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1 ABSTRACT

Background: The early diagnosis of infection or sepsis in burns are important for patient care. Globally, a large number of burn centres advocate quantitative cultures of wound biopsies for patient management, since there is assumed to be a direct link between the bioburden of a burn wound and the risk of microbial invasion. Given the conflicting study findings in this area, a systematic review was warranted.

Methods: Bibliographic databases were searched with no language restrictions to August 2015. Study selection, data extraction and risk of bias assessment were performed in duplicate using pre-defined criteria. Substantial heterogeneity precluded quantitative synthesis, and findings were described narratively, sub-grouped by clinical question.

Results: Twenty six laboratory and/or clinical studies were included. Substantial heterogeneity hampered comparisons across studies and interpretation of findings. Limited evidence suggests that (i) more than one quantitative microbiology sample is required to obtain reliable estimates of bacterial load; (ii) biopsies are more sensitive than swabs in diagnosing or predicting sepsis; (iii) high bacterial loads may predict worse clinical outcomes, and (iv) both quantitative and semi-quantitative culture reports need to be interpreted with caution and in the context of other clinical risk factors.

Conclusion: The evidence base for the utility and reliability of quantitative microbiology for diagnosing or predicting clinical outcomes in burns patients is limited and often poorly reported. Consequently future research is warranted.

Keywords: burns, infection, systematic review, quantitative microbiology, biopsies, wound swabs

2 BACKGROUND

Infection is a significant complication for patients who survive an initial burn injury. Although there are a variety of infection routes which may lead to systemic infection and sepsis in the thermally injured patient, a key route of infection is via the breached and burnt areas of the skin. Here infection typically starts as bacterial colonisation (with bacteria contained in a biofilm), with the source bacteria easily introduced onto this exposed and vulnerable surface via a number of exogenous and endogenous routes. Colonisation may then progress to systemic infection, where mortality rates range from 5-15% [1], with the majority of the mortality due to pneumonia (25%), sepsis (26%), urinary tract infections (22%), and acute burn wound infections (5%) [2].

The longer the colonisation persists, the greater the likelihood of systemic infection [3]. Furthermore, it is believed that the risks of bacterial invasion and systemic infection increase in proportion to the size of the skin breach [1]. Consequently, microbiological assessment of burn wounds particularly when clinical signs of infection are present, or if the wound is deteriorating, or has changed in appearance, is important in patient management [4,5], and forms the standard of care in most burns units. This can be achieved with qualitative (bacterial presence/absence), semi-quantitative (some form of bacterial enumeration conducted), or quantitative (full bacterial count provided) microbiological methods. In the UK, assessment of burn wounds is generally qualitative and semi-quantitative, and utilises swab cultures [6].

Various authors [7,8] have suggested that qualitative and semi-quantitative methods should be replaced by fully quantitative bacteriology of biopsies in order to improve patient management. The use of burn wound biopsies for histological and quantitative assessment of the burn wound originates from Teplitz *et al* [9], who stained and microscopically investigated tissue for bacteria, and provided an absolute measure of bacteria per unit of volume. Using a rat model, Teplitz *et al* [9]

found that increasing numbers of *Pseudomonas aeruginosa* on a burn wound were followed by invasion of the underlying viable tissue, and clinical infection.

A clinical method for quantitative biopsy in burns patients was first described by Loebl *et al* [10], and subsequently modified [11,12]. Consequently, there now exist a variety of quantitative methods, but no universally accepted 'gold standard'. These methods differ in a number of ways, such as the method of sample collection, biopsy collection and processing, and timing of collection.

The evidence for the utility of quantitative burn wound culture is inconsistent. Some animal and *in vitro* studies suggest an association between high bacterial counts and infection [13], delayed wound healing [14], and poor skin graft take [15]. Some clinical studies were unable to demonstrate a relationship between bacterial counts and subsequent sepsis or graft loss [11, 16].

The use of quantitative culture for the prediction of clinical outcomes is only one possible prognostic variable. Other prognostic factors could include the more traditionally used clinical factors, such as heart rate, temperature, and blood pressure [16], or newly developed novel tests such as neutrophil function [17]. The incremental utility of quantitative culture as a prognostic factor should therefore ideally be evaluated in the context of other known prognostic factors. Furthermore, any evidence on the prognostic utility of bacterial count (whether as a single prognostic factor or in conjunction with others), should ideally be evaluated in the context of the evidence on the accuracy and reliability of the counts obtained. Given the increased use of quantitative methods in some burns centres, and the varied and sometimes conflicting evidence base, a comprehensive systematic review of all existing evidence was warranted.

3 METHODS

A protocol detailing the methodology was registered (PROSPERO (CRD42015023903)) and published [18]. A summary of the methods is described here.

Bibliographic databases were searched to 3rd August 2015 (MEDLINE, PubMed, Embase, CINAHL, Cochrane Central Register of Controlled Trials (CENTRAL) and Scopus) using a combination of index and text words relating to the population (burns patients) and quantitative burn wound microbiology. There was no restriction by language, study design or outcome. A sample search strategy for MEDLINE is shown (Supplementary Figure S1). ZETOC (British library) and the Science Citation Index (Web of Science) were searched for conference proceedings. Abstracts from national and international burns and microbiology conferences were searched from 2012 onwards. Clinical trial registries were searched for ongoing trials and relevant articles were citation checked.

Prospective studies using any method(s) of quantitative burn wound microbiology, in patients of any age with a burn injury were eligible. Relevant outcomes included any measures of reliability or repeatability of a single method for obtaining bacterial counts, measures relating to the agreement between two or more methods, clinical outcomes (such as sepsis or mortality), and their association with bacterial counts and resource related outcomes (e.g. length of hospital stay). Animal and *in vitro* studies, and studies only examining qualitative or semi-quantitative methods, were excluded.

Study selection, data extraction and quality (risk of bias) assessment were performed in duplicate by two independent reviewers using pre-specified criteria and standardised forms. Disagreements were resolved through discussion or referral to a third reviewer. Data was extracted on study aims and design, patient characteristics, methods and timings of sample collection and culture, length of follow-up and outcomes.

As the review encompassed a range of study designs with different study aims, it was necessary to include risk of bias criteria from different tools. Risk of bias assessment therefore included, where relevant for individual studies, elements from the 'COnsensus-based Standards for the selection of health Measurement Instruments' (COSMIN) tool [19] (*e.g. were any samples taken in duplicate or was there >1 independent assessor?*); the Quality Assessment of Diagnostic Accuracy Studies (revised tool) (QUADAS-2) checklist [20] (*e.g. were samples for both tests collected at the same time?*); and the Quality in Prognosis Studies (QUIPS) tool [21] (*e.g. are important potential confounding factors appropriately accounted for?*). Items from the latter tool were important for assessing the prognostic validity of a study using bacterial count as a prognostic marker of future clinical outcomes such as sepsis. Items from the former tools related to the reliability and repeatability of different methods and any agreement between them. Full details of the quality assessment can be found in Supplementary Figure S2.

Synthesis was narrative with main findings (and any statistical significance) tabulated. Studies were grouped by clinical question, with some studies providing evidence for more than one question. Heterogeneity in population, sampling and culturing methods and reported outcome metrics precluded quantitative pooling, however similarities and differences between study findings were described. Where findings were dichotomised according to a threshold, this was considered when comparing studies. All findings were considered in the context of any risk of bias concerns, and gaps in the evidence highlighted where appropriate.

Formal assessment of publication bias was not possible. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines [22] were adhered to, and the study selection process documented using a PRISMA flow diagram.

4 RESULTS

4.1 Overall summary

Twenty-six studies were included (see Figure 1 for selection procedure). The studies were published between 1974 and 2013, but mostly conducted in the 1970s and 1980s. Twelve were laboratory comparisons of bacterial counts obtained from different sampling methods, and 14 incorporated both laboratory aspects and clinical outcomes.

4.2 Intra-and inter- observer repeatability of the different methods of obtaining bacterial counts

Only three small studies (46 patients in total) reported on duplicate sampling using duplicate swab collection [23], duplicate biopsies [24] and both duplicate swabs and biopsies [25]. All duplicate samples were processed using the same methods, therefore allowing assessment of reliability. No studies were identified that reported on inter-observer reliability. Details of sampling methods, main findings and methodological strengths and weaknesses are detailed in Table 1.

Levine *et al* [23] collected duplicate swabs to assess the variation in quantitative cultures from widely spaced areas on wounds of uniform clinical appearance. Twenty four patients, with large areas of exposed granulation tissue, were included and had swabs collected from four separate areas per wound. Assessment of variability showed that 95% of the counts obtained from the four swabs were ±1.7 logs from the mean count per sample set (95% confidence interval (CI)). Mean counts are not reported. It is also unclear whether the samples with no bacterial growth were included or excluded from the analysis as per Steer *et al* [25].

Volenec *et al* [24] collected 36 punch biopsies from four burns patients (27 pairs analysed), and assessed the variability in counts per gram between the duplicate samples. The 95% CI was ±1.31 $\log_{10}$ counts/g across all the samples. The results from these two studies suggest that a single sample may be able to provide a reliable approximation of the number of organisms present, without the need to collect duplicate samples.

Steer *et al* [25], collected duplicate biopsies and swabs (two of each per patient) from 18 patients. The authors found that there was a significant correlation between the log total bacterial counts obtained from two simultaneous biopsies ($p<0.002$), and from two swabs ($p<0.001$) collected from the same patient at the same time only if samples without growth were included. When samples without growth were excluded from the analysis (since by being negative, they are automatically concordant), the correlation between simultaneous biopsies was no longer significant, and only 29% of biopsies and 50% of swab counts agreed within the same log unit. The 95% CI ranges for biopsies and swabs respectively were ±5.4 $\log_{10}$ counts/g, and ±3.6 $\log_{10}$ counts/cm^2. This wide range may be due to the variation in sampling area for swabs. The authors conclude that single samples are not sufficient for measuring bacterial counts, and that one sample type cannot be used to predict the counts obtained from another sample type.

Comparisons across the three studies are difficult owing to the heterogeneity in terms of the samples collected (all studies involve different sampling methods and sites), and the populations studied. Several methodological weaknesses were also noted, especially the inadequate detail regarding how samples were processed. Whilst all studies provide measures of variation, there is no indication of reference values or guidance on clinical interpretation. Overall, there is insufficient evidence to draw conclusions on the reliability of the methods described.

4.3 Agreement between different methods

Twenty two studies [5,11,12,23,25–42] compared two or more methods of quantification, including charcoal swabs, biopsies (of a variety of types), and blood cultures. The methods for processing the samples (where stated) tended to be broadly similar, involving collection and plating onto solid agar (±quantitative counts) for the swabs, and homogenisation, serial dilution and plating (culture) for the biopsies, although there was a lack of reporting on the method of biopsy collection, or type of

biopsy for three studies [34,38,39]. Details of all the studies are shown in Tables 2 (A-D), and are described in the following text.

4.3.1 Agreement in bacterial counts with different sections from the same biopsy/biopsy site

Four studies investigated bacterial counts obtained from different sections of the same biopsy or biopsy sites [34,38–40], however there are several methodological weaknesses; only one study [40] provided detail on biopsy collection and processing method, and all provide only minimal detail on the patient population (Table 2A). Furthermore, each study investigated bacterial counts from different samples. In the Barret & Herndon [34] study, biopsies were collected from the eschar and excised wound bed, whereas in Mitchell *et al* [38] they were from 'adjacent sites'. The studies by McManus *et al* [39] and Woolfrey *et al* [40] collected a single biopsy that was then split for processing (transversely [40]; not stated in [39]). Counts are provided for three of the studies [34,39,40], but, given the paucity of information on the sampling methodology, it is hard to draw any conclusions regarding any differences observed between counts, as these appear to either represent distinct samples [34], or represent variation in sampling from the same/similar site [39,40]. Samples in Woolfrey *et al* [40] were analysed separately according to whether the same bacterial isolate was recovered from both split biopsy samples (paired), or whether the samples were discordant (unpaired). For 43% of the paired biopsies, the quantitative results were within the same log increment. Combining the paired and unpaired samples, 21% of the quantitative results were within the same log increments, 19% differed by ±1 log increment, and 60% differed by ±2 log increments or more. Although there are no reference ranges to guide interpretation, it appears that there is vast variation in different segments from the same biopsy specimen.

4.3.2 Agreement in bacterial counts between different processes used on single biopsies

Five studies compare quantitative counts from single biopsies processed using quantitative culture compared to a range of other methods (Table 2B). These include semi-quantitative culture from the

biopsy homogenate [26], acridine orange microscopy [27], histology [5], quantitative Gram stain [28], and absorbent discs [41]. The majority of these studies collected biopsies according to Loebl *et al* [10], but there is no detail on biopsy collection for the Pruitt & Foley [5] study, and no details provided on the processing of the sample for two studies [26,27]. The studies also vary in the skin preparation before biopsy collection, with three removing topical agents prior to sample collection [26,27,41], and no details on skin preparation for the other studies. This lack of detail makes it difficult to compare the robustness of study methodologies.

Three studies reported concordance between methods: this ranged from 96% for quantitative versus semi-quantitative [26], to 100% for quantitative versus acridine orange microscopy [27], but this latter result is misleading as this only relates to the culture positive samples, and 35% of the culture negative samples were positive on microscopy. Woolfrey *et al* [28] report a moderate positive association (correlation coefficient of 0.5) between quantitative and Gram stains, and using regression line analysis, indicate that the presence of $1.1x10^5$ stained microorganisms per slide preparation corresponds to the recovery of 10^6 cfu/gram on quantitative culture. Only a small proportion of samples (17%) were analysed in Pruitt & Foley [5], and no concordance data reported. Williams *et al* [41] also provide no summary concordance data for the bacterial counts obtained from the biopsies versus absorbent discs, instead reporting the correlation coefficients between the methods in terms of the frequency of isolation of the four most common organisms. Overall, the paucity of studies, and heterogeneity between study methodologies, precludes any conclusions relating to the best method for processing biopsies for obtaining reliable bacterial counts.

4.3.3 Comparison of bacterial counts obtained from swabs versus biopsies

Seven studies compared bacterial counts obtained from swabs versus biopsies (Table 2C). Biopsy types differed between studies (1-2 cm excision biopsy [29], punch biopsies of various sizes

[25,31,33], or skin slit [32]), or were not stated [23,30]. Only one of the seven studies [25] reported the swab type, and only three report on the surface area volume that was sampled [23,25,32].

The studies also differed according to whether or not quantitative counts were performed for both sample types. Three studies report their main findings as 'concordance between sampling methods in terms of positive and negative results'; in these studies quantitative counts were not performed on the swabs [29,30], or were not performed/reported for either of the sample types [31]. No quantitative results were reported, and the studies mention only that 'similar' bacteria were present between the two sample types.

Vural *et al* [31] compared biopsies and swabs in terms of the microorganisms that were isolated (i.e. biopsies collected but quantitative microbiology not performed), and found a 'moderate correlation' between the two methods, with a Kappa index value of 41%. Danilla *et al* [33], also found a moderate correlation (Kappa index value of 52%) between biopsies and swabs in terms of identification of bacterial species present. Although both studies used similar methods, and collected similar sized punch biopsies (3 and 5mm), it is hard to know what the relevance (for clinical practice) of a 'moderate' Kappa index is.

Three studies performed quantitative counts on swabs. Winkler *et al* [32] compared biopsies to swabs and other 'surface measures'. Detailed results are not provided, however there was no statistically significant correlation between the counts obtained from biopsies and swabs. These findings contrast with Levine *et al* [23] who found a 'good' positive correlation between counts from the seven sample pairs that were analysed, and Steer *et al* [25] who report a statistically significant correlation between bacterial counts obtained from both methods ($p<0.001$).

4.3.4 Comparison of bacterial counts obtained from swabs versus biopsies versus blood cultures

Six studies report the bacterial counts obtained from different samples collected from the same patient (Table 2D). There is a lack of detail on sample collection for swabs and/or blood cultures for five studies [11,35–37,42], and the type of biopsy sample collected is not detailed in Bahar *et al* [42] (although the weight of the biopsy is stated).

There is heterogeneity in both the types of biopsy that were collected (Loebl *et al* [10] method, or dermal punches), and the preparation of the skin prior to sampling. Only two of the six studies [36,37] mention that the sampling was performed aseptically. Without aseptic collection, biopsies may be positive because of translocation of the bacteria into the sample during sample collection rather than invasion of bacteria. This also applies to the collection of blood cultures and is a considerable methodological flaw of these studies.

Another limitation is the lack of detail on timing of sample collection. Three papers give a specific time for sample collection in terms of post-burn [12,35,36], e.g. samples collected on the 2nd, 4th and 7th days post burn [12]. The other studies are less clear, e.g. 'within the second week' [42], within 48 hours of admission [37], or at the start of a change of dressing [11]. It is also often not clear when the blood samples were collected in relation to the other samples. Furthermore, definitions of what constitutes a positive culture are only provided for two studies [35,36]. The overall lack of detail and/or heterogeneity thus hampers any comparisons of study findings.

Only four studies report concordance findings. The study by Sjoberg *et al* [37] is the most robust in terms of methodology. The study involved the collection (at stated time-points) and comparison of samples (surface swabs, tissue culture and blood culture), and included the regular disinfection of the skin surface (with 70% ethyl alcohol) prior to the collection of the dermal punch biopsies and

blood cultures. The authors found a poor correlation (29%) between swabs and biopsies in terms of no growth or identical bacterial growth.

This is in contrast to Bahar *et al* [42], where there was 'good' correlation (but no measure provided) between swabs and biopsies, Uppal *et al* [12], where the concordance between the swabs and biopsies was 95%, and Steer *et al* [11] where there was no significant difference in counts between the different sample types. These studies however were methodologically weaker owing to the lack of reporting or performing of skin asepsis.

4.3.5 Comparison of bacterial counts obtained from biopsies versus blood cultures

One study [43] (not tabulated) compared bacterial counts from biopsies versus blood cultures. Samples were collected (biopsies as per Loebl *et al* [10]) from 38 patients with >20% TBSA on the day of admission to the hospital, and every third day thereafter. They were processed to obtain quantitative counts, and positives defined by counts $\geq 10^4$ orgs/g. In terms of concordance between the samples, 92% of the biopsies were positive, but only 29% of these positives matched a simultaneously positive blood culture.

4.4 Association between quantitative microbiology, other measures and clinical outcomes

Thirteen studies [5,10,11,29–31,34–37,42–44] reported clinical outcomes such as sepsis or mortality Two additional studies were initially considered but subsequently excluded: Winkler *et al* [32] reported three case studies only, and Buchanan *et al* [26] investigated the impact of microbial counting methods on the decision for antimicrobial therapy, but did not relate this to outcomes such as sepsis or mortality.

4.4.1 Studies reporting sepsis

Sepsis was assessed in nine [5,10,11,29,34,35,37,43,44] of 13 studies. Definitions of sepsis (where described) varied across the studies (Table 3), and important threshold values for conditions such as leucopenia and tachypnoea have been omitted. Furthermore, none of the studies included a full definition of sepsis that would satisfy the definitions jointly developed by The American College of Chest Physicians (ACCP), the American Burn Association (ABA) and the Society of Critical Care Medicine (SCCM) [45,46], although it must be acknowledged that the majority of the studies predate these guidelines.

Additionally, positive culture thresholds were not defined for seven studies [5,11,30,31,34,42,44], and for the remainder, the threshold ranged from 10^4 to 10^5 bacteria/gram (Table 4). Patient populations varied between the studies in terms of the %TBSA.

4.4.2 Utility of different sample types and quantitative microbiology for predicting sepsis

Thirteen clinical studies investigated the ability of swabs, biopsies and/or blood cultures, and quantitative microbiology to predict a range of clinical outcomes (Table 4). Of the nine studies investigating sepsis, two investigated biopsies only [34,44], two compared biopsies to surface cultures [10,29], two compared biopsies to blood cultures [5,43], and the remaining three [11,35,37] used all three methods (biospies, surface cultures, and blood cultures). Barrett & Herndon [34] will not be mentioned further here since there was no definition of sepsis given.

All studies found that biopsies were more sensitive than swabs and/or blood cultures for diagnosing infection and predicting the likelihood of sepsis. Sjoberg *et al* [37] showed that the development of sepsis was better correlated to quantitative burn tissue biopsy cultures than surface swab cultures (but commented that the time needed for processing limits its predictive and therapeutic value), and Tahlan *et al* [29] found that surface swabs in general fail to accurately predict progressive bacterial colonisation or incipient burn wound sepsis. Additionally, Loebl *et al* [10] concluded that positive

wound biopsies performed better than surface cultures (Rodac plates) in terms of predicting the development of clinical sepsis. Of 210 patients included in this study, 117 had positive surface cultures, and 73 of these were also positive from the biopsy samples. From the biopsy samples, 48/73 (66%) of the patients became septic, and of these, 15/48 (31%) died. False positive results (i.e. patients who had positive biopsies but did not develop sepsis) were found to have either been treated with sub-eschar, or systemic antibiotics. No patients with sterile biopsies developed sepsis unless another source of infection was present. The overall conclusions need to be viewed cautiously however, as it is unclear how many patients with positive surface cultures died, or how many had positive biopsies in the absence of sepsis.

Eight studies [5,11,29,35–37,42,43] investigated the role of blood cultures in predicting clinical outcomes, with three [35,42,43] reporting a poor correlation between positive blood cultures and subsequent sepsis, two finding a positive correlation [29,36], and three [5,11,37] not providing any results or conclusions in this regard.

Bharadwaj *et al* [35] found that 16 patients who died of sepsis in their study cohort (and who had $>10^8$orgs/g in biopsies) had negative blood cultures. Bahar *et al* [42] found that blood culture positivity was not significantly different between patients who died and survivors (19% vs 18.8%, respectively), and Marvin *et al* [43] found blood cultures to be 'disappointing' as a means of diagnosing septic complications since only 30% of septic patients in their study had positive blood cultures. They also found a false positive rate of 10% (i.e. positive blood culture, but no simultaneous clinical signs of sepsis). This is higher than previously reported rates of 0.6-6.0% [47], but could be explained by a commensal being isolated from the blood, and delayed onset of sepsis in the patients the samples were collected from.

In contrast, two studies [29,36] found positive blood cultures to be associated with poor prognosis in burns patients (especially if they are positive within 24 hours of burn), and in many cases predicted impending mortality. The data regarding the sensitivity and specificity of blood cultures for the

diagnosis of sepsis from these studies have to be interpreted with caution. Many of these studies only required one positive blood culture for their analyses and as discussed previously their definitions of clinical sepsis were deeply flawed. The ABA criteria [45,46] define blood stream infection as a recognised pathogen cultured from two or more blood cultures, or one positive blood culture in the presence of sepsis. It is also important to consider the timing of blood culture collection. For example, blood cultures collected from pyrexial patients/episodes are more likely to be microbiologically positive than those collected from non-pyrexial patients/episodes.

In terms of quality assessment, all of the above studies have methodological limitations which affect the validity of the data. These include failure to define thresholds for positive cultures [5,11,34], small sample sizes (n<25) [29,34], and conclusions made in the absence of robust data analysis [10,29,35,43].

4.4.3 Utility of different sample types and quantitative microbiology for predicting mortality and other clinical outcomes

Three studies [30,36,42] investigated whether there was a correlation between quantitative counts and mortality alone, with a further seven investigating mortality alongside sepsis (Tables 3 & 4). All performed counts on different samples, and used different thresholds to define positivity, i.e. any growth 'considered positive' [42], threshold not stated [30], and $>10^5$ organisms per gram defined as positive for counts performed from biopsies [36].

Bahar *et al* [42] evaluated whether quantitative microbiology could predict the likelihood of mortality by looking at the association between counts from swabs and biopsies and mortality for 75 patients. There was no statistically significant difference in counts between those who died and those who survived; 59 patients died, of whom 48 had bacterial counts greater than $1x10^5$ cfus (units not stated), compared to 16/16 patients who survived but still had this high level of bacterial bioburden from the biopsy samples (Table 4). This was consistent with the findings from Pruitt & Foley [5] who performed quantitative counts on biopsies from 23 patients. There was no statistically

414 significant difference between the groups: 15/20 (75%) patients with >10^5 orgs/gram died (the
415 remaining five survived), whereas 1/3 pts with counts <10^5 orgs/g died (the remaining two survived).
416 Steer *et al* [11] analysed 69 swab and biopsy pairs from patients with 1-65% TBSA, and also
417 concluded that there was no statistically significant difference in total bacterial counts
418 (biopsy/surface) between patients judged as a clinical success or failure, and no variation in counts
419 according to whether patients underwent excision and grafting or change of dressings. However, the
420 authors also found a significant negative correlation between quantitative counts from swabs and
421 %TBSA (p=0.006) (i.e as TBSA increases, the counts decrease). This is in stark contrast to what would
422 be expected, and what is observed in clinical practice, and therefore suggests some error or serious
423 methodological flaws in the study.

424 Two studies [29,37] found that there was a difference in terms of bacterial counts in those with
425 sepsis compared to those without, and three studies [5,30,36] concluded that high bacterial load in
426 biopsies increased the risk of sepsis and mortality. Sjoberg *et al* [37] collected swabs, biopsies, and
427 blood cultures from 50 burns patients, whilst monitoring them (every 4 hours) for signs of sepsis.
428 The patients were then split into 'septic' (n=21) vs 'non septic' (n=29). Overall, bacterial load (from
429 biopsies) was significantly higher ($p<0.05$) in patients with signs of septicaemia compared to those
430 without (Table 4). In terms of mortality, 16 of the 21 septic patients had positive tissue cultures, and
431 8 of these died. There is however no information regarding possible deaths in the non-septic group.

432 Additionally, Bharadwaj *et al* [36] and Krupp *et al* [30] found that patients with higher bacterial
433 counts based on biopsies were more likely to die than those with low counts. In Bharadwaj *et al* [36],
434 23 of 50 patients died, with 18 deaths being attributed to infection. All 18 had counts >$1x10^8$
435 organisms per gram. Of note, the counts for the 27 who survived are not provided. Krupp *et al* [30]
436 reported that patients with burn wounds which showed >10^5 organisms/gram in biopsy tissue
437 seemed to be 'more likely' to die even with additional measures (e.g. aggressive wound care,

assisted ventilation etc). This finding is however based on only 10/21 patients, and a lack of information on the remaining patients means the findings should be viewed cautiously.

The majority of studies had some methodological limitations, particularly in terms of reporting outcomes for all patients (not just those with high counts) and there does not appear to be any correlation between the findings and how robust the studies were.

4.4.4 Quantitative counts, depth of invasion, and clinical outcomes (sepsis and mortality)

Only one study [5] looked at the relationship between depth of invasion and clinical outcome. Pruitt & Foley [5] used histology to grade infection (by depth of invasion) from 1 (burn surface) to 6 (most severe: microbial penetration into viable tissue beyond depth of original necrosis). There was a correlation between death and the histology grade, with grade 6 associated with high mortality. Furthermore, for 19 patients, two or more successive biopsies had shown evidence of increasing invasion, and 16 (85%) of these patients died. It is hard to draw meaningful conclusions from these observations in the absence of full results.

4.4.5 Impacts of quantitative counts on patient management

Two studies looked at the influence of microbiology results on clinical practice. Pruitt & Foley [5] reported that biopsy findings prompted therapy alteration in 25 patients (total number of patients unclear), whereas Buchanan *et al* [26] compared quantitative (Q) counts vs semi-quantitative (SQ), and found that bacterial counts changed the clinical practice for just two of 78 patients. The SQ method was therefore advocated over performing quantitative counts.

5 DISCUSSION

This systematic review was undertaken to clarify the evidence base around the use of quantitative microbiology (specifically from biopsy samples) for the management of burns patients. This is the first systematic review in this area and was warranted owing to the conflicting and varied reports of the clinical utility of quantitative counts in the literature. A sensitive search strategy meant that is it unlikely that any studies would have been missed, and detailed risk of bias assessment of included studies meant that any findings have been set in the context of the methodological quality of the primary studies.

Twenty six studies were included, of which twelve investigated clinical outcomes. There was substantial heterogeneity in terms of patient characteristics (%TBSA, type of burn injury, the time of presentation post burn), sample collection and processing (e.g. the type of biopsy collected, and whether skin was aseptically cleaned beforehand), the method for performing the counts, the analysis, and how the clinical outcomes were defined. This precluded any quantitative synthesis (e.g. meta-analysis), and hence findings are described narratively, sub-grouped by clinical question.

A key finding from this systematic review is that there is not a gold standard nor universally accepted method for monitoring a burn wound for bacterial colonisation and infection. Studies using any method of quantitative microbiology from biopsy samples were eligible for inclusion into the review resulting in a range of different types of biopsy collected, whilst in six studies [5,23,30,34,38,39] the authors failed to provide any information regarding the biopsy type. Furthermore, the comparator samples such as swabs, surface plates or blood cultures also varied between studies in how they were collected (e.g. the area of the skin swabbed), and how they were processed. The different methods of processing may be the reason why the critical bacterial concentrations necessary for burn wound sepsis vary so widely between studies. Freshwater *et al* [44] for example have theorised that the method of tissue homogenisation significantly impacts the critical number due to

488 less efficient means of homogenisation yielding less recoverable bacteria from tissue samples. It
489 remains unclear what the best method is to obtain bacterial counts from a burn wound.

490 In addition to variation in sample types, for some studies, the samples being investigated were not
491 collected at the same time per patient, or there was no information provided on when (post-burn
492 injury) the samples were collected. This is of utmost importance when the bacterial counts are being
493 compared from different sample types and across different studies.

494 Limited evidence does suggest that it is not sufficient to base clinical decisions on a single sample,
495 and that swabs (although a convenient sample type) generally only detect the surface flora, and
496 therefore do not reflect the invasion of the wound and potential progression to sepsis. In terms of
497 bacterial density and wound invasion, Winkler *et al* [32] hypothesised that the discrepancy between
498 surface swab and biopsy findings were linked to bacterial density (i.e. that when bacterial counts are
499 $<10^5$ organisms/g, deep invasion of wounds is not expected and biopsy results then correlate with
500 surface techniques). However, high bacterial density does not always lead to invasion, as reported
501 by McManus *et al* [39]. It is likely that the depth of invasion (especially involvement of healthy tissue
502 and vascular involvement) in combination with bacterial density on biopsy will be a more accurate
503 predictor of sepsis and mortality compared to just bacterial density alone.

504 Furthermore, it is clear that there are insufficient robust studies to fully investigate the utility of
505 blood cultures; only one study [43] specifically investigated this, but was methodologically weak
506 owing to biased selection of patients (those *'believed to be a high risk of septic complications'*), lack
507 of statistical testing, and incomplete reporting of results. All other studies investigating blood
508 cultures differed in time of sample collection, with some collected when a pyrexial spike was present
509 in the patient [29], at a pre-determined time not associated with clinical condition of the patient
510 [11,35–37], or at an unknown time (detail not provided) [5,42,43].

In terms of clinical outcomes, eight of nine studies (investigating sepsis) seem to suggest that biopsies performed better than swabs (or other comparators) in terms of correlation with sepsis. However the utility of quantitative analysis of biopsies is still not clear as three of 13 clinical studies [11,42,44] have also reported no correlation of biopsy results with clinical outcomes. These conflicting findings may be a result of differences in methodological quality between studies, or other sources of heterogeneity (e.g. population characteristics). Methodological flaws (or omissions in reporting) in the clinical studies include: the time of sample (biopsy) collection not being stated (bacterial density and antibiotic resistance has been shown to increase with longer time from burn [35,37,48,49]), the lack of a common definition of sepsis, and ambiguity surrounding when mortality is attributed to infection. For infection-attributed deaths, there is no clear definition or explanation on how this was decided (e.g. in some cases, patients may have died of other non-infection related cause). All of these factors may affect the robustness of the clinical findings, and these should therefore be interpreted very cautiously. Furthermore, none of the studies have adjusted their findings for potential confounding factors (i.e. other factors that might predispose a patient to adverse clinical outcomes). These include age, burn depth and severity, and inhalation injury, and may all lead to an inaccurate attribution of adverse clinical outcomes to high bacterial counts.

It may also be that sepsis is not a suitable clinical outcome to use for burns patients. It has been recognised for many years now that the SIRS and sepsis criteria do not apply well to burns patients due to their elevated systemic inflammatory response (e.g. a baseline temperature of 38.5^{o}C, and persistent tachycardia and tachypnoea). Many burns patients would thus trigger the criteria even when no infection is present thus making it difficult to detect true sepsis. Recognising this flaw, the American Burn Association (ABA) has published improved standardised definitions for sepsis and infection-related diagnoses for the burn population in 2007 [46]. Higher thresholds and some new criteria were introduced e.g. using temperature 39°C (versus ACCP and SCCM criteria of 38°C), tachycardia 110bpm (versus 90bpm), thrombocytopenia (3 days after initial resuscitation) and hyperglycaemia (>0.200mg/dl), instead of leucocytosis, as markers of infection. It is thus likely that

these historical papers (by using simple definitions of sepsis) have overestimated the incidence of true infection in their studied cohort.

A key question to address surrounds the relevance of bacterial counts to clinical outcomes. Out of the 13 clinical studies, ten [5,10,29–31,34–37,43] found that high bacterial counts were associated with a poorer prognosis, although the link between high bacterial density and adverse clinical outcomes is far from clear, as three studies [11,42,44] found no correlation between clinical outcomes and high bacterial load or density.

This discrepancy in findings may be due to the fact that the relationship between microbial colonisation and clinical outcomes is much more complicated and cannot be determined merely by bacterial load. Host susceptibility has a significant role in determining the result of a bacterial infection. The same microbes can cause a wide variety of clinical symptoms ranging anywhere from asymptomatic infection to fatal disease (dependent on endogenous and exogenous host factors such as genetic makeup and diet or antibiotic use which can alter their microbiota [48]). Ten of the 13 studies investigated the bacterial species present on the burn wound [5,11,29–31,34,36,42–44]. It is well known that certain species of bacteria (e.g. *Pseudomonas aeruginosa* and *Streptococcus pyogenes*) when present in the wound bed, increase the likelihood of graft failure [49,50] and additionally have a different propensity for invasiveness. Microbes cause skin graft failure by the production of plasmin and proteolytic enzymes that dissolve the fibrin scaffold that allows skin grafts to adhere to the wound bed and it is known that different bacteria have varying levels of efficiency in producing these enzymes [51].

It appears from the findings that in addition to bacterial density, the type of bacteria, depth of invasion (especially the invasion into healthy non-burned tissue,) and antibiotic resistance all need to be taken into account when analysing these biopsies and correlating them to clinical outcomes.

6 CONCLUSION

The evidence base on the utility and reliability of quantitative microbiology for diagnosing or predicting clinical outcomes in burns patients is limited and poorly reported. Although 26 studies have been conducted, substantial heterogeneity exists across studies in terms of study aims, population characteristics, sampling and processing methods, methodological quality and outcome metrics reported. This is further compounded by gaps in reporting of items/data that could indicate methodological robustness and other key characteristics. Such gaps include omitting to document the timing of sample collection in relation to injury, interpretation of data with certain results excluded (e.g. the exclusion of negative samples by a few of the studies leading to a skewed interpretation of concordance [25]), and poorly defined clinical outcomes including sepsis criteria. Furthermore, whilst all studies provide measures of variation, there is no indication of reference values or guidance on clinical interpretation. The substantial heterogeneity and methodological flaws make comparisons across studies difficult and hamper the interpretation of findings.

Limited evidence suggests that in order to obtain the most reliable bacterial counts (i) more than one sample is required, ideally from multiple anatomical areas [44] (due to the variability of bacterial counts from samples even in different segments of the same biopsy specimen); (ii) in terms of sensitivity, biopsies generally outperform swabs in diagnosis or predicting sepsis but have limited applicability due to the longer processing time; (iii) high bacterial loads may predict worse clinical outcomes (than low bacterial loads) but information on counts need to be combined with other factors such as depth of invasion and invasion into healthy tissue to be relevant; and (iv) both quantitative and semi-quantitative culture reports need to be interpreted with caution and not in isolation but alongside clinical findings.

There is a clear need for a robust study to be performed to fully address the question of whether quantitative microbiology (namely biopsies) are of clinical utility for the management of burns patients, and furthermore whether there is indeed a direct link between the bioburden of a wound

and the risk of microbial invasion. This systematic review has shown that there is currently no good evidence to prompt a change in practice, since, in additional to the methodological flaws and shortcomings, 77% of the included studies have been performed more than two decades ago, and in that period burn wound care has undergone significant changes. These include new treatments (negative pressure dressings and dermal substitutes), as well as improved burn unit set up and infection control protocols. Indeed, only one study addressed the utility of quantitative microbiology in making decisions regarding antimicrobial therapy. This represents an important omission, in light of the increasing levels of antimicrobial resistance, and the relevance of antimicrobial stewardship [52].

Similarly the microbiology field has also seen great advances such as the recognition of the role of biofilms and improved diagnostic techniques such as real time quantitative Polymerase Chain Reaction (PCR) identification of microbes [53] and metagenomic profiling of bacterial populations. These changes may mean that the findings from the older studies (where a limited selection of bacterial isolation media were used) may no longer be applicable to current clinical practice, and thus newer studies need to be performed.

Several areas however need to be addressed before such studies are performed. Firstly, faster and more reproducible techniques for the identification and quantification of bacteria need to be in place. In the absence of a gold standard method, studies need to be undertaken to check and improve the reliability/reproducibility of the chosen wound sampling method and as mentioned previously, multiple site sampling needs to be performed instead of single site sampling. Secondly, even if quantification is successful, it would only prove useful clinically if the results are available rapidly (in hours rather than days), and thus rapid techniques need to be tested or devised. In terms of clinical outcomes, a standardised minimum (or core) set of clinical outcomes needs to be devised and agreed upon by all stakeholders in advance, in order to allow comparison of trials across different centres.

611 It is hoped that once a carefully designed multi-centre study has been undertaken that the evidence
612 base on the utility and reliability of quantitative microbiology for diagnosing or predicting clinical
613 outcomes in burns patients can be clarified.

614

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7 REFERENCES

[1] Church D, Elsayed S, Reid W, Winston B, Lindsay R. Burn wound infections. Clin Micro Rev 2006;19:403–34.

[2] Sheppard NN, Hemington-Gorse S, Shelley OP, Philp B, Dziewulski P. Prognostic scoring systems in burns: a review. Burns 2011;37:1288–95.

[3] Ryssel H, Kloeters O, Germann G, Schäfer T, Wiedemann G, Oehlbauer M. The antimicrobial effect of acetic acid--an alternative to common local antiseptics? Burns J Int Soc Burn Inj 2009;35:695–700. doi:10.1016/j.burns.2008.11.009.

[4] Public Health England. Investigation of swabs from skin and superficial soft tissue infections. UK Stand Microbiol Investig 2016;B11.

[5] Pruitt BA, Foley FD. The use of biopsies in burn patient care. Surgery 1973;73:887–97.

[6] Papini RP, Wilson AP, Steer JA, McGrouther DA, Parkhouse N. Wound management in burn centres in the United Kingdom. Br J Surg 1995;82:505–9.

[7] Krizek TJ, Robson MC, Kho E. Bacterial growth and skin graft survival. Surg Forum 1967;18:518–9.

[8] Robson MC, Shaw RC, Heggers JP. Quantitative bacteriology and delayed wound closure. Surg Forum 1968;19:501–2.

[9] Teplitz C, Davis D, Mason AD, Moncrief JA. Pseudomonas burn wound sepsis. I: Pathogenesis of experimental pseudomonas burn wound sepsis. J Surg Res 1964;4:200–16.

[10] Loebl EC, Marvin JA, Heck EL, Curreri W, Baxter CR. The use of quantitative biopsy cultures in bacteriologic monitoring of burn patients. J Surg Resea 1974;16:1–5.

[11] Steer JA, Papini RPG, Wilson APR, McGrouther DA, Parkhouse N. Quantitative microbiology in the management of burn patients. II. Relationship between bacterial counts obtained by burn wound biopsy culture and surface alginate swab culture, with clinical outcome following burn surgery and change of dressings. Burns 1996;22:177–81.

[12] Uppal SK, Ram S, Kwatra B, Garg S, Gupta R. Comparative evaluation of surface swab and quantitative full thickness wound biopsy culture in burn patients. Burns 2007;33:460–3.

[13] Herruzo-Cabrera R, Vizcaineo-Alcaide MJ, Pinedo-Castilllo C, Rey-Calero J. Diagnosis of local infection of a burn by semiquantitative culture of the eschar surface. J Burn Care Rehabil 1992;13:639–41.

[14] Heggers JP, Haydon S, Ko F, Hayward PG, Carp S, Robson MC. Pseudomonas aeruginosa exotoxin A: its role in retardation of wound healing: the 1992 Lindberg award. J Burn Care Rehab 1992;13:512–8.

[15] Perry AW, Howard SS, Lawrence JG, Wayne KS, Thomas JK. Skin graft survival—the bacterial answer. Ann Plast Surg 1989;22:479–83.

[16] Mann-Salinas EA, Baun MM, Meininger JC, Murray CK, Aden JK, Wolf SE, et al. Novel predictors of sepsis outperform the American Burn Association sepsis criteria in the burn intensive care unit patient. J Burn Care Res Off Publ Am Burn Assoc 2013;34:31–43. doi:10.1097/BCR.0b013e31826450b5.

[17] Hampson P, Dinsdale RJ, Wearn CM, Bamford AL, Bishop JRB, Hazeldine J, et al. Neutrophil Dysfunction, Immature Granulocytes, and Cell-free DNA are Early Biomarkers of Sepsis in Burn-injured Patients: A Prospective Observational Cohort Study. Ann Surg 2016. doi:10.1097/SLA.0000000000001807.

[18] Kwei J, Halstead FD, Dretzke J, Oppenheim BA, Moiemen NS. Protocol for a systematic review of quantitative burn wound microbiology in the management of burns patients. Syst Rev 2015;4:1–7.

[19] COSMIN. COnsensus-based Standards for the selection of health Measurement INstruments. Cosm Checkl 2011. http://www.cosmin.nl/index.html (accessed May 5, 2016).

[20] Whiting PF, Rutjes AWS, Westwood ME. and the QUADAS-2 group. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–36.

[21] Hayden JA, van der Windt DA, Cartwright JL, Côté P, Bombardier C. Assessing Bias in Studies of Prognostic Factors. Ann Intern Med 2013;158:280–6.
[22] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ Clin Res Ed 2009;339:b2535.
[23] Levine NS, Lindberg RB, Mason ADJ, Pruitt BAJ, Colonel. The quantitative swab culture and smear: a quick, simple method for determining the number of viable aerobic bacteria on open wounds. J Trauma Acute Care Surg 1976;16:89–94.
[24] Volenec FJ, Clark GM, Mani MM, Humphrey LJ. Burn wound biopsy bacterial quantitation: a statistical analysis. Am J Surg 1979;138:695–7.
[25] Steer JA, Papini RPG, Wilson APR, McGrouther DA, Parkhouse N. Quantitative microbiology in the management of burn patients. I. Correlation between quantitative and qualitative burn wound biopsy culture and surface alginate swab culture. Burns 1996;22:173–6.
[26] Buchanan K, Heimbach DM, Minshew BH, Coyle MB. Comparison of quantitative and semiquantitative culture techniques for burn biopsy. J Clin Micro 1986;23:258–61.
[27] Husson MO, Galizia JP, Savage C, Fournier L, Pellerin P, Izard D, et al. Etude d'une méthode de dénombrement des bactéries par épifluoresccence à l'acridine orange. Presse Med 1988;17:1637–9.
[28] Woolfrey BF, Fox JMK, Quail CO. An Evaluation of Burn Wound Microbiology: The Quantitative Gram Stain. J Burn Care Res 1982;3:171–5.
[29] Tahlan RN, Keswani RK, Saini S, Miglani OP. Correlation of quantitative burn wound biopsy culture and surface swab culture to burn wound sepsis. Burns Incl Therm Inj 1984;10:217–24.
[30] Krupp S, Baechler M, Bille J. Assessment of burn wound sepsis. J Hosp Infect 1985;6 Suppl A:133–7.
[31] Vural MK, Altoparlak U, Celebi D, Akcay MN. Comparison of Surface Swab and Quantitative Biopsy Cultures Dependent on Isolated Microorganisms from Burn Wounds. Eurasian J Med 2013;45:34–8.
[32] Winkler M, Erbs G, König W, Müller FE. Vergleich von vier methoden zur keimzahlbestimmung auf verbrennungswunden. Zbl Bakt Hyg A 1987;265:82–98.
[33] Danilla S, Andrades P, Gómez ME, Chamorro M, Leniz P, Piñeros JL, et al. Concordance between qualitative and quantitative cultures in burned patients. Analysis of 2886 cultures. Burns J Int Soc Burn Inj 2005;31:967–71.
[34] Barret JP, Herndon DN. Effects of burn wound excision on bacterial colonization and invasion. Plast Reconstr Surg 2003;111:744—50.
[35] Bharadwaj R, Joshi BN, Phadke SA. Assessment of burn wound sepsis by swab, full thickness biopsy culture and blood culture--a comparative study. Burns Incl Therm Inj 1983;10:124–6.
[36] Bharadwaj R, Phadke SA, Joshi BN. Bacteriology of burn wound using the quantitative full thickness biopsy technique. Indian J Med Res 1983;78:337–42.
[37] Sjöberg T, Mzezewa S, Jönsson K, Robertson V, Salemark L. Comparison of Surface Swab Cultures and Quantitative Tissue Biopsy Cultures to Predict Sepsis in Burn Patients: A Prospective Study. J Burn Care Res 2003;24:365–70.
[38] Mitchell V, Galizia J-P, Fournier L. Precise Diagnosis of Infection in Burn Wound Biopsy: Specimens Combination of Histologic Technique, Acridine Orange Staining, and Culture. J Burn Care Res 1989;10:195–202.
[39] McManus AT, Kim SH, McManus WF, Mason AD, Pruitt BA. Comparison of quantitative microbiology and histopathology in divided burn-wound biopsy specimens. Arch Surg 1987;122:74–6.
[40] Woolfrey BF, Fox JM, Quall CO. An evaluation of burn wound quantitative microbiology. I. Quantitative eschar cultures. Am J Clin Pathol 1981;75:532–7.
[41] Williams HB, Breidenbach WC, Callaghan WB, Richards GK, Prentis JJ. Are burn wound biopsies obsolete? A comparative study of bacterial quantitation in burn patients using the absorbent disc and biopsy techniques. Ann Plast Surg 1984;13:388–95.

[42] Bahar MA, Manafi A, Bouduhi N, Karimi H, Hormadzi M, Esfandi S, et al. Assessment of burn wound infection by swab and biopsy culture: a comparative study. MJIRI 2008;22:80–5.
[43] Marvin JA, Heck EL, Loebl EC, Curreri PW, Baxter CR. Usefulness of blood cultures in confirming septic complications in burn patients: evaluation of a new culture method. J Trauma Acute Care Surg 1975;15:657–62.
[44] Freshwater MF, Su CT. Potential pitfalls of quantitative burn wound biopsy cultures. Ann Plast Surg 1980;4:216–8.
[45] Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644–55.
[46] Greenhalgh DG, Saffle JR, Holmes JH, Gamelli RL, Palmieri TL, Horton JW, et al. American Burn Association consensus conference to define sepsis and infection in burns. J Burn Care Res Off Publ Am Burn Assoc 2007;28:776–90. doi:10.1097/BCR.0b013e3181599bc9.
[47] Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev 2006;19:788–802. doi:10.1128/CMR.00062-05.
[48] Ribet D, Cossart P. How bacterial pathogens colonize their hosts and invade deeper tissues. Microbes Infect Inst Pasteur 2015;17:173–83. doi:10.1016/j.micinf.2015.01.004.
[49] Unal S, Ersoz G, Demirkan F, Arslan E, Tütüncü N, Sari A. Analysis of skin-graft loss due to infection: infection-related graft loss. Ann Plast Surg 2005;55:102–6.
[50] Bang RL, Gang RK, Sanyal SC, Mokaddas EM, Lari AR. Beta-haemolytic Streptococcus infection in burns. Burns J Int Soc Burn Inj 1999;25:242–6.
[51] Teh BT. Why do skin grafts fail? Plast Reconstr Surg 1979;63:323–32.
[52] Isbi Practice Guidelines Committee null, Steering Subcommittee, Advisory Subcommittee. ISBI Practice Guidelines for Burn Care. Burns J Int Soc Burn Inj 2016;42:953–1021. doi:10.1016/j.burns.2016.05.013.
[53] Cockerill FR. Application of rapid-cycle real-time polymerase chain reaction for diagnostic testing in the clinical microbiology laboratory. Arch Pathol Lab Med 2003;127:1112–20. doi:10.1043/1543-2165(2003)127<1112:AORRPC>2.0.CO;2.

8 LEGENDS FOR ILLUSTRATIONS

Figure 1: PRISMA flow diagram detailing the study selection process

Supplementary Figure S1: Sample search strategy for MEDLINE.

Supplementary Figure S2: Table showing the questions that formed the quality assessment

Figure 1

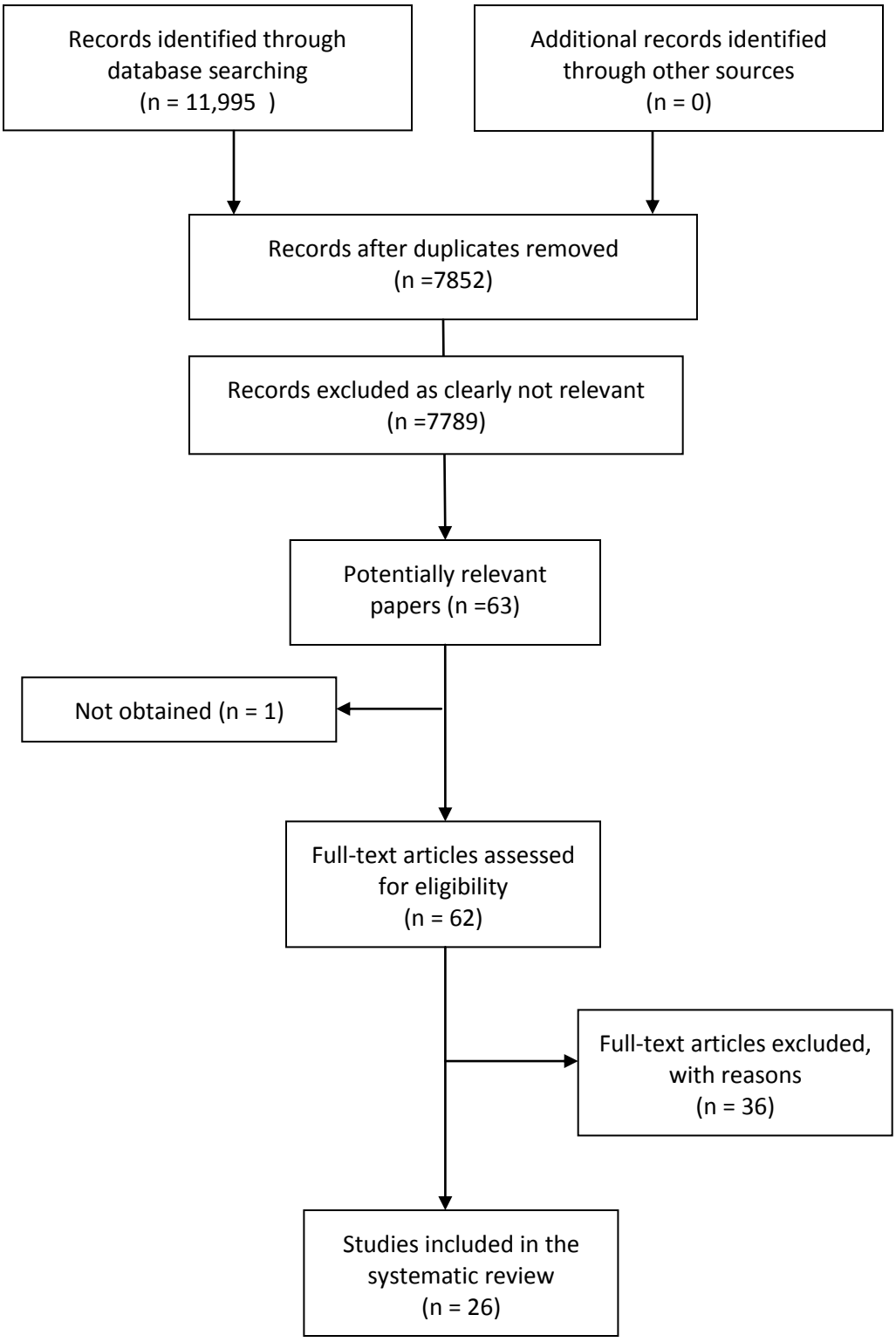
Records identified through database searching (n = 11,995)
Additional records identified through other sources (n = 0)
Records after duplicates removed (n =7852)
Records excluded as clearly not relevant (n =7789)
Potentially relevant papers (n =63)
Not obtained (n = 1)
Full-text articles assessed for eligibility (n = 62)
Full-text articles excluded, with reasons (n = 36)
Studies included in the systematic review (n = 26)

Table 1

Table 1: Studies reporting on intra- and inter-observer repeatability of the different methods of obtaining bacterial counts

Study	Population sample & any standard treatments	Type of biopsy	Type of swab	Main finding	Methodological strengths (+) and weaknesses (-)
Levine *et al* [23]^	24 patients with large areas of exposed granulation tissue. No detail on standard treatments	n/a	Not stated. Swabs (4 per patient) collected by twirling end on 1cm^2 section of the open wound for 5 seconds.	Four swabs were collected from 41 wounds and the mean log bacterial counts and standard error calculated per wound. The mean standard deviation was ±0.85 logs, and 95% of all results were ±1.7 logs from the mean per sample set (95% CI).	(-) Inadequate detail in terms of the sampling and the standard treatments. (-) Inadequate detail in terms of the processing of the swabs and how the quantitation was performed.
Volenec *et al* [24]	Four burn patients (unknown aetiology, 40-67% TBSA). Wounds washed free of topical antimicrobials before sampling (but no further details)	4 mm punch biopsy (exact method not stated) were collected on alternate days. Patients were sampled on a number of occasions (exact details not given)	n/a	36 paired samples collected and 27 analysed (8 pairs excluded as counts too low, and one excluded as counts too high). The counts per gram (in log) were compared between the samples in terms of range and standard deviation (SD). The mean range difference was low at 0.67 log, and mean SD was 0.64 log. 95% of all results were ±1.31 logs from the mean.	(+) Patients were sampled 2-3 times allowing the utility of biopsies to be assessed over time. (+) Topical antimicrobials removed from surface before sampling. (-) Only 4 patients included in the study design (-) Exact method of biopsy collection not stated (-) Timing of sample collection not stated (acute or delayed infection) (-) No removal of surface flora before sampling the wound via biopsy (-) 25% of the paired samples excluded from analysis
Steer *et al* [25]$^{\$}$	Population not stated but 18 patients in total. Topical antimicrobials (if visible) wiped	3mm biopsies (punch or collected by scalpel)	Alginate swabs collected from area adjacent to biopsy site.	Results reported in terms of counts per gram (biopsies), and per cm^2 (swabs). There was a significant correlation between the log total bacterial counts obtained from two simultaneous biopsies ($p<0.02$), and two swabs ($p<0.001$).	(+) Duplicate samples were collected from the same patient at the same time. (-) No removal of surface flora before sampling the wound via biopsy (-) Population not stated (-) Inconsistent treatment of wounds prior to sampling (-) Area of swabbing unclear: 4 cm^2 for some swabs, and

	away with sterile water-soaked gauze.			In samples with growth, only 29% of biopsies, and 50% of swab counts agreed within the same log unit. The biopsy correlation was no longer significant.	20 cm^2 for others.

^ Variation study $ Parallel cultures

Table 2A

TABLE 2A: Studies investigating the agreement between different test methods (different sections of single biopsy)

Study	Population sample & any standard treatments	Type of biopsy	Methods compared	Main finding	Methodological strengths (+) and weaknesses (-)
Barret & Herndon [34]	20 paediatric patients with 29-39% TBSA	2 biopsies collected from each patient (type of biopsy and method not stated)	QM on biopsies from eschar and excised wound bed	Compared counts of bacteria with the different biopsy segments – the eschars contained 10^4-10^6 cfu/g, compared to the excised wound bed (10^2-10^4 cfu/g). Difference was statistically significant.	(+) The patients were classified according to whether acute or delayed presentation. (-) The type of biopsy and method of collection not stated (-) Little information on processing of the biopsy
Mitchell *et al* [38]	Burns patients but no further details. No standard treatments	2 biopsies collected using 'conventional techniques' (exact method not stated)	Various methods performed on biopsies from adjacent sites: quantitative culture and acridine orange staining on one, histology on other.	Agreement between testing methods. Of 54 paired biopsy samples, 49 were negative by all methods. Very little data given in terms of quantitative counts.	(-) The type of biopsy and method of collection not stated (-) No skin prep before biopsy collection (-) Hard to compare methods when they are on different biopsies
McManus *et al* [39]	200 burns patients with mean TBSA of 54%	Single biopsy collected. Exact method not stated.	Biopsy split in half, one half for quantitative culture, the other for histology.	Correlation between methods in terms of positive and negative results (where $<10^5$ orgs/g). Good agreement for negative cultures, but poor correlation for positive samples.	(-) The type of biopsy and method of collection not stated (-) No skin prep before biopsy collection
Woolfrey *et al* [40]	56 biopsies, but no detail on number of patients. No standard treatments	Single biopsy collected using method similar to Loebl *et al* [10]	Single biopsy split in half transversely. Both segments processed by quantitative culture	Compared the counts between the two segments. For the paired isolates, 43% of the counts were within the same log increment, 29% differed by ±1 log increment, and 27% differed by ±2 log increments.	(+) Full details given for biopsy processing (+) Skin surface cleansed with an alcohol-soaked sponge before sampling (-) Patient population not stated.

TABLE 2B: Studies investigating the agreement between different test methods (same biopsies processed in different ways)

Study	Population sample & any standard treatments	Type of biopsy	Variables compared	Main finding	Methodological strengths (+) and weaknesses (-)
Buchanan *et al* [26]	Population not stated.	1-2cm excision biopsy (as per Loebl *et al* [10])	Single biopsy collected and processed using quantitative (Q) and semi-quantitative (SQ) methods	Methods compared according to concordance and Q count category. 96% agreement between the two methods.	(+) Topical agents removed prior to sample collection (-) Missing methodological details
Husson *et al* [27]	82 patients suffering from 2^{nd} and 3^{rd} degree burns	1-2cm excision biopsy (as per Loebl *et al* [10])	Single biopsy collected and processed using quantitative culture and acridine orange staining.	Concordance between processing methods in terms of counts and positive/negative (but no definitions given). 100% agreement for culture positive samples. 35% of culture negative samples were positive on microscopy.	(+) Topical agents removed prior to sample collection (-) Missing methodological details
Pruitt & Foley [5]	65 burn patients	Not stated	Single biopsy collected and processed using quantitative culture and histology.	No clear summary measure. Quantitative counts only performed for 23 of 132 biopsies. No concordance assessment made.	(-) The type of biopsy and method of collection not stated (-) No skin preparation before biopsy collection. (-) Positive histology result not defined.
Woolfrey *et al* [28]	112 biopsies collected but number of patients not stated.	1-2cm excision biopsy (as per Loebl *et al* [10])	Single biopsy collected and processed using quantitative culture and quantitative Gram stain.	Correlation coefficients measured between the counts obtained by culture and the Gram stain = 0.5 (mild positive association)	(+) Gram stains and culture performed from the same sample (-) No details on the population studied (-) No skin prep prior to biopsy
Williams *et al* [41]	228 samples collected from 'greater than' 50 patients with TBSA>20%	0.5 by 2cm excision biopsy (as per Loebl *et al* [10])	Single biopsy compared to an absorbent disc (of the same size) collected from the same area.	Correlation coefficients between methods in terms of bacteria isolated for the four most common organisms. Ranges from 0.66 (Enterococci) to 0.86 (*Pseudomonas aeruginosa*). No summary concordance data given for the bacterial counts.	(+) Sample processing details provided (+) Counts performed on both sample types (-) Skin prep performed before sampling (to remove topical agents) but no skin asepsis.

TABLE 2C: Studies investigating the agreement between different test methods (biopsies and swabs)

Study	Population sample & any standard treatments	Type of biopsy	Type of swab	Variables compared	Main finding	Methodological strengths (+) and weaknesses (-)
Krupp *et al* [30]	21 burns patients with 10 to >60% TBSA. Patients treated daily with topical antimicrobials.	Not stated	Not stated	Correlation between the methods in terms of bacteria recovered. No counts done for the swabs.	Some shared bacterial species between the methods but no relevant conclusions given in the paper.	(-) Type of biopsy and swab not stated (-) No counts performed on the swabs (-) No details on sample processing
Tahlan *et al* [29]	17 patients with 15-50% TBSA. Patients treated with topical and systemic antimicrobials.	1-2cm excision biopsy (as per Loebl *et al* [10])	Not stated	Correlation between the methods in terms of bacteria recovered. No counts done for the swabs.	The majority of samples (>85%) had similar bacterial species in both swabs and biopsies.	(-) No details on sample processing (-) No counts performed on the swabs
Vural *et al* [31]	160 patients. Regular treatments of burns not stated. Topical agents removed before biopsy.	5mm full thickness punch biopsy	Not stated.	Concordance between the methods in terms of bacteria recovered. No quantitative microbiology results given.	Classified concordance between the methods (in terms of bacteria recovered) in terms of the 'Kappa index'. 41% moderate agreement.	(+) Removal of topical agents before biopsy (-) Missing methodological details (-) Hardly any mention of quantitative bacterial counts in the paper
Danilla *et al* [33]	1443 paired samples from the Burns unit. Skin was surgically cleansed before sample collection	3mm punch biopsy	Not stated	Concordance between the methods in terms of the bacteria isolated and the counts. Swabs processed semi-quantitatively.	Concordance classified in terms of the Kappa Overall score of 52% (moderate).	(+) Large sample size (N=1443) (-) No detail on the timing of samples
Winkler *et al* [32]	12 patients with 2nd and 3rd degree burns of 20-70% TBSA. Burns regularly treated.	Type not stated although dimensions given (0.5cm long and 0.2cm thick)	Not stated but collected from a 1cm^2 area	Concordance between methods in terms of counts and standard deviations.	Poor and non-significant correlation between the bacterial counts from biopsies and swabs.	(+) Good detail given on the processing of the swabs and biopsies. (+) Counts performed on swabs (-) Small sample size (n=12) (-) Many results excluded from the analysis

Levine *et al* [23]	12 patients with 24 wounds. Regular treatment of burns not stated.	Not stated	Not stated but collected from a 1cm^2 area	Concordance between methods in terms of quantitative counts and R2 values	Good positive correlation between log biopsy cultures, and log swab cultures from the 7 pairs that could be analysed.	(+) Counts performed on swabs (-) Biopsy type not stated. (-) Small sample size
Steer *et al* [25]	74 patients but no further details	3mm punch or scalpel biopsy (topical antimicrobials removed prior to collection)	Alginate swabs collected from a 4 or 20cm^2 area	Correlation between methods in terms of quantitative counts and R2 values	Significant correlation between total bacterial count obtained by biopsy and by surface swab ($p<0.001$).	(+) Topical antimicrobials removed (if visible) (+) Quantitative counts performed on swabs (-) Non standardised methods for swab and biopsy collection (-) No skin asepsis prior to collection

TABLE 2D: Studies investigating the agreement between different test methods (biopsies, swabs and blood cultures)

Study	Population sample & any standard treatments	Type of biopsy	Type of swab	Type of blood culture	Variables compared	Main finding	Methodological strengths (+) and weaknesses (-)
Bahar *et al* [42]	75 burns patients with >20% TBSA. Regular treatment of burns not stated.	Collected from leading edge of wound and 1g in weight, but type not stated.	Not stated	Not stated (timing and condition of the patient not stated)	% agreement between the methods	Positivity rates: Swabs (100%), biopsies (89.3%), blood cultures (18.9%). Good correlation between swabs and biopsies	(+) Swabs and biopsies collected at the same time and timing is stated. (-) No skin asepsis prior to sample collection (-) Sample types and collection methods not stated (-) No definition for positive cultures
Bharadwaj *et al* [35]	50 burns patients, with burns >30-50% TBSA.	Quantitative full thickness as per Loebl *et al* [10]	Not stated	Not stated	Positivity rates between the methods.	Positivity rates of 94, 87.6, and 12% for swabs, biopsies, and bloods, respectively. No statistics or interpretation given.	(+) Timing of sample collection stated (-) Insufficient details on the processing methods. (-) No analysis performed in terms of concordance between the methods. (-) No skin asepsis prior to sample collection
Bharadwaj *et al* [36]	50 burns patients, with burns >30-50% TBSA.	Quantitative full thickness as per Loebl *et al* [10]	Not stated	Not stated	Positivity rates between the methods.	87.6% of the biopsies were positive. No results given for swabs or blood cultures.	(+) Skin aseptically cleaned prior to sample collection (-) Insufficient details on the processing methods. (-) Missing results for positivity rates of swabs and blood cultures.
Sjoberg *et al* [37]	50 burns patients, with burns >10% TBSA. Patients bathed daily in antimicrobial biocides and topical antibiotic creams applied.	8mm dermal punch taken from sites showing signs of infection	Not stated	Not stated	Positivity rates between the methods.	Poor correlation in between swabs and biopsies (no growth or identical bacterial growth) of only 29%. Poor correlation between organisms isolated from blood vs biopsies	(+) Skin aseptically cleaned prior to sample collection (+) Timing of sample collection stated (-) Definition of a positive culture not defined. (-) Indication for the collection of a blood culture not stated

Steer *et al* [11]	47 burns patients with 1-65% TBSA. Regular treatment of burns with topical biocides and antimicrobial creams.	3mm punch or scalpel biopsy (topical antimicrobials removed prior to collection)	Alginate swabs collected from a 4 or 20cm^2 area	Not stated.	Bacterial counts between the method types	No significant difference in counts between swabs and biopsy samples. No links in terms of counts to positivity of blood cultures.	(+) Topical antimicrobials removed (if visible) (-) No skin asepsis prior to sample collection (-) Time of sample collection not stated
Uppal *et al* [12]	100 burns patients with >30% TBSA. Numerous samples from each. Regular treatments of burns not stated.	5mm punch biopsy	Not stated but collected from a 4cm^2 area	5-10mls of blood collected and cultured using the BacTec automated system.	Positivity rates between the methods.	Concordance between swab and biopsy (95%). Blood cultures and biopsies both positive on 65 occasions, but many cases of discordance (biopsy positive and blood culture negative, and vice versa.	(+) Topical agents were removed from the sampling site with saline. (+) Timing of sample collection stated (+) Methods of sample collection and processing stated. (-) Indication for the collection of a blood culture not stated

Table 3

TABLE 3: Clinical outcomes investigated and definitions of sepsis utilised by studies

Study	Aim of study	Clinical outcomes studied	Sepsis definition
Bahar *et al* [42]	To evaluate whether QM can predict the likelihood of mortality	Mortality	n/a
Barret & Herndon [34]	To assess the efficacy of burn wound excision on decreasing burn wound colonisation	Burn wound infection, graft loss, sepsis	Not defined
Bharadwaj *et al* [35]	To evaluate QM methods in the diagnosis of burn wound sepsis	Sepsis, mortality	3 or more of: disorientation, tachypnoea, hypothermia, hyperpyrexia, thrombocytopenia, leucopenia, and paralytic ileus.
Bharadwaj *et al* [36]	Not clearly stated	Mortality	n/a
Freshwater & Su [44]	To examine the relationship between QM (biopsies) and sepsis	Sepsis	2 or more of: disorientation, hypothermia (<36.4^{o}C), hyperpyrexia (>39.2^{o}C), thrombocytopenia (<70,000 cells/m^{2}), leucopenia (<5,000 cells/m^{2}), tachypnea (>30 bpm), tachycardia (>140bpm), or paralytic ileus.
Krupp *et al* [30]	Not clearly stated, but to evaluate biopsies in predicting chances of survival	Mortality	n/a
Loebl *et al* [10]	To evaluate biopsies as an adjunct to the care of burns patients	Sepsis, mortality	2 or more of: hyperpyrexia, hypothermia, disorientation, leucopenia, thrombocytopenia, tachypnoea, tachycardia, or ileus
Marvin *et al* [43]	To evaluate the value of blood cultures for the diagnosis of sepsis	Sepsis, mortality	Presence of 3 or more significant alterations in physiologic parameters: disorientation, paralytic ileus, hyper/hyper thermia, sinus tachycardia, tachypnea, severe refractory hypotension, leukopenia, decreased platelets.
Pruitt & Foley [5]	To assess the utility of biopsies in burn patient management	Sepsis, mortality	No clear definition given but they do mention parameters such as temperature alteration, lethargy, disorientation, abdominal distention and ileus.
Sjoberg *et al* [37]	To evaluate whether QM is useful in predicting the possibility of septicaemia	Sepsis, mortality	Based on the following parameters (but did not state how many were required to be present for diagnosis): body temp (<36^{o}C or >39 ^{o}C), blood pressure (<90mm Hg or a reduction of 40mm HG or more), pulse rate (above 90 BPM), altered mental status.
Steer *et al* [11]	To examine the relationship between clinical outcome and bacterial densities	Use of antimicrobials within 72 hours of operation or dressing	Appearance of fever (>38^{o}C), rigors, hypotension, or graft loss (>5%)

		change, sepsis, and graft loss.	
Tahlan *et al* [29]	No clinical aims stated	Sepsis, mortality	3 or more of pyrexia, hypothermia, disorientation, leucopenia, thrombocytopenia, tachypnoea, tachycardia.
Vural *et al* [31]	No clinical aims stated	Length of stay	n/a

Table 4

Table 4: Summary of results of studies that have reported clinical outcomes and their methodological strengths and weaknesses.

Study	Quantitative Microbiology (number of patients; %TBSA)	Threshold for a positive culture	Main Finding	Methodological strengths (+) and weaknesses (-)	Conclusions
Bahar *et al* [42]	Swabs, biopsies and blood cultures (75; >20%)	Not defined; any growth considered positive	59/75 patients died. Bacterial counts of $>1x10^5$ cfu (no units) for 48/59 (81%), and $<1x10^5$ for 9/59. All of the 16 patients who survived had high bacterial counts ($>1x10^5$ cfu)	(+) Large sample size compared to other studies (-) The timing of sample collection is unclear with respect to the burn injury (-) Patients followed up until discharge or death, but unclear on readmission (-) No definition of infection	No statistically significant difference between bacterial load and positive cultures with mortality.
Barret & Herndon [34]	Biopsies: two different samples from the same patient (20; 29-39%)	Not defined; any growth considered positive	No patients with counts of less than 10^5 orgs/g experienced infection or graft loss, whereas patients with counts above this had a 50% chance of infection.	(+) Patients classified into acute or delayed in terms of excision (-) Delayed excision group received healthcare elsewhere (additional variable not explored)	Burn wounds that yield bacterial culture counts of more than 10^5 orgs/g should be considered at risk for invasive burn wound infection.
Bharadwaj *et al* [35]	Swabs, biopsies and blood cultures (50; 30-50%)	$\geq 10^4$orgs/g	In patients with sepsis, positivity of sample types varied: 62.5%, 82.5% and 100% for swabs, biopsies, and blood cultures, respectively. 23 patients died, and all had $>10^8$orgs/g in biopsies. 16 of these had negative blood cultures.	(+) Timing of sample collected stated. (-) No statistics performed (-) Poor definition of sepsis (-) Different numbers of samples collected from the patients (-) Unclear what the counts were in the patients who did not die.	Full thickness biopsy culture and bacterial counts were the best method for rapid diagnosis and assessing the progress of burn wound infection.
Bharadwaj *et al* [36]	Quantitative biopsy, swab and blood culture (50; 20-50%)	Positive biopsies defined as QM counts of $>10^5$ orgs/g	23/50 patients died. Deaths attributed to infection for 18/23 (78%), and for all, the bacterial counts were $>1x10^8$ orgs/g	(-) No statistical tests performed (-) No information of bacterial counts in survivors (-) Insufficient methodological details (-) Mentioned 'mortality due to infection', but do not state how this was decided or proven	Quantitative counts correlated well with the clinical condition of the patient

Freshwater & Su [44]	Quantitative full thickness biopsies (18; >20%)	Not defined; any growth considered positive	285 biopsies collected from 18 patients on 87 occasions. Bacteria per gram quantified and related to signs of sepsis. When bacterial counts were $>10^8$/g, 11 patients had 2 or more signs of sepsis compared to 24 with less than 2 signs of sepsis.	(+) Topical agents removed prior to sample collection (-) Patients treated with silver sulfadiazine cream (+) Definition of sepsis given	No apparent relationship between bacteria per gram of biopsy and clinical signs of sepsis
Krupp *et al* [30]	Swabs and biopsies (21; 10->60%)	Not defined; any growth considered positive	Burn deaths correlated with bacterial density on biopsy: 5/21 patients died. All 5 had counts $>1x10^5$ bacteria/g. 5/16 had counts $<1x10^5$ and survived.	(-) Results are incomplete – only 10/21 patients accounted for (-) Correlation is claimed, but no statistical tests have been performed (-) Small sample size	Biopsies have a diagnostic value for monitoring wound infection. Patients with burn wounds which showed $>10^5$ orgs/gram more likely to die even with additional measures, though this number was too small to reach statistical significance.
Loebl *et al* [10]	Surface cultures (not swabs) and biopsies (210; >20%)	Positive biopsies defined as QM counts of $\geq10^4$ orgs/g	73 patients had a positive biopsy. Of these, 48 became septic (25 did not), and 15 of these 48 died (33 survived).	(-) Counts not performed on the surface cultures (-) No stats performed to see if the relationships are significant (-) Unclear how sample population of 210 (from 270) was chosen (-) Unclear when the samples were collected	The authors conclude that 'biopsy cultures more accurately reflect burn wound colonisation than surface culture techniques', since a greater proportion of the biopsy positives progressed to sepsis than the surface cultures.
Marvin *et al* [43]	Blood cultures and biopsies (38; >20%)	Positive biopsies defined as QM counts of $\geq10^4$ orgs/g	35 patients had positive biopsies ($\geq10^4$ orgs/g). Sepsis occurred in 27/35 (71%), but only 11/27 also had positive blood cultures. 11/35 patients died from infection, but 4/11 had negative blood cultures.	(-) No stats performed to see if the relationships are significant (-) Result reporting is confusing (see text) (-) Blood cultures mostly taken in the absence of clinical indications (e.g. a pyrexial spike)	Blood cultures are disappointing for diagnosing septic complications, but a combination of QM from biopsies and clinical

					evaluation did allow early therapeutic interventions.
Pruitt & Foley [5]	Biopsies and blood cultures (65; not stated)	Not defined; any growth considered positive	65 patients, but QM only performed for 23. 20/23 with QM counts $>10^5$ orgs/g (15/20 died). 3/23 with counts $<10^5$ orgs/g (1/3 died)	(-) Unclear definition of sepsis (-) Samples collected at different times per patient, and varying number of samples collected per patient	The authors conclude that 'the severity of infection was related to the number of deaths'
Sjoberg *et al* [37]	Swabs, biopsies and blood cultures (50; >10%)	Positive biopsies defined as QM counts of $>10^5$ orgs/g	Patients split into septic (N=21) and non-septic (N=29). Biopsy QM counts statistically lower in the non-septic group ($6x10^8$ bacteria/g) compared to the septic group ($2x10^{11}$ bacteria/g; $p<0.05$)	(-) Unclear definition of sepsis (+) Sepsis parameters (e.g. blood pressure) recorded every 4 hours (-) Three different sample types collected at different times per patient (-) Limited analysis of the data wrt mortality	Significant difference in counts from biopsy in septic vs non-septic patients. Sepsis better correlated to biopsy QM counts than swabs or blood cultures.
Steer *et al* [11]	Swabs, biopsies and blood cultures (47; 1-65%)	Not defined; any growth considered positive	A total of 69 swab and biopsy pairs analysed. There was a significant negative correlation between QM count from the swab and %TBSA ($p=0.006$).	(-) Poor definition of clinical outcomes (-) Different number of samples collected per patient (-) Differential treatment of the wounds in terms of antimicrobial dressings. (-) Blood cultures not collected from each patient.	No significant difference in bacterial counts between patients judged to be a clinical success or clinical failure.
Tahlan *et al* [29]	Swabs and biopsies and blood cultures (17; 15-50%)	Positive biopsies defined as QM counts of $>10^5$ cfu/g	10/17 patients were not septic but had QM counts of $1x10^5$ cfu/g. 7/17 were septic with counts $\geq 1x10^8$ cfu/g. 3/7 died and had counts $7x10^8$ cfu/g.	(-) Small sample size (-) No stats performed	There was a difference in terms of QM count in those with sepsis compared to those without.
Vural *et al* [31]	Swabs and biopsies (160; <30->50%)	Not defined; any growth considered positive	Almost half (44%) of the patients hospitalised for more than 40 days had QM counts of $\geq 1x10^5$ CFU/g, compared to 5% in those hospitalised for less than 10 days.	(-) Bias in findings: Only 18 patients were in hospital for more than 40 days compared to 73 in for <10 days (-) No stats performed for outcome of interest (-) Limited clinical outcomes (-) Limited QM reported	QM counts increase with length of hospital stay

Supplementary Material S1

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Supplementary Material S2

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