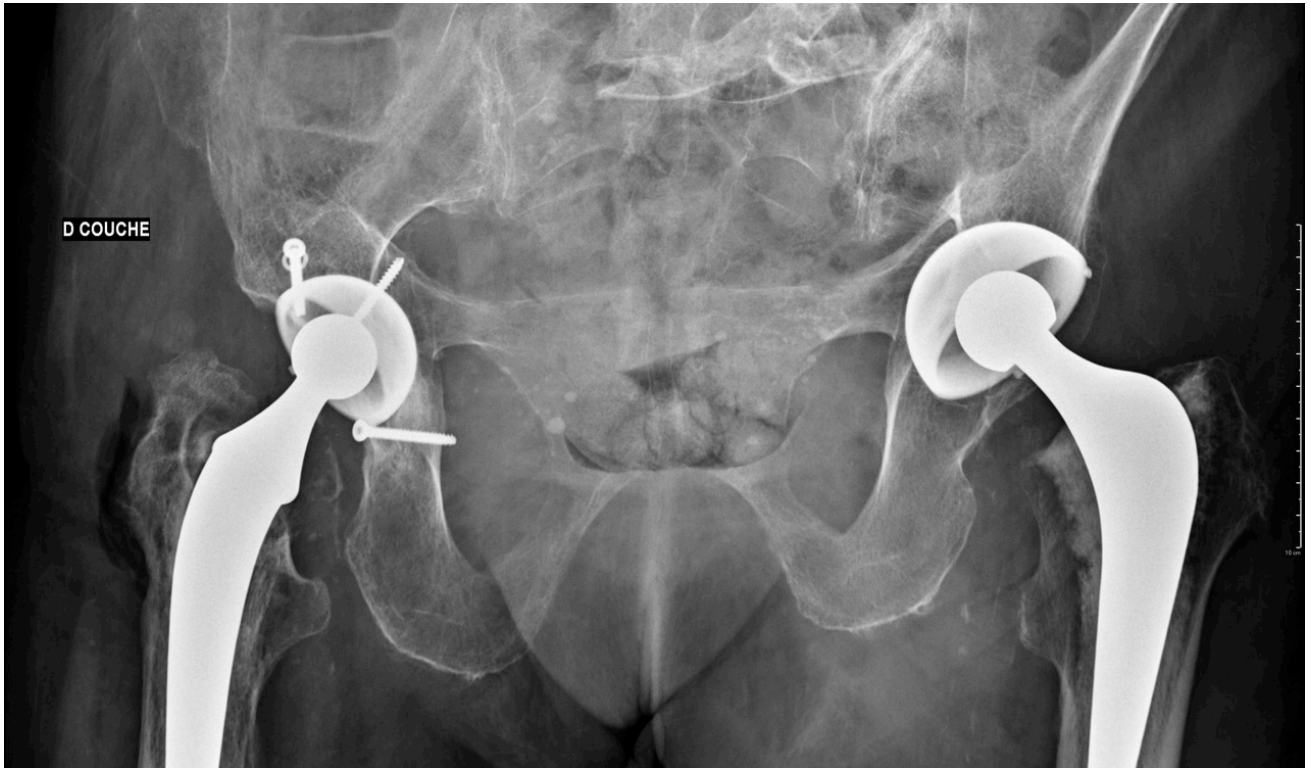
Mme B., 78 ans



Prothèse implantée il y a 11 ans, reprise il y a 4 ans

Insuffisance cardiaque majeure (FEVG 28%)

Gène fonctionnelle /Eruption sur la cicatrice (« bouton de moustique »)

Résultats

VS=21 / CRP=8

BJI Inoplex

Positif *Staphylococcus*

Négatif *Streptococcus agalactiae*

Négatif *Propionibacterium acnes*

Ponction True-cut

S. epidermidis 4/5

Fistule sur l'orifice de biopsie

Prélèvements peropératoires

S. epidermidis 5/5