

A systematic review of quantitative burn wound microbiology in the management of burns patients

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1 **A systematic review of quantitative burn wound microbiology in the management of burns**
2 **patients**

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39 **1 ABSTRACT**

40

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

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

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

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

59

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

61

62 **2 BACKGROUND**

63

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

72

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

82

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

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

90

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

95

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

100

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

111

112

113

114 **3 METHODS**

115 A protocol detailing the methodology was registered (PROSPERO (CRD42015023903)) and published
116 [18]. A summary of the methods is described here.

117

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

126

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

133

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

139

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

152

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

160

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

164

165

166 **4 RESULTS**

167 **4.1 Overall summary**

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

172

173 **4.2 Intra-and inter- observer repeatability of the different methods of obtaining bacterial**
174 **counts**

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

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

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

192 Steer *et al* [25], collected duplicate biopsies and swabs (two of each per patient) from 18 patients.
193 The authors found that there was a significant correlation between the log total bacterial counts
194 obtained from two simultaneous biopsies ($p < 0.002$), and from two swabs ($p < 0.001$) collected from
195 the same patient at the same time only if samples without growth were included. When samples
196 without growth were excluded from the analysis (since by being negative, they are automatically
197 concordant), the correlation between simultaneous biopsies was no longer significant, and only 29%
198 of biopsies and 50% of swab counts agreed within the same log unit. The 95% CI ranges for biopsies
199 and swabs respectively were $\pm 5.4 \log_{10}$ counts/g, and $\pm 3.6 \log_{10}$ counts/cm². This wide range may be
200 due to the variation in sampling area for swabs. The authors conclude that single samples are not
201 sufficient for measuring bacterial counts, and that one sample type cannot be used to predict the
202 counts obtained from another sample type.

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

209

210 **4.3 Agreement between different methods**

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

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

218 **4.3.1 Agreement in bacterial counts with different sections from the same biopsy/biopsy site**

219 Four studies investigated bacterial counts obtained from different sections of the same biopsy or
220 biopsy sites [34,38–40], however there are several methodological weaknesses; only one study [40]
221 provided detail on biopsy collection and processing method, and all provide only minimal detail on
222 the patient population (Table 2A). Furthermore, each study investigated bacterial counts from
223 different samples. In the Barret & Herndon [34] study, biopsies were collected from the eschar and
224 excised wound bed, whereas in Mitchell *et al* [38] they were from ‘adjacent sites’. The studies by
225 McManus *et al* [39] and Woolfrey *et al* [40] collected a single biopsy that was then split for
226 processing (transversely [40]; not stated in [39]). Counts are provided for three of the studies
227 [34,39,40], but, given the paucity of information on the sampling methodology, it is hard to draw any
228 conclusions regarding any differences observed between counts, as these appear to either represent
229 distinct samples [34], or represent variation in sampling from the same/similar site [39,40]. Samples
230 in Woolfrey *et al* [40] were analysed separately according to whether the same bacterial isolate was
231 recovered from both split biopsy samples (paired), or whether the samples were discordant
232 (unpaired). For 43% of the paired biopsies, the quantitative results were within the same log
233 increment. Combining the paired and unpaired samples, 21% of the quantitative results were within
234 the same log increments, 19% differed by ± 1 log increment, and 60% differed by ± 2 log increments
235 or more. Although there are no reference ranges to guide interpretation, it appears that there is vast
236 variation in different segments from the same biopsy specimen.

237 **4.3.2 Agreement in bacterial counts between different processes used on single biopsies**

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

240 biopsy homogenate [26], acridine orange microscopy [27], histology [5], quantitative Gram stain
241 [28], and absorbent discs [41]. The majority of these studies collected biopsies according to Loebel *et*
242 *al* [10], but there is no detail on biopsy collection for the Pruitt & Foley [5] study, and no details
243 provided on the processing of the sample for two studies [26,27]. The studies also vary in the skin
244 preparation before biopsy collection, with three removing topical agents prior to sample collection
245 [26,27,41], and no details on skin preparation for the other studies. This lack of detail makes it
246 difficult to compare the robustness of study methodologies.

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

260

261 **4.3.3 Comparison of bacterial counts obtained from swabs versus biopsies**

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

264 [25,31,33], or skin slit [32]), or were not stated [23,30]. Only one of the seven studies [25] reported
265 the swab type, and only three report on the surface area volume that was sampled [23,25,32].

266

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

273

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

281

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

288

289 **4.3.4 Comparison of bacterial counts obtained from swabs versus biopsies versus blood cultures**

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

294

295 There is heterogeneity in both the types of biopsy that were collected (Loebl *et al* [10] method, or
296 dermal punches), and the preparation of the skin prior to sampling. Only two of the six studies
297 [36,37] mention that the sampling was performed aseptically. Without aseptic collection, biopsies
298 may be positive because of translocation of the bacteria into the sample during sample collection
299 rather than invasion of bacteria. This also applies to the collection of blood cultures and is a
300 considerable methodological flaw of these studies.

301

302 Another limitation is the lack of detail on timing of sample collection. Three papers give a specific
303 time for sample collection in terms of post-burn [12,35,36], e.g. samples collected on the 2nd, 4th and
304 7th days post burn [12]. The other studies are less clear, e.g. ‘within the second week’ [42], within 48
305 hours of admission [37], or at the start of a change of dressing [11]. It is also often not clear when
306 the blood samples were collected in relation to the other samples. Furthermore, definitions of what
307 constitutes a positive culture are only provided for two studies [35,36]. The overall lack of detail
308 and/or heterogeneity thus hampers any comparisons of study findings.

309

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

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

316

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

322

323 **4.3.5 Comparison of bacterial counts obtained from biopsies versus blood cultures**

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

330

331 **4.4 Association between quantitative microbiology, other measures and clinical outcomes**

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

337

338 **4.4.1 Studies reporting sepsis**

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

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

349

350 **4.4.2 Utility of different sample types and quantitative microbiology for predicting sepsis**

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

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

363 wound biopsies performed better than surface cultures (Rodac plates) in terms of predicting the
364 development of clinical sepsis. Of 210 patients included in this study, 117 had positive surface
365 cultures, and 73 of these were also positive from the biopsy samples. From the biopsy samples,
366 48/73 (66%) of the patients became septic, and of these, 15/48 (31%) died. False positive results
367 (i.e. patients who had positive biopsies but did not develop sepsis) were found to have either been
368 treated with sub-eschar, or systemic antibiotics. No patients with sterile biopsies developed sepsis
369 unless another source of infection was present. The overall conclusions need to be viewed cautiously
370 however, as it is unclear how many patients with positive surface cultures died, or how many had
371 positive biopsies in the absence of sepsis.

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

376 Bharadwaj *et al* [35] found that 16 patients who died of sepsis in their study cohort (and who had
377 $>10^8$ orgs/g in biopsies) had negative blood cultures. Bahar *et al* [42] found that blood culture
378 positivity was not significantly different between patients who died and survivors (19% vs 18.8%,
379 respectively), and Marvin *et al* [43] found blood cultures to be ‘disappointing’ as a means of
380 diagnosing septic complications since only 30% of septic patients in their study had positive blood
381 cultures. They also found a false positive rate of 10% (i.e. positive blood culture, but no simultaneous
382 clinical signs of sepsis). This is higher than previously reported rates of 0.6-6.0% [47], but could be
383 explained by a commensal being isolated from the blood, and delayed onset of sepsis in the patients
384 the samples were collected from.

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

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

395 In terms of quality assessment, all of the above studies have methodological limitations which affect
396 the validity of the data. These include failure to define thresholds for positive cultures [5,11,34],
397 small sample sizes ($n < 25$) [29,34], and conclusions made in the absence of robust data analysis
398 [10,29,35,43].

399
400 **4.4.3 Utility of different sample types and quantitative microbiology for predicting mortality and**
401 **other clinical outcomes**

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

407 Bahar *et al* [42] evaluated whether quantitative microbiology could predict the likelihood of
408 mortality by looking at the association between counts from swabs and biopsies and mortality for
409 75 patients. There was no statistically significant difference in counts between those who died and
410 those who survived; 59 patients died, of whom 48 had bacterial counts greater than 1×10^5 cfus (units
411 not stated), compared to 16/16 patients who survived but still had this high level of bacterial
412 bioburden from the biopsy samples (Table 4). This was consistent with the findings from Pruitt &
413 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 $>1 \times 10^8$
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,

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

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

443

444 **4.4.4 Quantitative counts, depth of invasion, and clinical outcomes (sepsis and mortality)**

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

452

453 **4.4.5 Impacts of quantitative counts on patient management**

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

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464 **5 DISCUSSION**

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

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

478 A key finding from this systematic review is that there is not a gold standard nor universally accepted
479 method for monitoring a burn wound for bacterial colonisation and infection. Studies using any
480 method of quantitative microbiology from biopsy samples were eligible for inclusion into the review
481 resulting in a range of different types of biopsy collected, whilst in six studies [5,23,30,34,38,39] the
482 authors failed to provide any information regarding the biopsy type. Furthermore, the comparator
483 samples such as swabs, surface plates or blood cultures also varied between studies in how they
484 were collected (e.g. the area of the skin swabbed), and how they were processed. The different
485 methods of processing may be the reason why the critical bacterial concentrations necessary for
486 burn wound sepsis vary so widely between studies. Freshwater *et al* [44] for example have
487 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].

511 In terms of clinical outcomes, eight of nine studies (investigating sepsis) seem to suggest that
512 biopsies performed better than swabs (or other comparators) in terms of correlation with sepsis.
513 However the utility of quantitative analysis of biopsies is still not clear as three of 13 clinical studies
514 [11,42,44] have also reported no correlation of biopsy results with clinical outcomes. These
515 conflicting findings may be a result of differences in methodological quality between studies, or
516 other sources of heterogeneity (e.g. population characteristics). Methodological flaws (or omissions
517 in reporting) in the clinical studies include: the time of sample (biopsy) collection not being stated
518 (bacterial density and antibiotic resistance has been shown to increase with longer time from burn
519 [35,37,48,49]), the lack of a common definition of sepsis, and ambiguity surrounding when mortality
520 is attributed to infection. For infection-attributed deaths, there is no clear definition or explanation
521 on how this was decided (e.g. in some cases, patients may have died of other non-infection related
522 cause). All of these factors may affect the robustness of the clinical findings, and these should
523 therefore be interpreted very cautiously. Furthermore, none of the studies have adjusted their
524 findings for potential confounding factors (i.e. other factors that might predispose a patient to
525 adverse clinical outcomes). These include age, burn depth and severity, and inhalation injury, and
526 may all lead to an inaccurate attribution of adverse clinical outcomes to high bacterial counts.

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

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

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

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

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

560

561 **6 CONCLUSION**

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

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

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

586 and the risk of microbial invasion. This systematic review has shown that there is currently no good
587 evidence to prompt a change in practice, since, in addition to the methodological flaws and
588 shortcomings, 77% of the included studies have been performed more than two decades ago, and in
589 that period burn wound care has undergone significant changes. These include new treatments
590 (negative pressure dressings and dermal substitutes), as well as improved burn unit set up and
591 infection control protocols. Indeed, only one study addressed the utility of quantitative microbiology
592 in making decisions regarding antimicrobial therapy. This represents an important omission, in light
593 of the increasing levels of antimicrobial resistance, and the relevance of antimicrobial stewardship
594 [52].

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

601 Several areas however need to be addressed before such studies are performed. Firstly, faster and
602 more reproducible techniques for the identification and quantification of bacteria need to be in
603 place. In the absence of a gold standard method, studies need to be undertaken to check and
604 improve the reliability/reproducibility of the chosen wound sampling method and as mentioned
605 previously, multiple site sampling needs to be performed instead of single site sampling. Secondly,
606 even if quantification is successful, it would only prove useful clinically if the results are available
607 rapidly (in hours rather than days), and thus rapid techniques need to be tested or devised. In terms
608 of clinical outcomes, a standardised minimum (or core) set of clinical outcomes needs to be devised
609 and agreed upon by all stakeholders in advance, in order to allow comparison of trials across
610 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

615

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627

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629

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- 760
- 761

762 **8 LEGENDS FOR ILLUSTRATIONS**

763

764 **Figure 1: PRISMA flow diagram detailing the study selection process**

765

766 **Supplementary Figure S1: Sample search strategy for MEDLINE.**

767

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

769

770

Figure 1

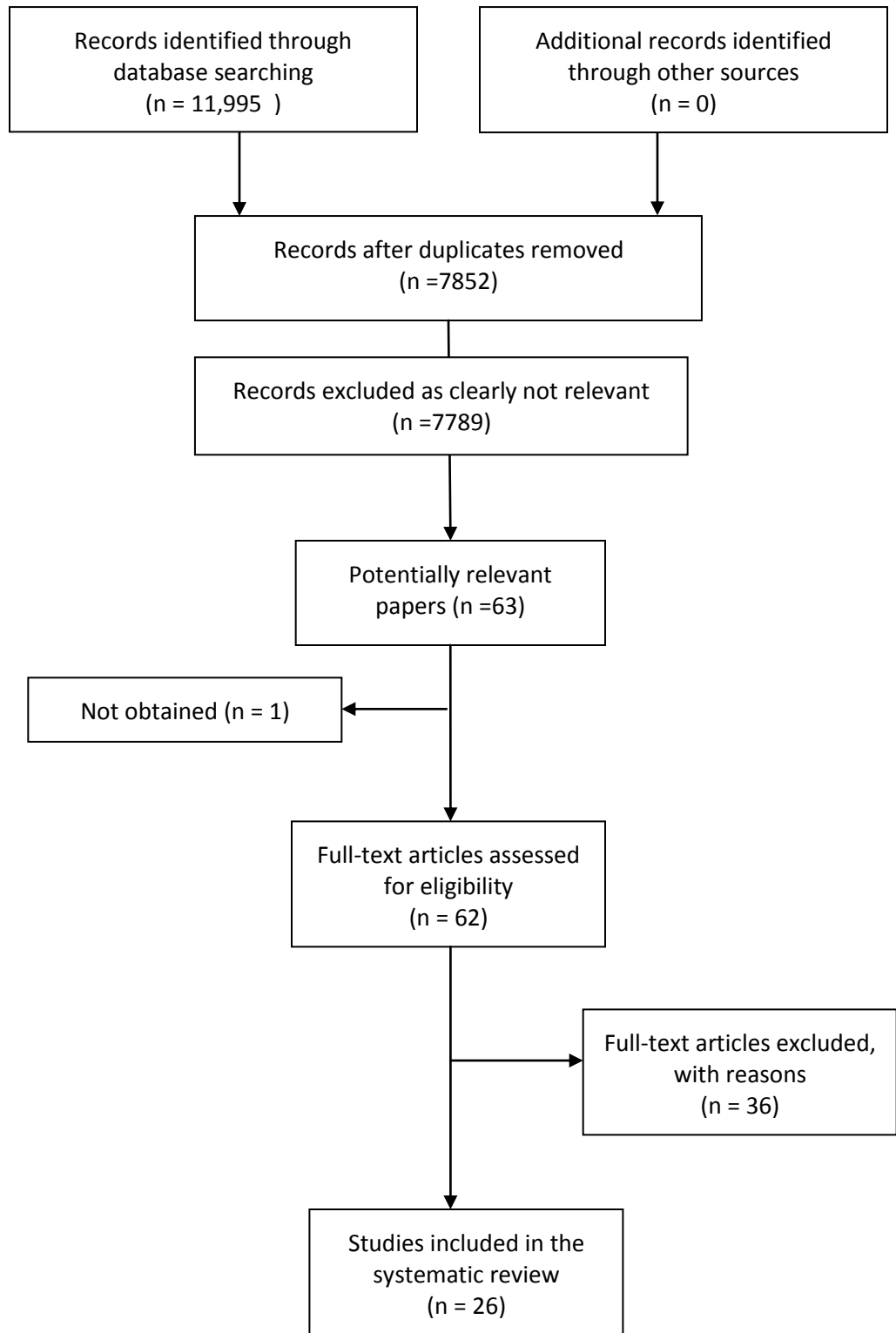


Table 1: Studies reporting on intra- and inter-observer repeatability of the different methods of obtaining bacterial counts

Study	Population sample & any standard treatments	Type of biopsy	Type of swab	Main finding	Methodological strengths (+) and weaknesses (-)
Levine <i>et al</i> [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.
Volenc <i>et al</i> [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 <i>et al</i> [25] ^s	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.
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^ Variation study § Parallel cultures

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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [40]	56 biopsies, but no detail on number of patients. No standard treatments	Single biopsy collected using method similar to Loebel <i>et al</i> [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 <i>et al</i> [26]	Population not stated.	1-2cm excision biopsy (as per Loeb <i>et al</i> [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 <i>et al</i> [27]	82 patients suffering from 2 nd and 3 rd degree burns	1-2cm excision biopsy (as per Loeb <i>et al</i> [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 <i>et al</i> [28]	112 biopsies collected but number of patients not stated.	1-2cm excision biopsy (as per Loeb <i>et al</i> [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 <i>et al</i> [41]	228 samples collected from 'greater than' 50 patients with TBSA>20%	0.5 by 2cm excision biopsy (as per Loeb <i>et al</i> [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 (<i>Enterococci</i>) to 0.86 (<i>Pseudomonas aeruginosa</i>). 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 <i>et al</i> [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 <i>et al</i> [29]	17 patients with 15-50% TBSA. Patients treated with topical and systemic antimicrobials.	1-2cm excision biopsy (as per Loebel <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [32]	12 patients with 2 nd and 3 rd 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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [35]	50 burns patients, with burns >30-50% TBSA.	Quantitative full thickness as per Loebel <i>et al</i> [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 <i>et al</i> [36]	50 burns patients, with burns >30-50% TBSA.	Quantitative full thickness as per Loebel <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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: Clinical outcomes investigated and definitions of sepsis utilised by studies

Study	Aim of study	Clinical outcomes studied	Sepsis definition
Bahar <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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°C), hyperpyrexia (>39.2°C), thrombocytopenia (<70,000 cells/m ²), leucopenia (<5,000 cells/m ²), tachypnea (>30 bpm), tachycardia (>140bpm), or paralytic ileus.
Krupp <i>et al</i> [30]	Not clearly stated, but to evaluate biopsies in predicting chances of survival	Mortality	n/a
Loebl <i>et al</i> [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 <i>et al</i> [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 <i>et al</i> [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°C or >39 °C), blood pressure (<90mm Hg or a reduction of 40mm HG or more), pulse rate (above 90 BPM), altered mental status.
Steer <i>et al</i> [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°C), rigors, hypotension, or graft loss (>5%)

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

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 <i>et al</i> [42]	Swabs, biopsies and blood cultures (75; >20%)	Not defined; any growth considered positive	59/75 patients died. Bacterial counts of $>1 \times 10^5$ cfu (no units) for 48/59 (81%), and $<1 \times 10^5$ for 9/59. All of the 16 patients who survived had high bacterial counts ($>1 \times 10^5$ 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 <i>et al</i> [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 <i>et al</i> [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 $>1 \times 10^8$ 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 <i>et al</i> [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 $>1 \times 10^5$ bacteria/g. 5/16 had counts $<1 \times 10^5$ 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 <i>et al</i> [10]	Surface cultures (not swabs) and biopsies (210; >20%)	Positive biopsies defined as QM counts of $\geq 10^4$ 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 <i>et al</i> [43]	Blood cultures and biopsies (38; >20%)	Positive biopsies defined as QM counts of $\geq 10^4$ orgs/g	35 patients had positive biopsies ($\geq 10^4$ 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 <i>et al</i> [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 (6×10^8 bacteria/g) compared to the septic group (2×10^{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 <i>et al</i> [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 <i>et al</i> [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 1×10^5 cfu/g. 7/17 were septic with counts $\geq 1 \times 10^8$ cfu/g. 3/7 died and had counts 7×10^8 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 <i>et al</i> [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 $\geq 1 \times 10^5$ 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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