Which « procedures » for an optimal Microbiological Diagnosis of Osteoarticular Infections?

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Microbiological Diagnosis of Osteoarticular Infections

- Microbiological procedures
  - conventional culture based
  - PCR based techniques
- Sampling procedures
  - superficial swab sampling
  - invasive surgical specimens

Microbiological Diagnosis of Osteoarticular Infections

Guided by epidemiological data that may differ

- Adult or pediatric population
- Primary arthritis or bone infections versus prosthetic device related infections
- Diabetic foot related osteomyelitis

Culture of Etiologic Agents in Cases of Septic Arthritis-Osteomyelitis

- Bacterial etiology of suspected septic arthritis remains unproven in one-third of cases:
  - patients received antimicrobial therapy
  - arthritis was of reactive nature
  - microorganisms are not detected by currently available techniques
  - concentrations of bacteria in synovial fluid may be of low magnitude
  - purulent exudates exert an inhibitory effect upon bacterial growth

Investigations in Pediatric Bone and Joint Infections

- White cell count: poor indicator of osteomyelitis elevated in only 35-40% of cases
- Erythrocyte sedimentation rate > 20 in 70-92% of cases
- CRP: most sensitive test: ↑ in up to 98% at admission
- Blood cultures: positive in 30-50% of patients
- Aspirations of affected bone: detection rate ↑ to 75-80%

Stott S J. Orthop Surg 2001; 9: 83-90

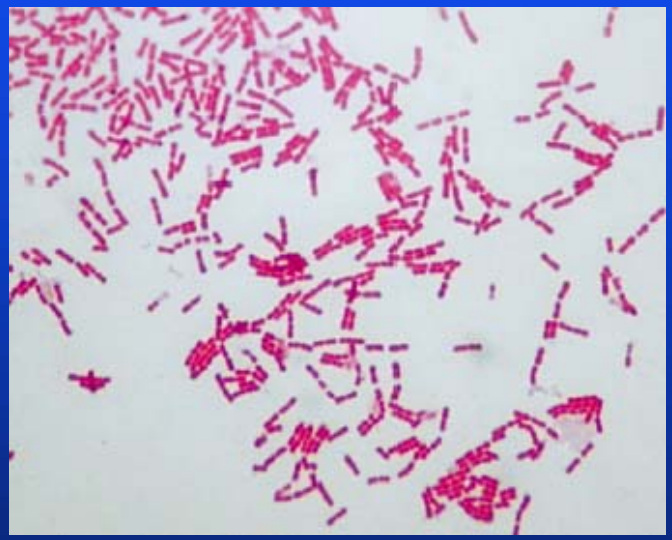
Kingella kingae: An Emerging Pathogen of Acute Osteoarticular Infections in Children

- Review of data of suspected osteoarticular infections in 406 children hospitalized during a 3.5-year period
- 74 bacteriologically proven cases: 38 septic arthritis, 36 bone infections
  - *Staphylococcus aureus* 44% most frequent ≥ 36m
    (including PVL+ CA-MRSA: 62% of all *S. aureus,* ↑ ERS and CRP)
  - *K. kingae* 14% most frequent ≤ 36m
  - *Streptococcus pyogenes* 10%
  - *Streptococcus pneumoniae* 10%
- Increasing number of reports of *K. kingae* OAI in young children, as result of improved isolation techniques: liquid media increases rate of positive cultures compared to standard media (P=0.0001)

Moumile K et al Acta Pediatr 2005; 94: 419-20
Bocchini CE et al Pediatrics 2006;117:433-40

Kingella kingae

- Small gram-negative nonencapsulated coccobacillus in pairs or short chains
- Fastidious aerobes
- Colonies on agar may be small, smooth and translucent or appear as larger colonies which look pitted
- Only species to produce beta–hemolysis but not all strains are beta-hemolytic



Yagupsky P et al. Lancet Infect Dis 2004; 4: 358-67

Pathogens Recovered from 100 Synovial Fluid Specimen in which Both Conventional (CC) and BACTEC (BC) Cultures were Performed

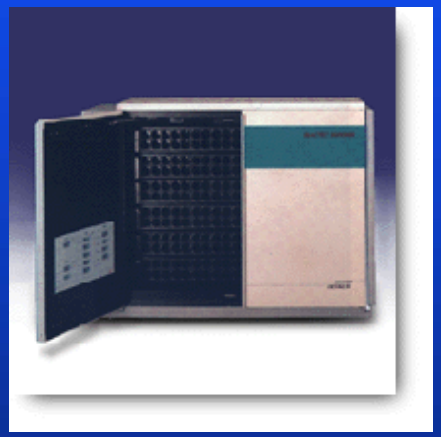
Organism	No. of specimens		
	CC+ BC+	CC+ BC-	CC- BC+
Staphylococcus aureus	7	1	3
Kingella kingae	**1**	**0**	**10**
Brucella melitensis	1	0	2
Streptococcus pyogenes	2	0	1
Streptococcus pneumoniae	0	2	0
Streptococcus group C	1	0	0
Haemophilus influenzae type b	3	0	0
Total	15	3	16

Yagupsky P et al. J Clin Microbiol 1992; 30: 1278-1281

Increased Recovery of *Kingella kingae* in Joint Fluid from Children with Septic Arthritis

- 10/11 *Kingella kingae* isolates recovered from BACTEC cultures only; for other pathogens recovery by BACTEC and conventional method comparable

  Yagupsky P et al. J Clin Microbiol 1992; 30: 1278-81



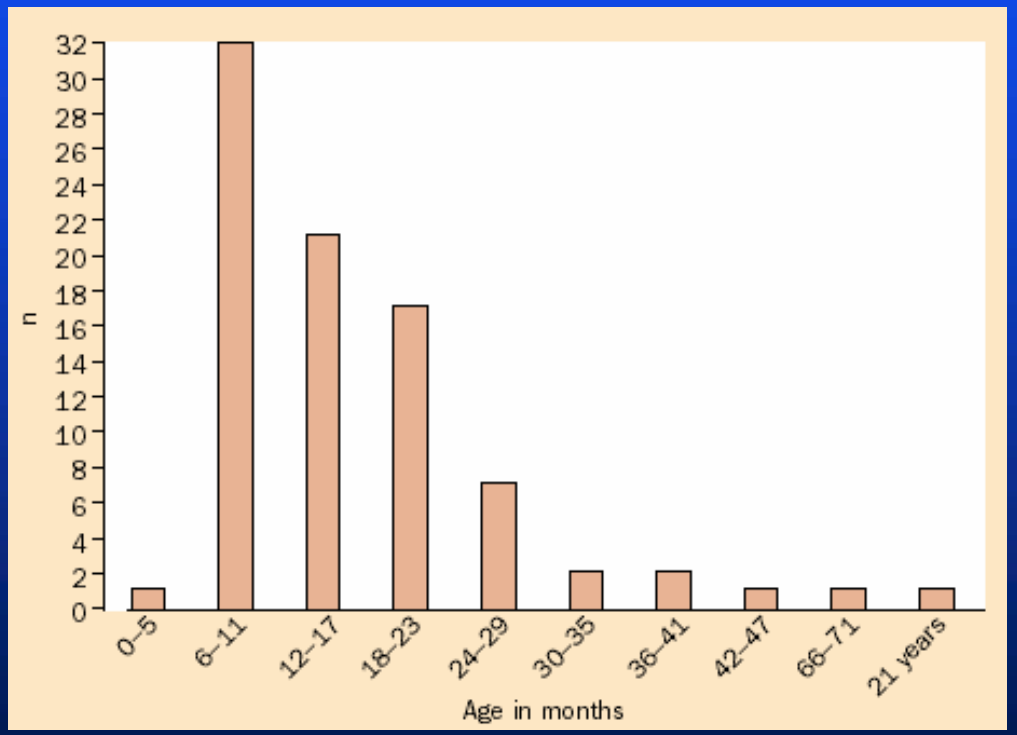
- *K. kingae* recovered from 2 cases of septic arthritis by using BacT/Alert, not from conventional cultures

  Lejbkowicz F et al. J Clin Microbiol 1999; 37:878

Age Distribution of 85 Patients with Invasive *K. kingae* Infections Diagnosed in Southern Israel in the 15-year Period 1988-2002

- *K. kingae* causesd 19/40 **(48%)** episodes of bacteriologically proven joint infections among children younger than 2 in southern Israel

- *K. kingae* was the most common bacterial isolate in children younger than 3 in Atlanta after the congugate *H.influenzae* vaccine



Yagupsky P et al. Lancet Infect Dis 2004; 4: 358-67

Increased Recovery of Organisms Causing Septic Arthritis Using Blood Culture Systems

- Prospective study on 137 adult and pediatric patients with clinical arthritis
- Inoculation of synovial fluid into an Isolator 1.5 microbial tube improves the recovery rate: total 21.5% positives :
  - 100% in Isolator
  - 78.9% in conventional cultures ($P<0.02$)
- Time to detection: similar 93.5% Isolator and 83.3% conventional cultures positive by the second day ($P>0.05$)
- Gram stain revealed causative organism in 56.0% culture

Yagupsky P et al. J Clin Microbiol 1997; 35: 2410-2412

Contribution of Broad Range PCR to Diagnosis of Osteoarticular Infections

- Patients
  - Prospective study:

    171 children with osteoarticular infections between 01/2001 – 02/2004
- Methods : osteoarticular fluids of biopsy samples:
  - Cultures on BA, chocolate agar
  - BACT ALERT 3D blood culture bottles
  - 16S rDNA amplification culture neg samples

Verdier I et al. Pediatr Infect Dis J 2005: 24: 692-96

Distribution of Bacterial Species Detected by Culture or PCR in Children with Primary OAI

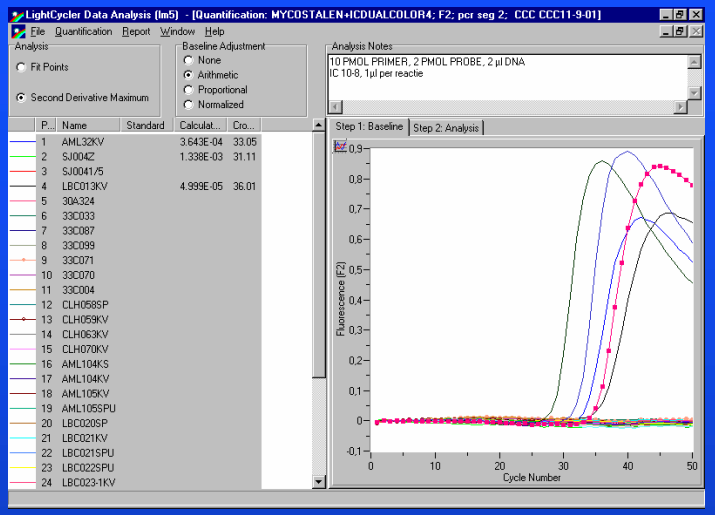
Species	Culture		Culture and PCR	
	Number	% of positive OAI (n=64)	Number	% of Positive OAI (n=79)
Staphylococcus aureus	30	47	30	38
ß-Hemolytic streptococci, group A (n=7), group B (n=2), group C (n=1)	10	16	10	13
Kingella kingae	**9**	**14**	**24**	**30**
Coagulase-negatieve staphylococci	7	11	7	9
Streptococcus pneumoniae	4	6	4	5
Propionibacterium acnes	2	3	2	3
Moraxella spp.	1	1.5	1	1
Gemella morbillorum + *Streptococcus gordonii*	1	1.5	1	1

Verdier I et al. Pediatr Infect Dis J 2005: 24: 692-96

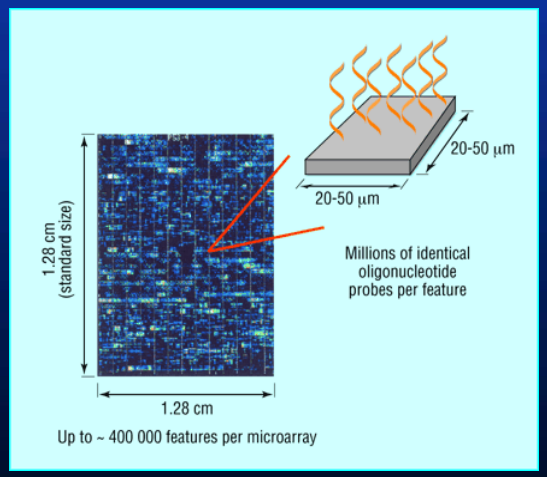
Contribution of Broad Range PCR to Diagnosis of Osteoarticular Infections : CONCLUSIONS

- Only 79/171 (46%) of cases of OAI were bacteriologically documented by culture on PCR.
- The use of blood culture bottles strongly increases the diagnostic yield
- *Kingella kingeae* accounted for 14% of culture-positive cases.
- Molecular methods increased the identification of *Kingella kingeae* in OAI up to 30%

Verdier I et al. Pediatr Infect Dis J 2005: 24: 692-96



Do we need PCR for the Diagnosis of Osteoarticular Infections ?



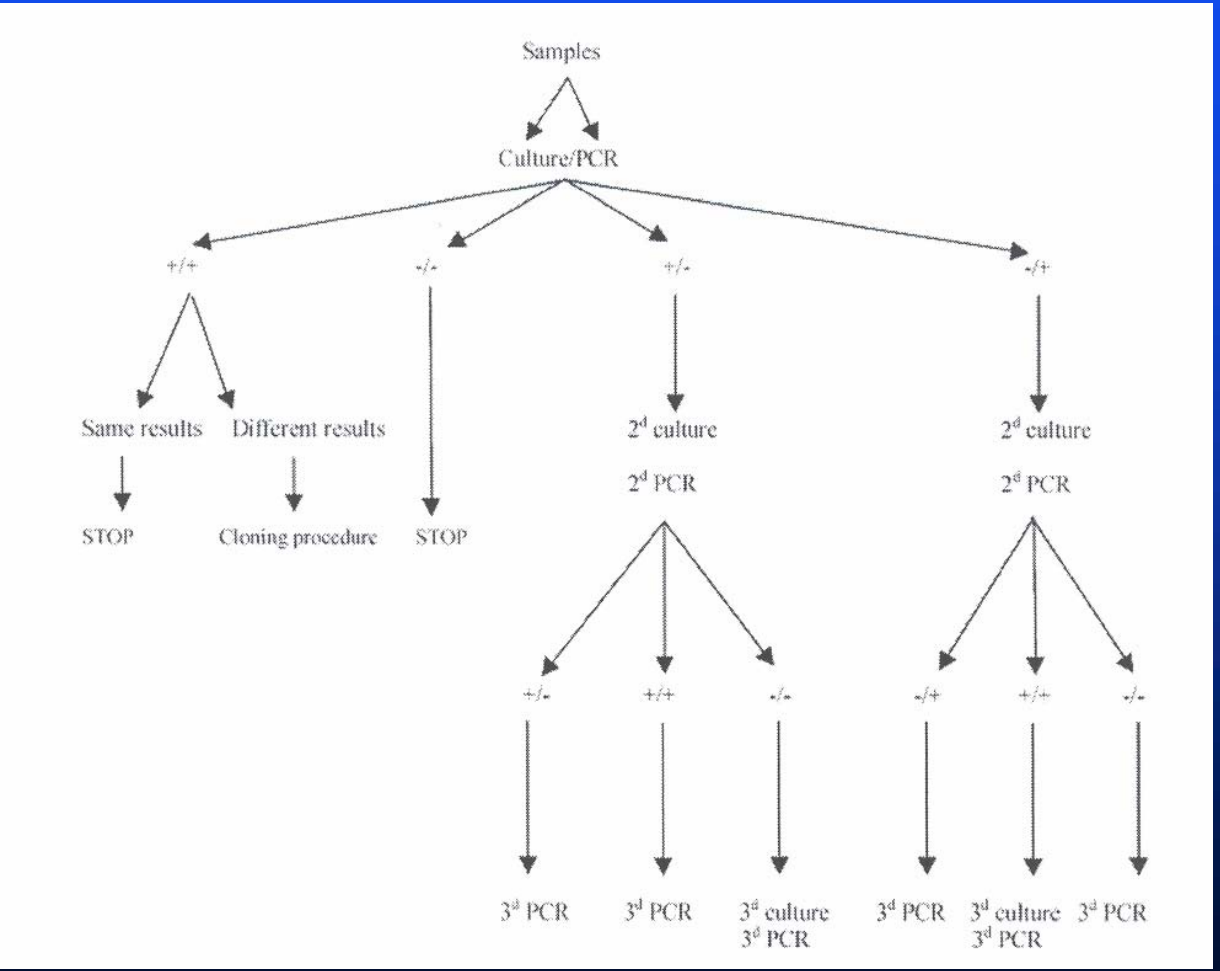
Usefulness of PCR for the Diagnosis of Bone and Joint Infections ?

- 525 bone and joint samples collected by needle aspiration or by surgical biopsy
- Conventional culture and 16S rRNA PCR followed by sequencing
- Interpretation of results:
  - Isolation of the organism at least twice
  - If negative culture: true PCR positive if same microorganism at least twice with different PCR's

⇒ **Very strict criteria**

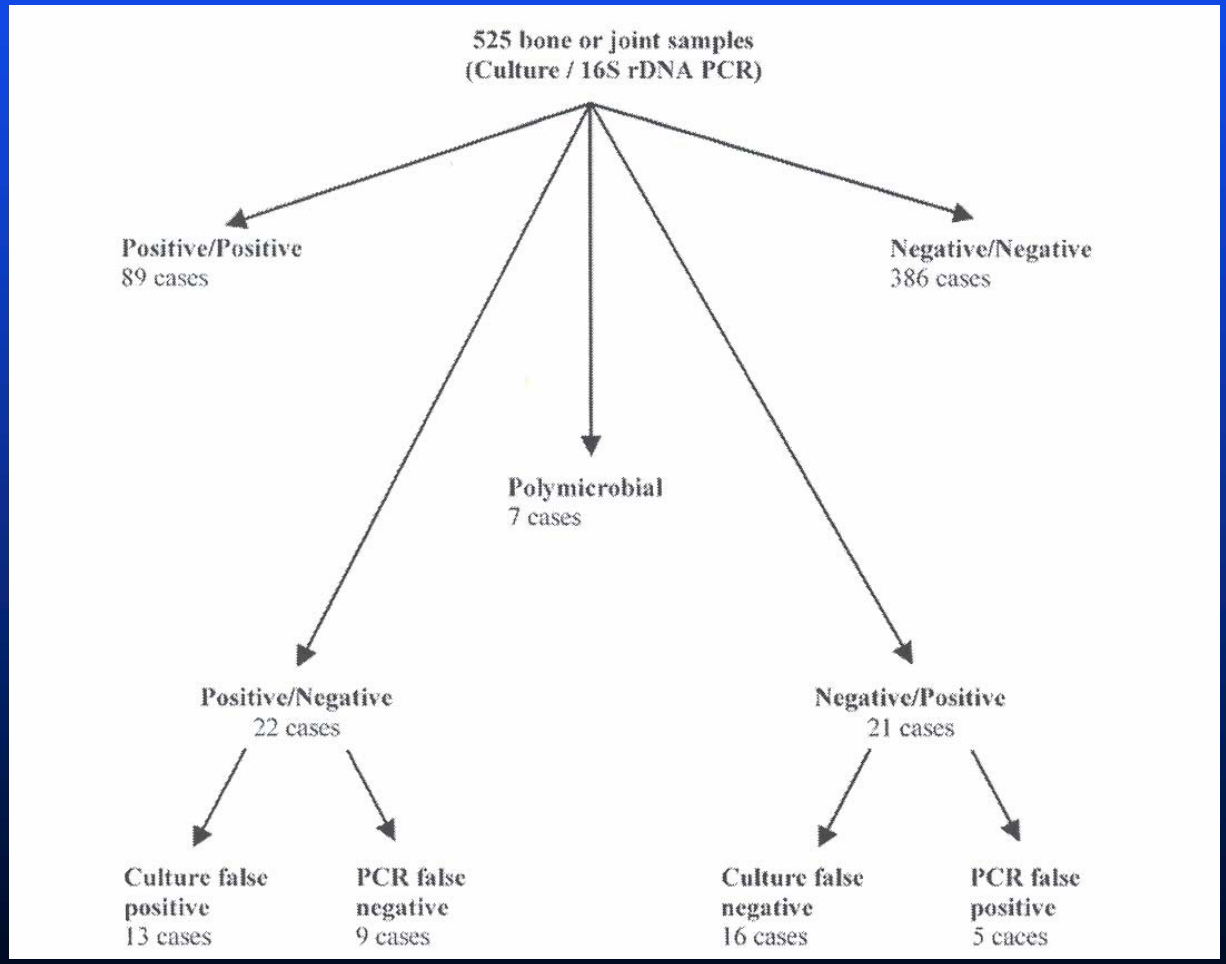
Fenollar F. et al. J Clin Microbiol 2006; 44: 1018-28

Schematic Strategy for the Reconciliation of Conventional Culture and PCR Assays



Fenollar F. et al. J Clin Microbiol 2006; 44: 1018-28

Comparison of the Data Obtained for the Analysis of 525 Bone or Joint Samples Using Conventional Culture and 16S rRNA gene PCR followed by sequencing



Fenollar F. et al. J Clin Microbiol 2006; 44: 1018-28

Usefulness of PCR for the Diagnosis of Bone and Joint Infections ? Results

- 89 concordant results:
  - 40% *S. aureus*
  - 20% CNS
  - 12% Streptococci
  - 12% Enterobacteriaceae
  - 6% *Ps. aeruginosa*
  - 7% anaerobes, HACEK, *M.tuberculosis*
- Culture false negative (n=16): 44% due to antibiotic treatment:
  - 25% *S. aureus*
  - 50% Streptococci
  - 25% Gram negatives and fastidious organisms
- PCR false negative (n=9): most S. aureus and CNS : inhibitors
- Polymicrobial (n=7): rarely or previously unreported: most anaerobes

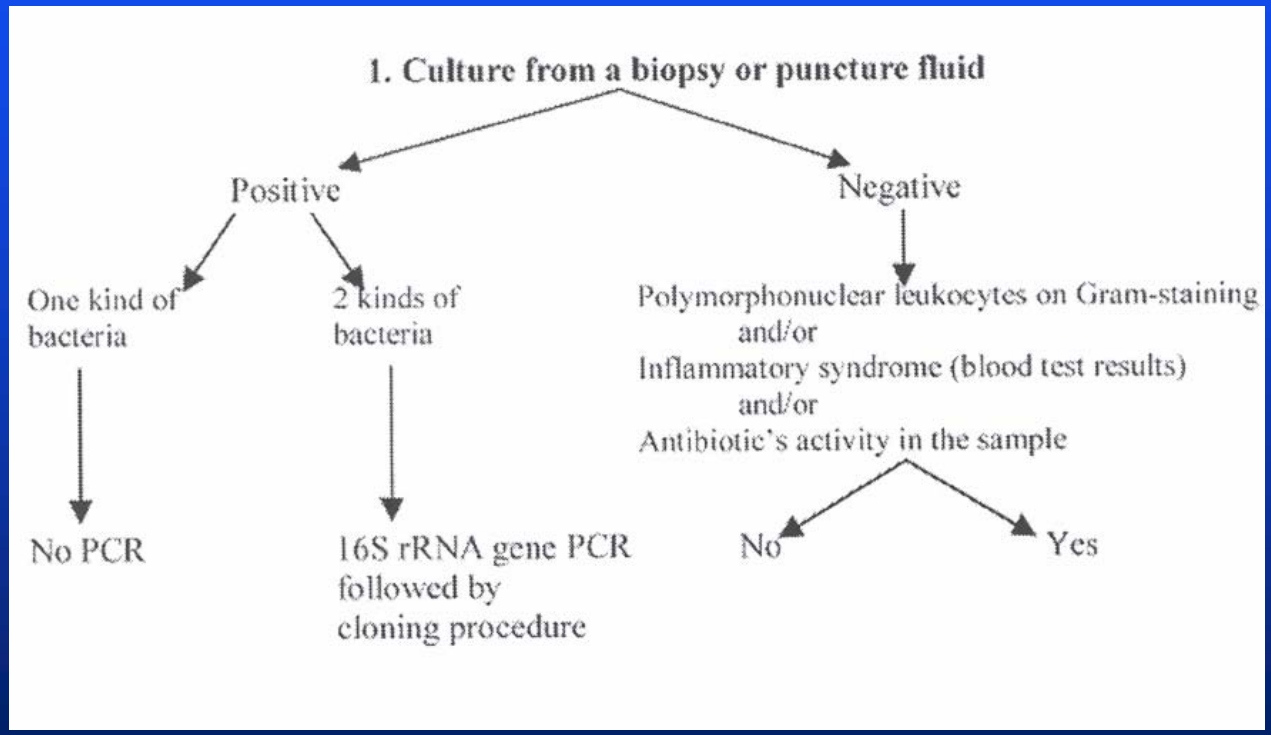
Comparison of Sensitivity, Specificity, Positive Predictive Value, and Negative Value between Culture and 16S rRNA gene PCR Followed by Sequencing

Test	Result	No. of samples with definite conclusion		% sensitivity[a]	% specificity[b]	Predictive value	
		Positive	Negative			% Pos.	% Neg
Culture	Positive	105	13	86.7	89	89	96
	Negative	16	391				
PCR	Positive	112	5	92.5	95.7	95.7	97.7
	Negative	9	389				
Total		121	424				

[a] *P* = 0.13 (chi-square test).
[b] *P* = 0.06 (chi-square test).

Fenollar F. et al. J Clin Microbiol 2006; 44: 1018-28

Strategy for the Use of 16S rRNA gene PCR for the Diagnosis of Bone or Joint Infections



PCR is **complimentary to culture** and should be used in patients **highly suspected for whom culture was negative** or in cases of **suspected polymicrobial infections**

Fenollar F. et al. J Clin Microbiol 2006; 44: 1018-28

Osteomyelitis in Diabetic Foot Infections

Difficult and controversial

- Lack of consensus on the diagnosis of foot osteomyelitis
- Many available diagnostic tests, but they often yield equivocal results
- Osteomyelitis ↑ the likelihood of surgical intervention, including amputation
- Osteomyelitis impairs healing of the overlying wound and acts as a focus for recurrent infections

⇒ **Diagnosis is important**

Lipsky B et al. Clin Infect Dis 2004; 39: 889

Percutaneous Bone Biopsy versus Swab Cultures for Diagnosis of Diabetic Foot Osteomyelitis

Pathogen	No. of instances in which culture yielded the specified pathogen				
	Total	From bone biopsy sample only	From swab sample only	From both bone biopsy and swab samples	concordance %
Staphylococcus aureus	49	13	15	21	42.8
CNS	35	**30**	**4**	**1**	2.8
Streptococci	31	11	12	8	25.8
Enterococci	15	9	5	1	6.67
Corynebacteria	10	2	8	0	0
Gram-negative bacillii	42	12	18	12	28.5
Anaerobes	9	6	3	0	0
Total	191	79	65	43	22.5

Senneville E et al. Clin Microbiol Infect Dis 2006; 42: 57-62

Percutaneous Bone Biopsy versus Swab Cultures for Diagnosis of Diabetic Foot Osteomyelitis

- Largest population of consecutive patients with diabetic foot osteomyelitis studied with surgical percutanous bone biopsy
- Overall concordance for all isolates was 22.5%: 42.8% for *Staphylococcus aureus.*
- Distribution in bone and swab cultures were similar, except for CNS which were more prevalent in bone samples ($P<0.001$)

⇒ **Superficial swab cultures do not reliably identify bone bacteria**

Senneville E et al. Clin Microbiol Infect Dis 2006; 42: 57-62

Microbiology versus MRI and Labelled Leucocyte Scanning in the Diagnosis of Osteomyelitis of the Diabetic Foot

- 31 patients with foot lesions ≥ grade 3 Wagner classification
- Histopathological examination as gold standard
- *Pseudomonas aeruginosa* (33%) most common, MRSA (24%), *Acinetobacter* spp (12%); anaerobic cultures only *Peptostreptococcus* spp (3%)

	Sensitivity	Specificity	PPV	NPV
Microbiology	92%	60%	92%	60%
Leucocyte scanning	91%	67%	95%	50%
MRI	78%	60%	90%	37.5%

⇒ Microbiology is effective and less costly

Ertugrul MB et al. Diab Med 2006; 23: 649-53.

Needle Puncture vs Superficial Swab in Infected Diabetic Foot with Osteomyelitis

- Prospective study in 21 diabetic patients
- Mean number of microorganisms isolated by needle puncture compared to superficial swabbing: 1.09 vs 2.04 ($P<0.02$)
- *S. aureus* : 70% of cases
- In 76% of patients, microbiological confirmation with needle aspiration
- Needle puncture

  = more specific

  = safe, minimally invasive

Kessler L et al. Diab Med 2005; 23: 99-102

IDSA Grading System for ranking Recommendations in Clinical Guidelines

Strength of recommendation	
A	Good evidence to support a recommendation for use
B	Moderate evidence to support a recommendation for use
C	Poor evidence to support a recommendation; optional
D	Moderate evidence to support a recommendation against use
E	Good evidence to support a recommendation against use
Quality of evidence	
I	Evidence from at least one properly randomized, controlled trial
II	Evidence from at least 1 well-designed clinical trial without randomization, f rom cohort or case-controlled analytic studies, from multiple time-series studies, or from dramatic results in uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Lipsky B et al, Clin. Infect. Dis. 2004; 39:885-909

Guidelines on Diagnosis of Osteomyelitis in Diabetic Foot Infections

- **Bone biopsy** is valuable for establishing the diagnosis of osteomyelitis : **B II**
  - for defining the pathogenic organism
  - for determining the antibiotic susceptibility
- **Further research is much needed**: adequately powered prospective studies should be undertaken to elucidate and validate: **A III**
  - systems for classifying infection
  - diagnosis osteomyelitis
  - defining optimal antibiotic regimes
  - clarifying the role of surgery in treating osteomyelitis

Lipsky B et al. Clin Infect Dis 2004; 39: 889-910

Simple Puncture versus Notch Needle Biopsy

- Prospective study on 54 patients with deep infections of knees, ankels, elbows, shoulder
- surgical prelevement was gold standard

	Sensitivity	Specificity	PPV
Simple puncture	31.25%	97%	83%
Notch needle biopsy	69%	100%	100%

$\Rightarrow$ Notch needle biopsy is superior to simple puncture

Piriou P et al. Rev Chir Orthop 1998; 84: 685-88

Osteoarticular Tuberculosis

- Review of 26 patients over last 10 years
- Positive skintest in 15/18 patients: sensitivity 83.3%
- Positive, negative tuberculin test does not rule out skeletel tuberculosis
- Yield in different samples:
  - bone tissue: 86%
  - synovial tissue: 69%
  - abscess: 66%
  - synovial fluid: 66%

⇒Tissue samples yield best results and should be available

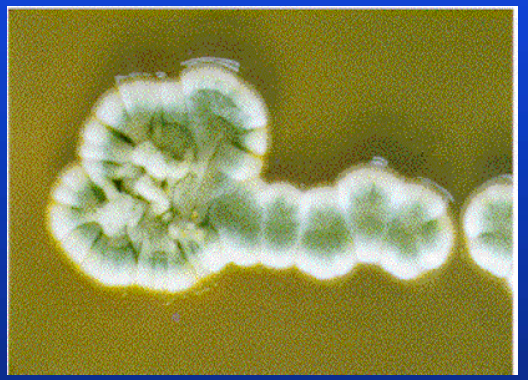
⇒Case reports of PCR proven MTb bone infections

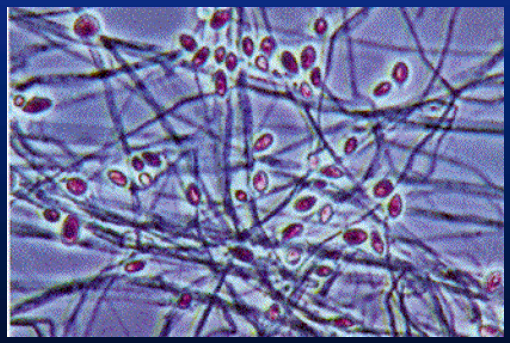
Ruiz G et al. Clin Microbiol Infect 2003; 9: 919-923

Fungal Osteoarticular Infections

- Most studies consist of case reports or small case series
- Increasing with growing use of prosthetic joints and ↑ immunosuppressed patients
- Largest review → in cancer patients
- Moulds are most common pathogens: 24/28 (86%)
  - *Aspergillus* spp (n=10)
  - *Fusarium* spp. (n=6)
  - Zygomycetes (n=5)
  - *Scedosporium apiospermum* (n=2)

⇒ Diagnosis by **culture of bone tissue**





Kumashi PR et al. Clin Microbiol Infect Dis 2006; 12: 621-26

Osteoarticular Involvement in Brucellosis

- Documented extensively from Middle East and Spain, infrequently in this region
- In 42% of cases of acute brucellosis: osteoarticular involvement
- 95% of patients: positive serology with culture proven brucellosis
- Overall, 82% of bloodcultures and 100% of other body fluid cultures positive

Andriopoulos P Int J Infect Dis 2006; 29: ????.

Prosthetic Osteoarticular Infections

- 60% by direct contamination during operative procedure
- 50% of all isolates: *S. aureus* and *S. epidermidis*
- ESR and CRP: non specific
- Gram staining of synovial fluid and periprosthetic tissue: low sensitivity: 25-30%
- Culture from synovial fluid of periprosthetic tissue = GOLD STANDARD: sensitivity : 65-94%
- PCR for rRNA: experimental but ↑ sensitivity

Barberan J. Clin Microbiol Infect Dis 2006; 12: 93-101

Osteoartritis and Anaerobes:

- *Peptostreptococcus magnus*
  - After orthopaedic prothesis or inplantation of fixation devices

Felten et al. Pathol Biol 1998; 46: 442-48

- *Pripionibacterium acnes*
  - Spondylodiscitis following lumbar punction

Hammann C Schweiz Med Wochensch 1999; 9: 129: 1456

  - Spontaneous infection or secondary to implantation of foreign material

Crouzet J Rev Rhum Engl Ed 1998; 65: 68-71

⇒ **ANAEROBIC CULTURE SHOULD BE CONSIDERED**

Which « procedures » for an optimal Microbiological Diagnosis of Osteoarticular Infections: CONCLUSIONS

- **ESR and CRP**: quite sensitive but low specificity
- **Correct sampling is crucial**: percuteneous or surgical biopsy material required in case of bone infections, synovial fluid for arthritis
- Inoculation of samples into **blood culture systems** increases significantly the diagnostic yield
- PCR may be considered in culture negative cases but so far not introduced into routine management