How effective is wound swabbing? A clinimetric assessment of wound swabs

KEY WORDS
- Clinimetric assessment
- Validity
- Wound swabbing

The swabbing of wounds is routinely carried out in a number of healthcare environments to determine the level of bioburden present in wounds of various aetiologies. The test is carried out as wound bioburden is widely recognised as a threat to wider systemic infection, as well as a barrier to healing. Clinimetrics is the assessment of medical tests in regards to the validity, reliability and robustness of any test and how effectively the results recommend effective medical interventions. This article explores the published literature relating to how effectively wound swabbing satisfies different aspects of clinimetrics and what might better satisfy these requirements in the future.

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Human skin acts as a physical and immunological barrier to bacteria (Nemes and Steinert, 1999). Once this barrier is lost — for instance, through injury or surgery — the wound bed can be colonised by bacteria (Percival and Bowler, 2006). In some cases, sufficient virulence of the invading organism leads to systemic infection (Cutting and White, 2004). However, further infection does not always occur and bacteria persist in the wound bed, stalling the wound in the inflammatory phase of healing (Schultz et al, 2004).

It is widely accepted that the presence of increased bioburden in the wound bed contributes directly to impaired wound healing (Ayton, 1985; Bowler et al, 2001). Yet no wound is completely free of bacteria and there is much debate as to what level of bioburden will predict delayed healing or infection (Breidenbach and Trager, 1995; Robson, 1997: Gardner et al, 2007). The European Wound Management Association (EWMA) suggests the level is likely to vary between patients based on their underlying conditions, as well as the type of bioburden present and the status of the wound and periwound area (EWMA, 2005).

Tests to determine the presence of bioburden are required as it cannot be directly observed. Dealey (2000) proposed only indications of infection are communicable/observable, which are traditionally considered to be pain, inflammation, serous exudate and cellulitis, with bioburden itself being invisible to the naked eye. However, Halloran and Slavin (2002) also suggested these signs are not definitive. The World Union of Wound Healing Societies (WUWHS) has suggested that impaired healing may indicate the presence of bioburden, and to overcome the inherent complexity and clinician subjectivity, laboratory tests for bioburden should be used in order to determine an effective treatment plan as part of a holistic assessment, which includes wound, periwound area and patient history (WUWHS, 2008).

There are a number of ways of determining the bioburden in a wound, with taking a wound biopsy being considered the gold standard (Heggers and Robson, 1991). However, obtaining a biopsy can cause pain and bleeding, as well as further damage to the wound. It also requires healthcare practitioner (HCP) training which may not be readily available (Bowler et al, 2001; Ratliff and Rodeheaver, 2002). More commonly wound swabs are used to determine bioburden, to provide either a quantitative or semi-quantitative indication of the presence of bacteria (Bowler et al, 2001). This involves the HCP taking the swab sample, transporting to the laboratory, recovering bacteria from the swab, culturing of the bacteria on specific media in incubators and laboratory interpretation of the plates, which can all impact the reliability and accuracy of the result (Ratliff and Rodeheaver, 2002).

A quantitative measure gives the number of colony forming units (CFUs) recovered per swab, with a level of 10^5 CFUs typically being indicative of infection (Bowler et al, 2001). This method requires several steps, including the manual counting of the colonies that requires extra time and skill and, therefore, cost, on the part of the laboratory (Ratliff and Rodeheaver, 2002). A

simpler approach is the semi-quantitative culture, whereby the bacteria recovered from the swab are cultured onto one quadrant of a single agar plate, and the sample is then diluted by using individual sterile loops to serially dilute the sample over the four quadrants. After incubation, each quadrant is assessed with no bioburden present being reported if all quadrants are free of CFU, and heavy bioburden being reported if quadrant four exhibits any CFU, with two intermediate levels (Ratliff and Rodeheaver, 2002). This simple, semi-quantitative approach is most often employed in wound clinics and in the community due to its accessibility in regards to its ease of use, relatively low cost and ease of interpreting the results (Gardner et al, 2007). In this article, the evidence for the validity and reliability of the swab and culture methods will be examined.

CLINIMETRICS

The subject of clinimetrics was first introduced by Feinstein (1983). As quantitative tools were developed to eliminate the subjective nature of clinical decision making, Feinstein recognised the importance of being able to assess the robustness of these tests and the measurements they give in specific relation to patient care. The concept was further elaborated by Guyatt et al (1993), stating that the only way for objective, evidence-based conclusions to be made, on which to recommend future clinical interventions, is to ensure the robustness of data, and specifically the measurements of this data through methodical assessment of techniques. Two key themes of clinimetrics are the validity and reliability of any test (de Vet et al, 2003).

VALIDITY

The validity of any assessment tool is based on its ability to accurately establish the parameter it is designed to determine and comprises a number of aspects, such as construct validity, criterion validity and predictive validity (Guyatt et al, 1993). Face validity assesses the overall judgement of adequacy (de Vet et al, 2003), and given the widespread use of swab tests, with wide acceptance from tester, subject and medical institutes (Bowler et al, 2001) this suggests the technique does have face validity. However, in recent years various aspects of the validity have been brought into question and will now be explored.

CONSTRUCT VALIDITY

One aspect of construct validity relates to the level of evidence built up over time (de Vet et al, 2003), and given the continuous use of the swab since 1893 (Councilman, 1893) — originally for the detection of diphtheria — this would suggest the swab method satisfies this aspect of construct validity. Construct validity also relates to how the instrument or test under study relates to other methods of measuring the same construct (de Vet et al, 2003). In determining wound bioburden, swabs provide additional information on bioburden that cannot be derived from patient history and wound examination alone (WUWHS, 2008), and so satisfies this facet of construct validity, however, construct validity should really consider the tests that detect the same signal (i.e. bioburden, rather than related indicators, such as inflammation).

Tissue biopsy is regarded as the gold standard in determining bioburden (Heggers and Robson, 1991). Gardner et al (2007) took simultaneous tissue samples and swabs from 44 patients and demonstrated that semi-quantitative determinations do not correlate well with tissue biopsy or quantitative determinations. This could be due to the use of a single agar plate, which means the colonies are competing for space and nutrients, leading to reduced CFUs and, therefore, produces results that do not accurately reflect the reality of the situation.

Conversely, in a study of 124 chronic wounds of various aetiologies, a study by Ratliff et al (2002) showed a good correlation between quantitative and semi-quantitative measurements ($p<0.001$). Based on previous research by Bill et al (2001), who had demonstrated a 79% correlation between quantitative swabbing and biopsy, Ratliff et al concluded that the semi-quantitative method was a useful adjuvant for HCPs, that directly measures wound bioburden and therefore has construct validity. One problem with this conclusion is that the semi-quantitative method was not directly compared to simultaneous wound biopsy samples, so the assumption that the semi-quantitative method is directly comparable may not be accurate.

The study by Ratcliff et al used an externally valid and clinically relevant criteria of 10^5 CFU or quadrant III and/or IV being positive for CFU to compare the determinations, but it is difficult to compare the findings to the Gardner et al study as they used a 10^6 CFU cut-off point (Gardner et al, 2007). Furthermore, in the Ratliff et al study, in instances of a false negative

(21% of the results) by the semi-quantative method, — when high levels of bioburden were determined by the fully quantitative method — the semi-quantitative still reported growth in quadrant II (equivalent to 10^4 CFU) and would have indicated that bioburden was approaching significant levels, and was, therefore, still measuring sufficient bioburden to alert the HCP.

Despite this discrepancy in performance, Ratliff et al considered the semi-quantitative method to be directly measuring bioburden sufficiently to direct a treatment pathway, when balanced against other practical constraints, such as cost, ease-of-use and patient acceptability encountered in a real clinical situation. However, the limitations and discrepancies discussed highlight why the construct validity of semi-quantative swabbing has been questioned, with the common use of swabs justified on usability and acceptability, rather than specifically the construct validity.

CONTENT VALIDITY

Content validity relates to how comprehensively a test actually measures what it is designed to measure and is generally considered for composite assessments that give an overall diagnostic output (Guyatt et al, 1993). It is often not used to assess direct, analytical tests like swabbing, although there are some aspects that may affect the content validity of such tests. Culture-based methods are known to have bacterial attrition from those that are actually sampled from the wound, as only bacterial species amenable to the culture media and conditions are reflected in the results — demonstrated by comparing culture with molecular techniques (Dowd et al, 2008; Wolcott and Dowd, 2008).

This finding is significant with regards to the content validity of the test when it is considered that this attrition could be eliminating those bacteria that are responsible for impaired wound healing, or significant bioburden — particularly anaerobic bacteria which contribute up to 38% of the total number of isolates in chronic wounds (Bowler et al, 2001). This impacts on the content validity if the presence and amount of each species is considered to be an individual test. The composite CFU result does not fully reflect the actual type and total number of bacteria in the wound which impairs the ability to effectively influence treatment plans (WUWHS, 2008).

Specific organisms, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa,* are suggested to be detrimental to healing (Bowler et al, 2001), while other authors suggest that no individual bacteria is detrimental and it is the combination of bacteria (four or more species) that influences healing outcomes (Trengove et al, 1996). Either way, the semi-quantitative method does not attempt to isolate individual species, which would also help identify the most appropriate antibiotic, bringing into question the content validity as it may not be fully measuring the clinically important data, and so it is often supplemented with specific instructions from the HCP to aid identification of specific bacteria of interest (Bowler et al, 2001).

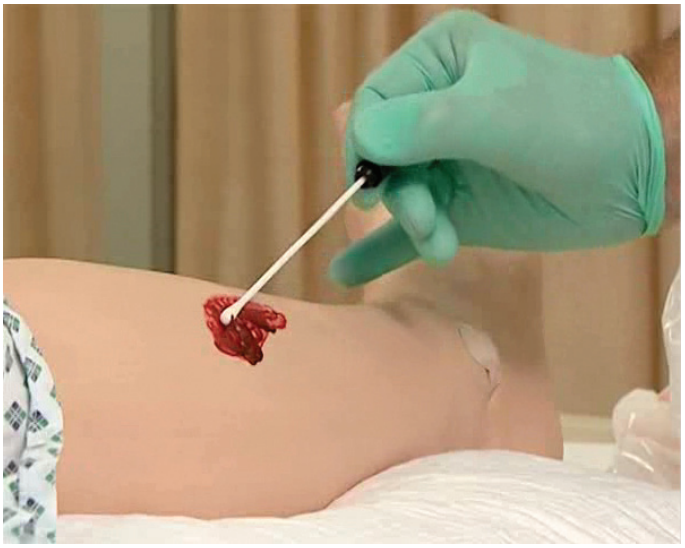
CONCURRENT AND CRITERION VALIDITY

Concurrent and criterion validity asks the question to what extent does this measurement technique relate to existing tests (concurrent) or 'gold standard' tests (criterion), and has been argued to be the most powerful type of validity (Guyatt et al, 1993; Streiner and Norman, 2008). Evidence suggest the semi-quantitative method does not perform, as well as the other commonly available methods (Heggers and Robson, 1991). Using a semi-quantative method, Gardner et al (2007) only recovered 57% of the bacterial species compared to tissue biopsy.

Similarly, all swab and culture methods are outperformed by more advanced molecular techniques (Dowd et al, 2008; Frank et al, 2009), which provide additional information about all bioburden present in the wound bed, including all bacterial typing and indicating whether the bacteria in the wound are in the planktonic or biofilm phenotype. This information is of clinical importance when determining treatment plans and predicting outcomes (James et al, 2008), however, these new techniques are relatively expensive and are not widely deployed, available or even well known in clinical practice so would constitute criterion rather than concurrent tests.

PREDICTIVE VALIDITY

The predictive validity of a measurement relates to how well the test predicts the future condition based on the present measurement (de Vet et al, 2003). In this instance, the question to ask is to what extent do wound swab samples accurately predict either the presence or absence of bioburden indicative of impaired healing. Current methods do not fulfil these aspects alone, and are supplemented by HCP observation and patient history, but even then are not



The Levine technique consists of rotating a swab over a 1 cm² area with sufficient pressure to express fluid from within the wound tissue.

fully predictive of clinical outcome (WUWHS, 2008).

In order to test the predictive validity of using wound swabs, Breidenbach and Trager (1995) examined quantitative cultures to predict infection in 50 postoperative lower extremity wounds. Their results suggest that quantitative culture had a similar ability as semi-quantitative swabbing with regard to positive predictive validity. Using a cut off of 104, a sensitivity was achieved of 89% and 83% respectively for the two methods. The quantitative method was, however, much less effective at predicting an absence of infection, having a specificity of only 20%, compared to 95% for the semi-quantitative method. This would leave the HCP potentially trying to control bioburden when this was not the cause of the impaired healing, leaving the underlying cause or comorbidity potentially untreated.

RELIABILITY

The reliability of any test relates to how consistent and reproducible results are over time and between user/rater, as well as how well the test responds to changes in the subject it is testing (Fette, 2006). There are inherent difficulties in assessing the reliability/reproducibility in respect to changes in the subject, as the wound and bioburden interact dynamically and change over relatively short time periods (Frank et al, 2009), meaning there is not a controlled standard. *In vitro* laboratory tests can provide this standard, but do not accurately reflect the complex nature of a wound (Gottrup, 2000, Seth et al, 2012). There are, however, a number of demonstrable issues relating to the reliability of swabs and swabbing technique, which will now be discussed.

SWABBING TECHNIQUE — INTER-RATER RELIABILITY

The reproducibility of any test can be controlled by using explicit methods (de Vet et al, 2003), yet the sampling at the wound with swabs did not have a single accepted standard, while much of the available evidence was being generated (Gilchrist, 1996). Various suggestions exist in the literature from using the Levine technique, which involves swabbing a 1 cm^2 area, while applying pressure to ensure bacteria are extracted from the wound bed (Levine et al, 1976) to using a zig-zag method over the whole of the wound surface (Lawrence, 1999) — both of which could impact the inter-rater reliability of the test, where consistency is required to ensure meaningful results downstream of the sampling.

Currently, the UK Standards Unit of the Health Protection Agency (HPA) provides guidance to address these issues (HPA, 2012), while it must be understood that much of the cited evidence was not generated using this standard approach.

Firstly, it is advised that the wound be cleansed prior to taking the sample (Gilchrist, 1996; Lawrence, 1999) to ensure surface debris are not reflected in the culture results, which could impact the accuracy and usefulness of the result to individual cases, but also the clinimetric reliability of data if this is not controlled between patients, institutions and regions.

The Levine swabbing technique is widely accepted as the most appropriate technique (Angel et al, 2011), yet this limits the area sampled (to 1 cm^2) even though evidence suggests bioburden is not uniformly distributed within the wound, causing Schneider et al (1983) to question the value and reliability of any single determination. It has been suggested that this is not a problem as the pressure extracts fluid from the deeper wound bed, which is reflective of the wound bed as a whole (Bowler et al, 2001) — i.e. the heterogeneous distribution of bacteria that Schneider observed being limited to the surface only — however, no data are reported to verify this assumption.

SWABS AND TRANSPORTATION

Inconsistencies exist in practice in terms of the

range of different swabs being used in practice, which collect and culture different numbers of bacteria (Gilchrist, 1996), to variances in absorptive properties of swabs themselves leading to the suggestion that wounds should be cleansed and swabs moistened prior to sampling to improve reproducibility (Lawrence and Ameen, 1998). The transportation of the swabs from patient sampling to microbiology laboratory, including the duration and transport medium have been shown to influence the numbers of bacteria decreasing or increasing between sampling and culturing (Yrios et al, 1975) depending on the species present, however, duration of transport and condition during transportation is often a factor that is difficult to control in clinical practice (Lawrence, 1999).

Assessing the reliability of the swab test has been complicated by the number of swab products available (Gilchrist, 1996), methods used, as well as advice available in the literature (Levine et al, 1976; Lawrence, 1999; Angel et al, 2011), yet this aspect of the clinimetric reliability can be addressed by adopting consistent techniques (HPA, 2012).

CONCLUSION

Despite debate over whether swabs have equivalent construct validity to biopsy sampling (Gardner et al, 2007) and evidence suggesting any culture methods are limited from a technical perspective to be able to fulfil content validity (Wolcott and Dowd, 2008), the semi-quantitative method remains a valid, useful, cost-effective and simple to use diagnostic tool in determining the presence or absence of clinically relevant levels of bacteria (Ratliff and Rodeheaver, 2002) as part of a full patient assessment (EWMA, 2005).

An important consideration for all clinimetric studies, including those cited in this article, is that they are carried out by experienced clinical researchers, in controlled studies, so any findings may not reflect the true levels of validity (and reliability) observed in real clinical practice (de Vet et al, 2003).

The concurrent validity benefits of modern molecular techniques over the swab and culture methods (Wolcott and Dowd, 2008) suggest that these techniques could become more prevalent in clinical practice once access and cost implications have been addressed. WUK

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