Review

Optimizing culture methods for diagnosis of prosthetic joint infections: a summary of modifications and improvements reported since 1995

Lone Heimann Larsen,¹ Jeppe Lange,^{2,3} Yijuan Xu^{4,5}
and Henrik C. Schønheyder¹

Correspondence
Lone Heimann Larsen
lone.l@rn.dk

¹Department of Clinical Microbiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark

²Department of Orthopaedics, Aarhus Hospital, Aarhus University Hospital, Aarhus, Denmark

³Department of Orthopaedics, Regional Hospital Silkeborg, Silkeborg, Denmark

⁴Section of Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Aalborg, Denmark

⁵Life Science Division, The Danish Technology Institute, Aarhus, Denmark

Improving diagnosis of prosthetic joint infections (PJIs) has become an increasing challenge due to a steadily rising number of patients with prosthetic implants. Based on a systematic literature search we have ascertained the evidence base for improvement of culture diagnosis. We searched PubMed/MEDLINE using the medical subject heading (MeSH) 'prosthesis-related infections' 1995 through 2010 without further restrictions. An analogous search was conducted for ISI Web of Knowledge. A total of 1409 reports were screened for original results, obtained by methods described in sufficient detail to make replication possible. We gave priority to methods for sample preparation, culture media, culture methods and incubation time. Clinical sensitivity and specificity were calculated where possible. We found evidence to support superiority of cultures obtained from the diluent after sonication of prosthetic implants in comparison with culturing tissue biopsies. Sonication parameters and accessory steps have been studied extensively, and thresholds for significant growth have been defined. Conversely, methods for processing of soft tissue biopsies have been studied to a limited extent. Culture of synovial fluid in blood culture vials has been shown to be more sensitive (90–92 %) than intraoperative swab cultures (68–76 %) and tissue cultures (77–82 %). Formal evaluation of agar media for culturing PJI specimens seemed to be lacking. The polymicrobial nature of PJIs supports the routine use of an assortment of media suitable for recovery of fastidious, slow-growing, anaerobic and sublethally damaged bacteria. A number of studies supported an incubation period for up to 14 days. Although we identified evidence-based improvements of culture methods, there is a need for more studies especially with regard to tissue biopsies. Culturing remains an important means to identify and characterize pathogenic micro-organisms and supplements the increasing number of culture-independent assays.

Introduction

Joint replacement has become one of the most common surgical procedures in industrialized countries and contributes significantly to the mobility and quality of life of elderly people. Even with the best precautions, prosthetic joint infections (PJIs) do occur and they have become a significant burden on orthopaedic services due to the sheer

number of patients with hip and knee prostheses. The clinical spectrum of PJIs is variable and includes both overt and silent infections, and bacterial pathogens are envisaged to have a role in 'aseptic' loosening (Zimmerli *et al.*, 2004; Zappe *et al.*, 2008; Pedersen *et al.*, 2010).

For these reasons, diagnostic methods have received increasing attention. A range of molecular techniques have been introduced primarily as research tools and a new concept of PJIs is gradually emerging (McDowell & Patrick,

A supplementary table is available with the online version of this paper.

2005; Achermann *et al.*, 2010). Bacteria common in PJIs are typically organized in a biofilm, which is a microbial community enclosed within an extracellular matrix (Donlan, 2002). The normal microbiota of the skin is the most common source of bacteria detected in PJIs, and 16S rRNA gene sequence-based methods have revealed infections to be polymicrobial in line with results achieved by standard culture methods in some studies (Tunney *et al.*, 1999; Moojen *et al.*, 2007).

Despite the increased utilization of molecular techniques, culture methods are indispensable for determination of antibiotic susceptibility and they are an important means of confirming results obtained by culture-independent methods. Kamme & Lindberg (1981) were the first to report separate sampling and processing of multiple tissue biopsies taken in proximity to hip prostheses as a means of increasing the accuracy of infection diagnosis. Over the years, a number of studies have addressed different methodological issues that may have an impact on the yield of positive cultures. However, to our knowledge, few attempts have been made to systematize and critically assess such methods (Gollwitzer *et al.*, 2006).

Several overviews of the biochemistry, clinical diagnosis and treatment of PJIs have recently been published (Senthil *et al.*, 2011; Gomez & Patel, 2011a, b). Still, there is a need for a more detailed assessment of methods for sample preparation, culture media, culture methods and incubation time. The aim of this review was to determine the best practices for improvement of culture diagnosis of PJIs.

Methodological approach

A search of ISI Web of Knowledge and the US National Library of Medicine's MEDLINE database was conducted for relevant articles. Access to MEDLINE was through PubMed, using the medical subject heading (MeSH term) 'prosthesis-related infections' (introduced 1992) with the subheading 'microbiology' and a restriction to the years 1995–2010 (date of search 15 January 2011). No restriction was made to orthopaedic infections because methodological studies addressing other prosthetic infections were seen as potentially relevant.

A total of 1409 articles were screened initially by title and subsequently by the contents of the abstract and the section on material and methods. We specifically sought information on modification, improvement or optimization of diagnostic methods as well as detailed descriptions of sample preparation and culture methods, including incubation time. Further references were obtained from reference lists.

For each eligible article, we collected the following information: year of publication, researcher/research group, design (observational study vs clinical trial, prospective vs retrospective study, comparative vs non-comparative study), diagnostic criteria for PJI, method(s) evaluated, reference method ('gold standard') if any, unit of observation (patients, samples, bacterial isolates), report format

(cross tables or aggregated figures) and accuracy [sensitivity, specificity, positive and negative predictive values (PPV and NPV)].

Few studies did fulfil basic criteria for a systematic review. The most pertinent problems were lack of a gold standard and independent evaluation of results. Statistical analyses of results were often unclear and units of observation differed between (and sometimes within) studies. Where possible, we assessed test performance based on numbers of patients, and we defined the sensitivity of a given method as the number of PJI patients with a positive culture divided by the number of PJI patients examined by that method. Likewise, specificity was defined as the number of patients without PJI who had a negative culture divided by the number of non-PJI patients examined by the method. In order to compare the accuracy of methods, we calculated exact 95% confidence limits for proportions [(#/#)] (Stata 11, College Station, Texas, USA).

Current approaches

Transportation systems

We found no studies evaluating the performance of transport media with orthopaedic samples. A non-comparative study used broad-necked containers with Stuart transport medium for surgical biopsies from patients undergoing prosthetic joint revision (Mikkelsen *et al.*, 2006). However, several studies evaluated the performance of transport media with cultures of fastidious and robust aerobic and anaerobic bacteria according to the M40-A standard (Clinical and Laboratory Standards Institute) (CLSI, 2003; Rishmawi *et al.*, 2007; Van Horn *et al.*, 2008; Stoner *et al.*, 2008; Tano & Melhus, 2011). Performance differed depending on temperature, holding time and bacterial strains. In general, good preservation was reported for media held at 4 °C but the results varied at room temperature. Results obtained with simulated polymicrobial samples were less predictable (Tano & Melhus, 2011). Transport systems with Amies medium or variations thereof maintained viability better than Stuart medium, but promoted growth of some bacteria (Tano & Melhus, 2011).

Tunney *et al.* (1998, 1999) used anaerobic jars for transportation of prosthetic components from the surgical theatre to the laboratory followed by strict anaerobic processing of samples. Anaerobes accounted for a high proportion (62%) of isolates from the prosthetic components.

Sample preparation

PJI samples comprise frank pus, purulent fluids, synovial fluid, synovia and/or other soft tissue samples, bone biopsies, prosthetic components or entire prostheses.

Different treatments are applied to the samples before inoculation takes place, either to dislodge bacteria from a matrix or to increase the density. Several studies have addressed such preparatory steps.

Synovial fluid. Techniques primarily developed for blood culturing have been pivotal for processing of synovial fluid in PJIs. Paediatric blood culture vials were reported to detect more pathogens than agar plate methods (62 vs 51 pathogens; $P=0.001$) with fewer contaminants (1 vs 11 contaminants; $P=0.006$) (Hughes *et al.*, 2001). The authors related the contaminants to handling and inspection of agar plates.

Two studies compared culturing of synovial fluid with surgical swabs or tissue biopsies in patients with PJIs using direct inoculation of aerobic and anaerobic blood culture vials for synovial fluid and agar plates for swabs or biopsies (Levine & Evans, 2001; Font-Vizcarra *et al.*, 2010). Both studies showed higher sensitivity and specificity for direct inoculation although the precision was low (Table 1).

Melhus & Tjernberg (2000) evaluated three different blood culture vials (two anaerobic and one paediatric) for recovery of anaerobic bacteria in a simulation study with 10 bacterial strains. They found differential growth of anaerobic bacteria in blood culture vials and thioglycolate broth whereas chopped meat broth (a traditional anaerobic medium) performed well.

Swab cultures. Culturing of superficial swabs from wound drainage has a traditional place in the diagnosis of deep bacterial infections (Mackowiak *et al.*, 1978). Cuñé *et al.* (2009) evaluated such cultures in patients with acute postoperative PJI and found isolation of *Staphylococcus aureus* and enteric rods highly predictive of the aetiological organism (PPV >86 %, NPV >94 %).

As shown in Table 1, intraoperative swab cultures had a lower sensitivity compared with culturing of synovial fluid and tissue biopsies according to the study by Font-Vizcarra *et al.* (2010). Of note, we did not find studies that addressed the performance of different types of swabs in orthopaedic infections.

Soft tissue samples. The literature search did not reveal comparative studies on the preparation of tissue samples for microbial cultures. Three different preparation methods were applied for tissue samples: (1) partitioning into smaller pieces with a surgical knife (Mikkelsen *et al.*, 2006), (2) grinding with a mortar and pestle or (3) stomaching.

Homogenization by use of mortar and pestle was applied in several studies (Günthard *et al.*, 1994; Levine & Evans, 2001). Günthard *et al.* (1994) reported two cases of endocarditis with negative cultures by direct plating of cardiac tissue, but positive cultures matching previous blood cultures after homogenization with the Ten Broeck tissue grinder.

The Stomacher technique was applied for PJI samples in a study addressing *Propionibacterium acnes* in particular (Butler-Wu *et al.*, 2011). The release of cultivable bacteria by this technique has recently been evaluated in food microbiology (Hannah *et al.*, 2011).

Table 1. Comparison of three culture methods for synovia and synovial fluid from patients with infected hip or knee prostheses

	No. of patients	Swab culture (intraoperative)		Synovial fluid culture (blood culture vials)		Tissue culture (sample set)	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Levine & Evans (2001) 95 % CI	32/34/20	76 % (19/25) (55; 91)	86 % (6/7) (42; 100)	92 % (23/25) (74; 99)	100 % (9/9) (66; 100)	77 % (10/13) (46; 95)	100 % (7/7) (59; 100)
Font-Vizcarra <i>et al.</i> (2010) 95 % CI	150	68 % (59/87) (57; 77)	99 % (62/63) (91; 100)	90 % (78/87) (81; 95)	100 % (63/63) (94; 100)	82 % (71/87) (72; 89)	81 % (51/63) (89; 90)

Solid samples and prosthetic components. Culturing of bone biopsies has rarely been reported in studies on PJIs. Conversely, there is an extensive literature on processing of prosthetic materials by sonication. Tunney *et al.* (1998) applied mild sonication [5 min, 50 kHz, corrected by McDowell & Patrick (2005)] to dislodge bacteria adherent to the implants and reported an improved detection of infective agents in hip PJIs. A number of subsequent studies confirmed a higher yield by this method (Table 2) (Trampuz *et al.*, 2006, 2007; Bjerkan *et al.*, 2009; Monsen *et al.*, 2009; Piper *et al.*, 2009). Sonication was evaluated in experiments with bacterial cultures relevant to PJIs, indicating, especially for Gram-negative bacteria, a trade-off between dislodgement of bacteria and decreased viability. According to Monsen *et al.* (2009), the duration of sonication and the material of the tube were critical parameters for the cultivability of bacteria after treatment. They recommended the following parameters for sonication: 40 kHz for 7 min at 22 °C in order to provide maximum effect and preserve viability of Gram-negative bacteria. No further improvement was observed by lowering the temperature. Thin-walled glass tubes provided an increased effect compared to plastic tubes. Furthermore, Monsen *et al.* (2009) compared sonication in tubes deeply submerged in the sonication bath with sonication in tubes only partially submerged (~25%), and they found a similar effect as long as the sample in the tube was completely submerged in the diluent.

By sonication bacteria are released into the diluent, and a centrifugation step can be required for concentrating bacteria into a smaller volume. When processing entire prostheses the volume of diluent makes centrifugation of aliquots the most practical way to proceed (Monsen *et al.*, 2009; Piper *et al.*, 2009).

Newly published studies indicated that vortexing of the sample for 30 s before and after sonication may increase the yield of positive cultures (Trampuz *et al.*, 2007; Kobayashi *et al.*, 2009; Sampedro *et al.*, 2010).

In a thorough study of a patient with PJI, Sendi *et al.* (2010) observed phenotypic variation of *Escherichia coli* recovered from periprosthetic biopsies and from the diluent after sonication of the entire prosthesis. These small-colony variants (SCVs) were clonally indistinguishable from *E. coli* with normal morphological features. Cultures from synovial fluid showed colonies with the normal phenotype only. With further subcultivation the SCV *E. coli* adapted to the normal phenotype. The authors concluded that the different phenotypes most likely originated from different niches, i.e. the biofilm on the prosthesis and the synovial fluid. Proctor *et al.* (2006) did also bring attention to this issue with reference to *S. aureus* isolates surviving within mammalian cells: SCV *S. aureus* has an impaired growth rate and unusual biochemical characteristics that may hamper its correct identification.

Culture media

The media commonly used for PJI samples are non-selective and enriched with a content of blood or blood

Table 2. Comparative studies of sonication and yield of positive cultures in patients with PJIs

	No. of patients	+ Sonication (prosthesis)		- Sonication (tissue)		Comments
		Sensitivity	Specificity	Sensitivity	Specificity	
Piper <i>et al.</i> (2009) 95 % CI	134	67 % (22/33) (48; 82)	98 % (99/101) (93; 100)	55 % (18/33) (36; 72)	95 % (96/101) (89; 98)	PJIs of the shoulder
Esteban <i>et al.</i> (2008) 95 % CI	31	94 % (16/17) (71; 100)	50 % (7/14) (23; 77)	88 % (15/17) (64; 99)	100 % (8/8) (63; 100)	PJIs of hip, knee and miscellaneous
Trampuz <i>et al.</i> (2007) 95 % CI	331	78 % (62/79) (68; 87)	99 % (249/252) (97; 100)	61 % (48/79) (49; 72)	99 % (250/252) (97; 100)	PJIs of hip and knee
Trampuz <i>et al.</i> (2006) 95 % CI	78	75 % (18/24) (53; 90)	87 %* (47/54) (75; 95)	54 % (13/24) (33; 74)	98 %* (53/54) (90; 100)	Contamination due to leakage of sonication bags

*Excluding the patients who received antibiotics, the specificity was 100 % for all types of samples.

products. However, studies rarely provided detailed information on culture media. In Supplementary Table S1 in JMM Online, we have listed papers which provided sufficient details as well as a list of bacterial isolates. To our knowledge, no studies have formally evaluated the performance of different agar media in the diagnosis of PJIs.

A differential effect of the culture medium was noted by van Kats *et al.* (2010) in a study of heart valve biopsies. The transport medium was inoculated both into blood culture vials (FA and FN blood culture media, BacT/Alert; bioMérieux) and thioglycolate broth. Coagulase-negative staphylococci and *Propionibacterium* species were isolated in higher frequencies with thioglycolate broth than with blood culture vials, and vice versa for *S. aureus*. Similarly, thioglycolate broth was reported to promote growth of a broader range of anaerobic bacteria than anaerobic blood culture vials in an experimental study mentioned previously (Melhus & Tjernberg, 2000). The use of pre-reduced culture media and strict anaerobic techniques has been emphasized in some studies addressing optimization of diagnosis of PJI (Tunney *et al.*, 1998, 1999; McDowell & Patrick, 2005).

Nevertheless, not only can the type of medium have an effect on bacterial cultivability but also the viscosity impacts on the spectrum of micro-organisms isolated (Wyatt & Archer, 1988).

Incubation

The papers listed in Supplementary Table S1 reported incubation periods from 2 to 14 days and this broad range applied to both aerobic and anaerobic cultures. In the majority of studies, the incubation period was in the order of 5 days for aerobic cultures and 7 days for anaerobic cultures (Trampuz *et al.*, 2007; Piper *et al.*, 2009; Achermann *et al.*, 2010). According to Schäfer *et al.* (2008), prolongation of the incubation period was associated with an increase in the proportion of positive samples and diversity of bacterial isolates (Table 3). In line with this, Schäfer *et al.* (2008) recommended an incubation period of up to 14 days based especially on late recovery of aerobic Gram-positive rods, *Propionibacterium* species and *Peptostreptococcus* species. In the aforementioned study by Günthard *et al.* (1994), growth of *P. acnes* was reported to require more than 8 days, and, similarly, Butler-Wu *et al.* (2011) found a 29 % increase in cultures positive for *P. acnes* when comparing a 13 day incubation period with 7 days. Likewise, an incubation period of 14 days was used by Sendi *et al.* (2010) for recovery of SCV *E. coli*.

Skovby *et al.* (2011) described a practical scheme for extending the incubation of PJI cultures beyond day 5 by subculturing of visually negative semi-solid thioglycolate media onto appropriate agar plates, which were evaluated on day 12. The scheme resulted in a 10 % increase in bacteriological findings deemed to be clinically significant.

Quantitative aspects of cultures

In their study on multiple biopsies in prosthetic hip infections, Kamme & Lindberg (1981) were primarily concerned with contamination during sampling, transportation and handling in the laboratory. The biopsies ($n=5$) were taken from the same area adjacent to the cement or prosthesis guided by suspicion of infection or bone resorption. In 10 of 31 control arthroplasties, one or two biopsies were positive per set [*P. acnes* accounted for 9 (69 %) of 13 bacterial isolates]. On this basis, the authors defined $\geq$ three positive biopsies as significant growth. To our knowledge, this criterion has only been evaluated once, namely in a retrospective Danish study comprising 118 patients with knee prosthetic joints [specificity 100 % (94; 100); sensitivity 46 % (27; 67)] (Mikkelsen *et al.*, 2006).

Atkins *et al.* (1998) undertook a prospective evaluation of microbiological diagnostic criteria of PJIs and found growth of an indistinguishable micro-organism from cultures of at least three independent specimens (biopsies and synovial fluid included) to be highly predictive of infection (sensitivity 65 %, specificity 99.6 %). A caveat to this study was the fact that the sampling strategy varied somewhat between patients. By mathematical modelling, $\geq$ five specimens was found to be the optimal number of samples in order to minimize false-negative outcomes.

In accordance with the two former studies, Schäfer *et al.* (2008) obtained sets of five biopsies of both periprosthetic tissue and the synovial membrane in PJIs (hips and knees included). They deemed $\geq$ two biopsies with indistinguishable growth to indicate infection, but made a concession to one positive culture in cases with acute inflammation diagnosed by histopathological examination.

A different aspect of multiple samples was highlighted by Zappe *et al.* (2008), who investigated the role of *Propionibacterium* species in PJIs. The study analysed eight patients who formed a subset from a larger study, and all but one had multiple positive samples (median proportion of positive samples 39 %, interquartile range 18–55 %). The mean number of biopsies was 9.5 in cases with positive

Table 3. Proportion of samples with early and late growth during prolonged incubation

	No of samples; patients	Incubation period (days)	Early detection of growth (≤ 7 days)	Late detection of growth (> 7 days)
Schäfer <i>et al.</i> (2008)	S: 284; P: 110	14	73 %	27 %
Butler-Wu <i>et al.</i> (2011)	S: 557; P: 173	13	71 %	29 %

cultures and the authors stressed the importance of multiple samples, especially for patients treated with antibiotics before surgery.

Towards more efficient diagnostics

Review of the literature has identified a number of options for improvement of culture diagnosis in patients with PJI. During the last 15 years, there has been a growing understanding of the role of polymicrobial infection and biofilm formation in PJIs and this has been a challenge to time-honoured culture methods. The causative microbiota originates predominantly from the skin, and many of these micro-organisms show different phenotypes with varying cultivability (Donlan, 2002; Proctor *et al.*, 2006).

The key areas for evidence-based improvements were the following:

- collection of multiple samples from the site of infection;
- selection of the transportation system;
- inoculation of synovial fluid directly into blood culture vials;
- culture of sonication diluent after sonication of prosthetic components if necessary combined with a centrifugation step;
- prolongation of incubation of cultures for up to 14 days.

The crucial number of positive specimens is still debatable as Kamme & Lindberg (1981) and Atkins *et al.* (1998) both settled for three independent specimens to confirm a diagnosis of PJI, but Schäfer *et al.* (2008) required only two.

The importance of transportation of specimens from the operation room to the laboratory seems to have been underestimated but so far surrogate studies with bacterial cultures have indicated Amies medium and variations thereof to be reliable within a time frame of 24 h (Rishmawi *et al.*, 2007; Van Horn *et al.*, 2008; Stoner *et al.*, 2008; Tano & Melhus, 2011). A cool (4 °C) transport chain may not be vital as the studies quoted showed acceptable preservation of viability at room temperature.

An effective protocol has been devised for sonication of prosthetic material (Fig. 1) and significant growth has been defined in terms of c.f.u. per entire implant or per volume of sonication diluent.

The direct inoculation of synovial fluid in blood culture vials has been proven to increase rates of positive cultures deemed clinically significant and to reduce contamination as compared with plate cultures (Hughes *et al.*, 2001). However, the optimal choice of blood culture medium and aerobic versus anaerobic conditions is open for discussion (Melhus & Tjernberg, 2000).

In a review of culture techniques for biofilms, Høgdall *et al.* (2010) emphasized that many bacteria may be inactive,

Fig. 1. Effective protocol for sonication of prosthetic material. Limits for interpretation of growth: ≥ 20 c.f.u. per plate [≥ 20 c.f.u. (10 ml Ringer's solution) $^{-1}$]. Ringer's solution was used by Trampuz *et al.* (2007), Monsen *et al.* (2009) and Sampedro *et al.* (2010). Ringer's solution (25 %, v/v) containing L-cysteine (0.05 %, w/v) as a reducing agent for optimal isolation of *P. acnes* was used by Tunney *et al.* (1998, 1999). According to Tunney *et al.* (1998, 1999), Trampuz *et al.* (2007), Monsen *et al.* (2009), Sampedro *et al.* (2010), Gomez & Patel (2011b).

dormant or damaged by sonication during sample preparation. The time range until colonies can be recognized on agar plates can therefore be very prolonged (Høgdall *et al.*, 2010). These observations are compatible with an incubation period of up to 14 days as suggested by both Schäfer *et al.* (2008) and Butler-Wu *et al.* (2011).

Conclusions and perspective

Only a few diagnostic studies on PJIs fulfilled rigid criteria for a systematic review. The most pertinent problems were lack of an independent reference standard and inclusion of patients because their samples had been processed with the evaluated method (STARD Statement 2008). 'Best evidence' was therefore based primarily on concordant results from different researchers. The robustness and accuracy of methods coming from the research laboratory should be confirmed in the routine clinical setting before they become standard practice. With the increased handling of PJI samples, one of the concerns is the increased risk of contamination. Various precautions can be taken including handling of samples and cultures in a laminar air flow bench (Schäfer *et al.*, 2008), separate incubators for prolonged incubation and, in general, keeping inspections to a minimum.

In future studies, it will be important to correlate results obtained by culture with those obtained by culture-independent methods (Tunney *et al.*, 1999; Panousis *et al.*, 2005; McDowell & Patrick, 2005; Achermann *et al.*, 2010) and thereby possibly define a new reference standard.

Techniques for processing of biopsies of soft or solid tissue should be studied in the same diligent way as sonication of prosthetic implants, as the studies showed that the effective dislodgement of bacteria can increase the sensitivity. Tissue grinding has proved useful for other areas of microbiological research (Günthard *et al.*, 1994) and should be tested preferably with single use equipment.

The time-honoured concept of broad-range bacteriological media seems still to be valid considering the multiple bacterial species associated with PJI. Whether supplementary or new media can augment the yield of cultures is not clear. The polymicrobial nature of many PJIs makes control of overgrowth by, for example, coagulase-negative staphylococci an important consideration. The use of selective media must be given due consideration.

As mentioned briefly, the viscosity of the medium may have an impact on the culturability of planktonic and biofilm-adapted bacterial phenotypes. This speaks in favour of semi-solid media such as thioglycolate agar and semi-solid nutrient agar (Tittsler & Sandholzer, 1936).

Both antibiotic treatment before surgery and the preparatory steps can lead to sublethal damage of bacteria and thereby have a negative impact on culture results. Culturing techniques drawing on experience from food microbiology (Wu, 2008) together with culture-independent techniques may help improve diagnosis under these circumstances.

Attention to strict anaerobic precautions during transportation and processing of samples is a key area for improvement of diagnosis of PJIs (Summanen *et al.*, 1993; Tunney *et al.*, 1998, 1999). Based on the current literature, it is tempting to see the isolation rate of *P. acnes* as the best indicator of the proficiency of anaerobic cultures.

Ultimately, one must bear in mind that many bacteria are not cultivable despite all attempts to optimize culture methods; the cultivable fraction from human skin or oral cavity is around 16–20 % of the total diversity and even lower for environmental bacteria (<1 %) (Amann *et al.*, 1995; Kroes *et al.*, 1999; Gao *et al.*, 2007). To complicate matters further, growth of some bacteria may depend on other species and therefore they cannot be isolated in pure culture (Amann *et al.*, 1995).

Acknowledgements

This review was done within the framework of the 'Prosthetic-Related Infection and Pain' (PRIS)-Innovation project. The PRIS-Innovation project is funded by The Danish Agency for Science, Technology and Innovation, Ministry of Science, Technology and Innovation. We are especially indebted to Trine Rolighed Thomsen, Jan Lorenzen and Peter Lüttge Jensen for their constructive criticism.

References

- Achermann, Y., Vogt, M., Leunig, M., Wüst, J. & Trampuz, A. (2010). Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. *J Clin Microbiol* **48**, 1208–1214.
- Amann, R. I., Ludwig, W. & Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* **59**, 143–169.
- Atkins, B. L., Athanasou, N., Deeks, J. J., Crook, D. W., Simpson, H., Peto, T. E., McLardy-Smith, P., Berendt, A. R. & The OSIRIS Collaborative Study Group (1998). Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. *J Clin Microbiol* **36**, 2932–2939.
- Bjerkkan, G., Witsø, E. & Bergh, K. (2009). Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro. *Acta Orthop* **80**, 245–250.
- Butler-Wu, S. M., Burns, E. M., Pottinger, P. S., Magaret, A. S., Rakeman, J. L., Matsen, F. A., III & Cookson, B. T. (2011). Optimization of periprosthetic culture for diagnosis of *Propionibacterium acnes* prosthetic joint infection. *J Clin Microbiol* **49**, 2490–2495.
- CLSI (2003). *Quality Control of Microbiological Transport Systems*; Approved Standard M40-A. Wayne, PA: Clinical and Laboratory Standards Institute.
- Cuñé, J., Soriano, A., Martínez, J. C., García, S. & Mensa, J. (2009). A superficial swab culture is useful for microbiologic diagnosis in acute prosthetic joint infections. *Clin Orthop Relat Res* **467**, 531–535.
- Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerg Infect Dis* **8**, 881–890.
- Esteban, J., Gomez-Barrena, E., Cordero, J., Martín-de-Hijas, N. Z., Kinnari, T. J. & Fernandez-Roblas, R. (2008). Evaluation of quantitative analysis of cultures from sonicated retrieved orthopedic implants in diagnosis of orthopedic infection. *J Clin Microbiol* **46**, 488–492.
- Font-Vizcarra, L., García, S., Martínez-Pastor, J. C., Sierra, J. M. & Soriano, A. (2010). Blood culture flasks for culturing synovial fluid in prosthetic joint infections. *Clin Orthop Relat Res* **468**, 2238–2243.
- Gao, Z., Tseng, C. H., Pei, Z. & Blaser, M. J. (2007). Molecular analysis of human forearm superficial skin bacterial biota. *Proc Natl Acad Sci U S A* **104**, 2927–2932.
- Gollwitzer, H., Diehl, P., Gerdesmeyer, L. & Mittelmeier, W. (2006). Diagnostische Strategien bei Verdacht auf periprosthetische Infektion einer Kniegelenkttotalendoprothese. *Orthopade* **35**, 906–916.
- Gomez, E. & Patel, R. (2011a). Laboratory diagnosis of prosthetic joint infection, Part I. *Clin Microbiol News* **33**, 55–60.
- Gomez, E. & Patel, R. (2011b). Laboratory diagnosis of prosthetic joint infection, Part II. *Clin Microbiol News* **33**, 63–70.
- Günthard, H., Hany, A., Turina, M. & Wüst, J. (1994). *Propionibacterium acnes* as a cause of aggressive aortic valve endocarditis and importance of tissue grinding: case report and review. *J Clin Microbiol* **32**, 3043–3045.
- Hannah, J. F., Cason, J. A., Richardson, J. R., Cox, N. A., Hinton, A., Jr, Buhr, R. J. & Smith, D. P. (2011). Effect of stomaching on numbers of bacteria recovered from chicken skin. *Poult Sci* **90**, 491–493.
- Høgdall, D., Hvolris, J. J. & Christensen, L. (2010). Improved detection methods for infected hip joint prostheses. *APMIS* **118**, 815–823.
- Hughes, J. G., Vetter, E. A., Patel, R., Schleck, C. D., Harmsen, S., Turgeant, L. T. & Cockerill, F. R., III (2001). Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. *J Clin Microbiol* **39**, 4468–4471.

- Kamme, C. & Lindberg, L. (1981). Aerobic and anaerobic bacteria in deep infections after total hip arthroplasty: differential diagnosis between infectious and non-infectious loosening. *Clin Orthop Relat Res* (154), 201–207.
- Kobayashi, H., Oethinger, M., Tuohy, M. J., Procop, G. W. & Bauer, T. W. (2009). Improved detection of biofilm-formative bacteria by vortexing and sonication: a pilot study. *Clin Orthop Relat Res* 467, 1360–1364.
- Kroes, I., Lepp, P. W. & Relman, D. A. (1999). Bacterial diversity within the human subgingival crevice. *Proc Natl Acad Sci U S A* 96, 14547–14552.
- Levine, B. R. & Evans, B. G. (2001). Use of blood culture vial specimens in intraoperative detection of infection. *Clin Orthop Relat Res* (382), 222–231.
- Mackowiak, P. A., Jones, S. R. & Smith, J. W. (1978). Diagnostic value of sinus-tract cultures in chronic osteomyelitis. *JAMA* 239, 2772–2775.
- McDowell, A. & Patrick, S. (2005). Evaluation of nonculture methods for the detection of prosthetic hip biofilms. *Clin Orthop Relat Res* (437), 74–82.
- Melhus, A. & Tjernberg, I. (2000). Blood culture bottles for transportation and recovery of anaerobic bacteria from non-blood samples. *APMIS* 108, 453–458.
- Mikkelsen, D. B., Pedersen, C., Højbjerg, T. & Schønheyder, H. C. (2006). Culture of multiple peroperative biopsies and diagnosis of infected knee arthroplasties. *APMIS* 114, 449–452.
- Monsen, T., Lövgren, E., Widerström, M. & Wallinder, L. (2009). In vitro effect of ultrasound on bacteria and suggested protocol for sonication and diagnosis of prosthetic infections. *J Clin Microbiol* 47, 2496–2501.
- Moojen, D. J., Spijkers, S. N., Schot, C. S., Nijhof, M. W., Vogely, H. C., Fleer, A., Verbout, A. J., Castelein, R. M., Dhert, W. J. & Schouls, L. M. (2007). Identification of orthopaedic infections using broad-range polymerase chain reaction and reverse line blot hybridization. *J Bone Joint Surg Am* 89, 1298–1305.
- Panousis, K., Grigoris, P., Butcher, I., Rana, B., Reilly, J. H. & Hamblen, D. L. (2005). Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases. *Acta Orthop* 76, 341–346.
- Pedersen, A. B., Svendsen, J. E., Johnsen, S. P., Riis, A. & Overgaard, S. (2010). Risk factors for revision due to infection after primary total hip arthroplasty. A population-based study of 80,756 primary procedures in the Danish Hip Arthroplasty Registry. *Acta Orthop* 81, 542–547.
- Piper, K. E., Jacobson, M. J., Cofield, R. H., Sperling, J. W., Sanchez-Sotelo, J., Osmon, D. R., McDowell, A., Patrick, S., Steckelberg, J. M. & other authors (2009). Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. *J Clin Microbiol* 47, 1878–1884.
- Proctor, R. A., von Eiff, C., Kahl, B. C., Becker, K., McNamara, P., Herrmann, M. & Peters, G. (2006). Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat Rev Microbiol* 4, 295–305.
- Rishmawi, N., Ghneim, R., Kattan, R., Ghneim, R., Zoughbi, M., Abu-Diab, A., Turkuman, S., Dauodi, R., Shomali, I. & other authors (2007). Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. *J Clin Microbiol* 45, 1278–1283.
- Sampedro, M. F., Huddleston, P. M., Piper, K. E., Karau, M. J., Dekutoski, M. B., Yaszemski, M. J., Currier, B. L., Mandrekar, J. N., Osmon, D. R. & other authors (2010). A biofilm approach to detect bacteria on removed spinal implants. *Spine* 35, 1218–1224.
- Schäfer, P., Fink, B., Sandow, D., Margull, A., Berger, I. & Frommelt, L. (2008). Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. *Clin Infect Dis* 47, 1403–1409.
- Sendi, P., Frei, R., Maurer, T. B., Trampuz, A., Zimmerli, W. & Graber, P. (2010). *Escherichia coli* variants in periprosthetic joint infection: diagnostic challenges with sessile bacteria and sonication. *J Clin Microbiol* 48, 1720–1725.
- Senthi, S., Munro, J. T. & Pitto, R. P. (2011). Infection in total hip replacement: meta-analysis. *Int Orthop* 35, 253–260.
- Skovby, A. L., Bjørnslev, N., Andersen, M., Hvolris, J. J. & Friss-Møller, A. (2011). Improving the diagnosis of prosthetic joint infections by prolonged culture of periprosthetic tissues. In *Proceedings of the 21st ECCMID/27th ICC, Milan, Italy, 7–10 May 2011*. P1628.
- STARD Statement (2008). Standards for the Reporting of Diagnostic Accuracy Studies. www.stard-statement.org. 8–4-2008.
- Stoner, K. A., Rabe, L. K., Austin, M. N., Meyn, L. A. & Hillier, S. L. (2008). Quantitative survival of aerobic and anaerobic microorganisms in Port-A-Cul and Copan transport systems. *J Clin Microbiol* 46, 2739–2744.
- Summanen, P., Baron, E. J., Citron, D. M., Strong, C. A., Wexler, H. M. & Finegold, S. M. (1993). *Wadsworth Anaerobic Bacteriology Manual*, 5th edn. California: Star Publishing.
- Tano, E. & Melhus, A. (2011). Evaluation of three swab transport systems for the maintenance of clinically important bacteria in simulated mono- and polymicrobial samples. *APMIS* 119, 198–203.
- Tittsler, R. P. & Sandholzer, L. A. (1936). The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* 31, 575–580.
- Trampuz, A., Piper, K. E., Hanssen, A. D., Osmon, D. R., Cockerill, F. R., Steckelberg, J. M. & Patel, R. (2006). Sonication of explanted prosthetic components in bags for diagnosis of prosthetic joint infection is associated with risk of contamination. *J Clin Microbiol* 44, 628–631.
- Trampuz, A., Piper, K. E., Jacobson, M. J., Hanssen, A. D., Unni, K. K., Osmon, D. R., Mandrekar, J. N., Cockerill, F. R., Steckelberg, J. M. & other authors (2007). Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med* 357, 654–663.
- Tunney, M. M., Patrick, S., Gorman, S. P., Nixon, J. R., Anderson, N., Davis, R. I., Hanna, D. & Ramage, G. (1998). Improved detection of infection in hip replacements. A currently underestimated problem. *J Bone Joint Surg Br* 80, 568–572.
- Tunney, M. M., Patrick, S., Curran, M. D., Ramage, G., Hanna, D., Nixon, J. R., Gorman, S. P., Davis, R. I. & Anderson, N. (1999). Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. *J Clin Microbiol* 37, 3281–3290.
- Van Horn, K. G., Audette, C. D., Sebeck, D. & Tucker, K. A. (2008). Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. *J Clin Microbiol* 46, 1655–1658.
- van Kats, J. P., van Tricht, C., van Dijk, A., van der Schans, M., van den Bogaerd, A., Petit, P. L. & Bogers, A. J. (2010). Microbiological examination of donated human cardiac tissue in heart valve banking. *Eur J Cardiothorac Surg* 37, 163–169.
- Wu, V. C. (2008). A review of microbial injury and recovery methods in food. *Food Microbiol* 25, 735–744.
- Wyatt, G. M. & Archer, D. B. (1988). Response of populations of human faecal bacteria to viscosity in vitro. *J Appl Bacteriol* 64, 163–167.
- Zappe, B., Graf, S., Ochsner, P. E., Zimmerli, W. & Sendi, P. (2008). *Propionibacterium* spp. in prosthetic joint infections: a diagnostic challenge. *Arch Orthop Trauma Surg* 128, 1039–1046.
- Zimmerli, W., Trampuz, A. & Ochsner, P. E. (2004). Prosthetic-joint infections. *N Engl J Med* 351, 1645–1654.