

# Real-time bacterial fluorescence imaging accurately identifies wounds with moderate-to-heavy bacterial burden

**Objective:** Clinical evaluation of signs and symptoms (CSS) of infection is imperative to the diagnostic process. However, patients with heavily colonised and infected wounds are often asymptomatic, leading to poor diagnostic accuracy. Point-of-care fluorescence imaging rapidly provides information on the presence and location of bacteria. This clinical trial (#NCT03540004) aimed to evaluate diagnostic accuracy when bacterial fluorescence imaging was used in combination with CSS for identifying wounds with moderate-to-heavy bacterial loads.

**Methods:** Wounds were assessed by study clinicians using NERDS and STONEES CSS criteria to determine the presence or absence of moderate-to-heavy bacterial loads, after which the clinician prescribed and reported a detailed treatment plan. Only then were fluorescence images of the wound acquired, bacterial fluorescence determined to be present or absent and treatment plan adjusted if necessary.

**Results:** We examined 17 VLUs/2 DFUs. Compared with CSS alone,

use of bacterial fluorescence imaging in combination with CSS significantly improved sensitivity (22% versus 72%) and accuracy (26% versus 74%) for identifying wounds with moderate-to-heavy bacterial loads ( $\geq 10^4$  CFU/g,  $p=0.002$ ). Clinicians reported added value of fluorescence images in >90% of study wounds, including identification of wounds incorrectly diagnosed by CSS (47% of study wounds) and treatment plan modifications guided by fluorescence (73% of study wounds). Modifications included image-guided cleaning, treatment selection, debridement and antimicrobial stewardship.

**Conclusion:** Findings from this pilot study suggest that when used in combination with CSS, bacterial fluorescence may: (1) improve the diagnostic accuracy of identifying patients with wounds containing moderate-to-heavy bacterial loads and (2) guide more timely and appropriate treatment decisions at the point-of-care.

**Declaration of interest:** This clinical trial was sponsored by MolecuLight Inc., Toronto, Canada.

bacterial fluorescence imaging • MolecuLight • wound assessment • wound infection

**C**hronic wounds frequently harbour moderate-to-heavy levels of bacteria, which can challenge the accuracy of clinical diagnosis and contribute to poor patient outcomes.<sup>1</sup> Wounds with moderate-to-heavy bacterial loads take longer to heal<sup>2–4</sup> and reduce patients' quality of life (QoL), while increasing health-care costs.<sup>5</sup> Left untreated, these wounds can cause local or systemic infections and, in some cases, patient loss of limbs.<sup>3,6</sup> While wound cultures remain a routine part of the standard of care (SoC), tools enabling real-time visualisation of bacteria in the wound remain an unmet need. Accurate bacterial cultures rely on accurate wound sampling, and culture results are often delayed by days and therefore have limited use in real-time assessment or informing treatment selection during a patient's visit.<sup>7</sup> Accordingly, numerous detection schemes, mnemonics and checklists have been developed around the 'classic signs and symptoms' (CSS) of the bacterial-infection continuum such as pain, lack of healing, purulent exudate, erythema, heat and oedema.<sup>1,8–14</sup> Examples are

the NERDS and STONEES mnemonics,<sup>8</sup> developed to evaluate the presence or absence of clinical signs of critical colonisation (NERDS) or infection (STONEES).<sup>8,9</sup> Under this mnemonic NERDS is — non-healing, exudate, red and bleeding surface or granulation tissue, debris, smell or unpleasant odour; and STONEES — size is bigger, temperature is increased, osteomyelitis probe to or exposed bone, new or satellite areas of breakdown, exudate, erythema/oedema, smell.<sup>8,9</sup> Note that the most recent International Wound Infection Institute (IWII) guidelines for assessing a wound for infection have replaced the term critical colonisation with local or covert infection.<sup>1</sup> While these solutions have been found to be useful, their widespread adoption has been limited and inconsistent across the world.<sup>1</sup>

The CSS of wound infection reflect the response of the host to the presence of elevated bacterial levels in wounds along with underlying comorbidities and do not detect the bacteria themselves.<sup>1</sup> This is problematic for several reasons:

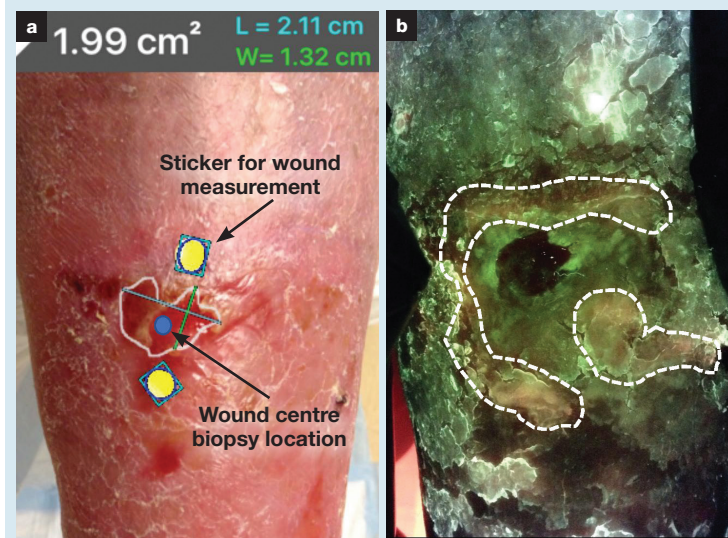
- Host response to bacteria varies widely and is often entirely absent (i.e. patient is asymptomatic)<sup>15–18</sup>
- Observation of signs and symptoms is subjective and inter-observer variability is high<sup>19,20</sup>
- Signs and symptoms, when present, fail to show the clinician where the bacteria are, as they are invisible to the unaided eye.

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**Fig 1.** Example standard mode (a) and fluorescence mode (b) images of a venous leg ulcer. Yellow calibration stickers were required for wound measurement (area of 1.99cm<sup>2</sup> outlined in white, length 'L' and width 'W' shown as green and blue lines across the wound bed). The square surrounding each sticker indicates that they have been detected by the measurement tool. Blue circle denotes the wound centre as identified by study clinician, where a biopsy would be taken. Hatched white outlines on fluorescence image denote regions of red-fluorescence identified by the study clinician



A meta-analysis of 15 clinical studies<sup>9,15,18,21–25</sup> evaluating the effectiveness of various CSS in 1056 chronic wounds found that pain was the only useful sign or symptom in diagnosing infection.<sup>17</sup> Other 'classic signs' of infection such as purulent exudate, erythema, heat and oedema had no predictive value in diagnosing infection,<sup>17</sup> which most studies classified as a bacterial load of 10<sup>5</sup> colony forming units/gram (CFU/g) or higher.<sup>15,17,18,22</sup> However, the absence of pain cannot rule out infection, and the high prevalence of diabetic foot ulcer (DFU) patients with neuropathy<sup>26</sup> greatly reduces the usefulness of pain alone as an infection predictor. Moreover, identifying wounds with bacterial colonisation presents an even greater challenge using the standard of care, as colonisation can occur in asymptomatic patients.<sup>4</sup> Gardner et al.<sup>16</sup> have shown that no individual signs of infection are adequate predictors of bacterial loads greater than 10<sup>6</sup>CFU/g in DFU wounds.<sup>16</sup> Furthermore, various combinations of individual signs and symptoms obtained using CSS checklists also have poor predictive value for identifying wounds with significantly elevated bacterial loads.<sup>16,17</sup> Real-time information about bacterial presence and location in wounds, obtained from fluorescence images, may increase the predictive value of these checklists in identifying wounds with elevated bacterial burden.

A handheld fluorescence imaging device can be used at the point-of-care to visualise tissue and bacterial fluorescence within and around wounds in real-time without contact with the patient or need for contrast agents.<sup>27–34</sup> While most wound tissues fluoresce green

(mainly due to connective tissues and other endogenous fluorophores), bacteria emit characteristic red or cyan fluorescence when illuminated by the device's safe violet excitation light (40nm).<sup>34</sup> The device visualises these red and cyan coloured bacterial fluorescence signals, which are emitted by endogenous porphyrins<sup>35–38</sup> and pyoverdines,<sup>39,40</sup> respectively. Porphyrins are a naturally occurring, red-fluorescing by-product of bacterial haem production<sup>37,38</sup> and are produced by a majority of bacterial species commonly found in wounds.<sup>41</sup> In contrast, pyoverdines are fluorophores specific to the pseudomonads,<sup>39</sup> most notably *Pseudomonas aeruginosa*, a common wound pathogen requiring early identification and tailored treatments.<sup>42</sup> Cyan-fluorescing pyoverdines are produced endogenously by these bacteria as part of the pseudomonad iron acquisition process.<sup>40</sup> Multi-centre clinical trials have previously established that the positive predictive value of red and/or cyan fluorescence observed in wounds with this handheld imaging device for detecting bacteria is 100%, i.e. no false positives were detected.<sup>33,43</sup> Based on these results, we conducted a single centre clinical trial to test our hypothesis that real-time knowledge about wound bacterial fluorescence could improve the identification of wounds containing moderate-to-heavy bacterial loads that were not evident with standard CSS wound assessment alone.

## Methods

### Trial recruitment and documentation

Adult patients presenting at an advanced outpatient wound research clinic with a variety of wound types, DFUa, venous leg ulcers (VLU), pressure ulcers (PU) and surgical wounds, of unknown infection status were consented for a prospective, single-blind, single centre clinical trial (clinicaltrials.gov #NCT03540004). Patients were excluded if they had been treated with an investigational drug within the last month, had recently (<30 days) had their wound biopsied or curettaged, used antibiotics (topical, oral or intravenous) within the previous two-weeks, were not able to consent, or had any contraindication to routine wound care and/or monitoring. Additionally, wounds that could not be completely imaged by the study device because of inaccessibility due to anatomical location were ineligible for the study.

All procedures were performed in compliance with the Declaration of Helsinki as well as local laws and guidelines. This study was approved by an external institutional review board (IRB), Veritas. All patient participants provided written consent on an IRB-approved informed consent form (ICF). An IRB-approved ICF checklist was used to ensure the patient was provided with all required study information and was given sufficient time to ask questions and make an informed decision regarding their participation. Before the trial started, study clinicians were trained on use of the device, trial procedures and conduct — as per good clinical practices — and were tested for colour blindness. Study clinicians were also provided on-site

training in fluorescence image interpretation<sup>34</sup> by the device manufacturer (MolecuLight Inc.)<sup>44</sup> and were required to pass a standardised test (passing grade:  $\geq 80\%$ ) evaluating fluorescence image interpretation before trial participation. Previously obtained fluorescence images were used for image interpretation training and testing of study clinicians and were representative of the wound types and fluorescence characteristics that study clinicians would encounter during the trial.

### CSS assessment alone

The patient and their wound were assessed for clinical signs and symptoms using the NERDS and STONEES checklist (NERDS: non-healing, exudate, red and bleeding surface or granulation tissue, debris, smell or unpleasant odour; STONEES: size is bigger, temperature is increased, osteomyelitis probe to or exposed bone, new or satellite areas of breakdown, exudate, erythema/oedema, smell).<sup>8,9</sup> Any patient exhibiting  $\geq 3$  NERDS or  $\geq 3$  STONEES was considered positive for moderate-to-heavy bacterial growth based on clinical signs and symptoms (CSS+). The information was recorded on the case report form. The wound was rinsed with normal saline and gauze, if required, after which a standard light image of the wound was taken and wound measurement obtained using the wound measurement feature on the MolecuLight i:X device (QuickSize, MolecuLight Inc., Toronto, Canada). On the standard image, the clinician electronically recorded the wound centre, where a biopsy would later be obtained for microbiological confirmation (Fig 1a). The clinician had the option to record one additional area on the standard image, suspicious for bacterial presence, where a second, optional CSS+ biopsy could be obtained. A treatment plan was devised for the wound by the study clinician and recorded on the case report form before any fluorescence imaging.

### Fluorescence image assessment

The examination room was made dark and a fluorescence image was acquired of a wound using the previously described imaging device.<sup>2,9,31,33</sup> In brief, this device emits a safe violet light (405nm) and uses specialised optical filters to capture the resulting, relevant fluorescence from tissues and bacteria.<sup>34</sup> That information appears on the device screen in real-time allowing localisation of bacterial fluorescence, which appears red (most species) or cyan (*Pseudomonas aeruginosa*).<sup>27–34</sup> Bacterial fluorescence is spectrally and visually different than background tissue fluorescence, which appears in various shades of green (Fig 1b).<sup>34</sup> When red or cyan fluorescence was identified on the fluorescence image the wound was considered positive for moderate-to-heavy bacterial growth (FL+). Specifically, the clinician was required to verbally respond 'yes' or 'no' to the question 'is there moderate or heavy levels of bacteria in the wound according to your interpretation of the fluorescence image?' and the response was recorded. If 'yes', the location of a bacterial

fluorescence positive region (i.e. red or cyan) where a FL+ biopsy would be taken was noted electronically on the image. A treatment plan, which incorporated bacterial fluorescence positive or negative results, was devised for the wound by the clinician and recorded on the case report form. Additionally, clinicians were asked after all study procedures whether the fluorescence images changed their wound assessment for each patient in any way and whether the information provided on fluorescence images improved patient care. This information was recorded.

Note that, in this clinical setting, achieving the darkness required for fluorescence imaging was achieved simply by turning off the room lights. A room with windows would require use of the device's disposable dark drape attachment or use of blackout curtains.

### Wound sampling

Up to three punch biopsies (3mm diameter) were obtained from the wound under local anaesthetic:

- CSS+ biopsy at wound centre
- Optional CSS+ from a region of particular concern
- Biopsy from a region positive for bacterial fluorescence (FL+), if applicable.

If a wound was deemed CSS– and FL–, a single control biopsy was obtained from the centre of the wound. Samples were cut to a depth of 2mm, halved along the long axis and flash-frozen before shipment to an accredited clinical laboratory for advanced microbiological analysis (RTLGenomics, Lubbock, Texas). All laboratory staff were blinded to the wound's CSS and FL assessment outcomes.

### 16S qPCR and rDNA pyrosequencing

Total bacterial load (CFU/g) was determined via quantitative polymerase chain reaction (qPCR) analysis using a primer specific to the highly conserved 16S region of all bacteria, as previously described.<sup>45</sup> The minimum limit of detection with this analysis is  $10^2$  CFU/g. Loads  $\geq 10^4$  CFU/g were considered moderate-to-heavy. Additionally, advanced 16S rDNA pyrosequencing was performed to survey all prevalent bacterial taxa present in a given biopsy, as previously described in detail.<sup>46,47</sup> The obtained bacterial sequences were processed through the RTLGenomics taxonomic analysis pipeline to determine both the taxonomic classifications (down to genus and species when possible) and the relative abundance for each sample.<sup>46,47</sup> Relative to traditional, culture-based analysis, 16S rDNA pyrosequencing yields a much larger and robust report of bacterial species present within a wound.<sup>48,49</sup> Therefore, to simplify this information, bacterial species representing less than 2% of the entire sample were not reported in this study. Sequences for which a genus could not be determined were also not reported.

### Analysis

The addition of FL+/FL– information to wound assessments was analysed in two ways:

- As an addition to the NERDS and STONEES checklist, increasing the likelihood of a patient exhibiting three or more CSS
  - CSS+ FL, in which the presence of bacterial fluorescence anywhere within or around the wound was considered positive for moderate-to-heavy bacterial loads, regardless of the CSS determination.
- True positives (TP), false positives (FP), true negatives (TN) and false negatives (FN) for CSS alone and in conjunction with fluorescence imaging were determined from microbiological assessment of biopsies. Total bacterial loads  $\geq 10^4$  CFU/g were considered microbiologically positive. Standard diagnostic accuracy measures were calculated for CSS alone and CSS in conjunction with fluorescence imaging. Diagnostic measures included accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

#### Statistical analysis

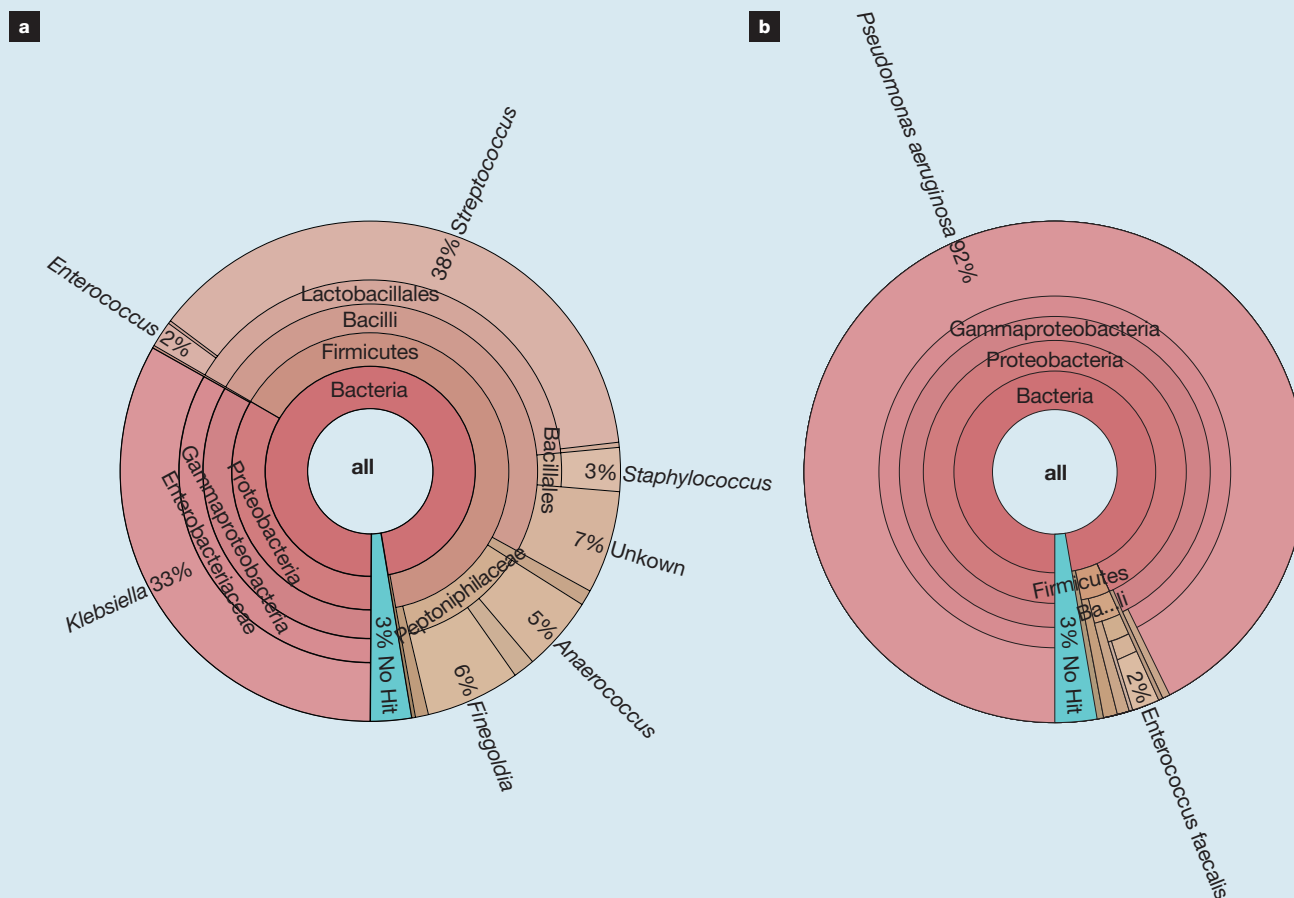
One-sided McNemar's tests were used to compare accuracy and sensitivity between the assessment methods.

Note that PPV was identical across all groups, so could not be statistically tested. Out of the 19 wounds assessed, only one wound did not have moderate-to-heavy load of bacteria, as determined by microbiology. Therefore, due to the small sample size ( $n=1$ ), statistical tests comparing specificity could not be performed. Statistical testing of NPVs was performed using the method proposed by Leisenring et al.<sup>50</sup> Note that Leisenring's asymptotic test is also limited by the small true negative sample size in this population; NPV results should therefore be interpreted with caution. All tests were performed using R statistical software (version 3.3.3). To account for multiple comparisons, a significance level of  $p \leq 0.0125$  (i.e.  $0.05/4$ ) was chosen for all tests.

#### Clinician questionnaire

In addition to the primary study outcomes, after all study procedures were complete the clinicians were asked to fill in a structured questionnaire regarding how the fluorescence imaging results supported their real-time wound care decision-making. Specifically, the case report forms required the clinician to identify the

**Fig 2.** Example of DNA pyrosequencing data is shown from two individual patient wounds. In this VLU with notable regions of red bacterial fluorescence (wound c, Fig 3), biopsy data revealed the presence of five confirmed bacterial species, as well as additional bacteria that could not be classified down to the genus or bacterial level (a). This venous leg ulcer with widespread cyan/white fluorescence (wound d, Fig 3) was predominantly monomicrobial; 92% of the bacteria present ( $10^8$  colony forming units/g) was *Pseudomonas aeruginosa* (b).



specific areas of wound care in which fluorescence imaging guided their assessment and treatment plan decision-making (e.g. cleaning, sampling, debridement, treatment selection, antimicrobial administration, documentation), whether the information in the fluorescence images increased their confidence in their wound assessment or treatment decision and whether the information provided by fluorescence imaging improved patient care overall. The questionnaire also required the clinicians to indicate whether fluorescence imaging aided the implementation of the T.I.M.E framework, a checklist used world wide to guide wound bed preparation (T.I.M.E: tissue management, infection/inflammation, moisture balance, edge of wound).<sup>51,52</sup>

## Results

### Patient population

We examined 19 patients with wounds of unknown infection or bacterial burden status (17 VLU and two DFU wounds) were enrolled in this study by two clinicians from a single centre. Patients ranged in age from 34 to 87 years old and were 58% male and 42% female. Average wound area was 10.81cm<sup>2</sup> (range: 1.29–47.27cm<sup>2</sup>) at the time of study enrolment. Wound duration before this single visit study was as follows: <3 months (16% of wounds), 3–6 months (21%), 6–12 months (21%), >12 months (42%).

### Bacterial load and diversity

qPCR analysis of biopsies found that 95% (18/19) of patients were positive for moderate-to-heavy bacterial loads ( $\geq 10^4$ CFU/g). Overall, bacterial loads in this patient group were high ( $\sim 10^6$ – $10^{10}$ CFU/g) at the time of study visit. Pyrosequencing data for 18/19 study wounds that were positive for moderate-to-heavy bacterial loads revealed 52 unique bacterial species from 39 bacterial genera across all the wounds. More than one predominantly abundant bacterial species was detected in 17/18 wounds. Examples of species and taxonomy data observed are shown in Fig 2a. Species identified in study wounds varied widely between wounds, as expected. Moreover, bacterial species varied widely from wound to wound. Only four species were observed in  $\geq 4$  wounds: *Staphylococcus aureus* (eight wounds), *Corynebacterium striatum* (seven wounds), *Propionibacterium acnes* (seven wounds), and *Pseudomonas aeruginosa* (four wounds).

### CSS assessment

Only 21% (4/19) of patients were identified as positive (for moderate-to-heavy bacterial loads) based on clinical signs and symptoms alone (Table 1). This assessment was based on exhibiting  $\geq 3$  of the symptoms on the NERDS and/or STONEES checklists. Approximately half of the patients in this study exhibited either none or one symptom, including some patients with bacterial loads of  $>10^9$ CFU/g (Table 1). This resulted in a CSS specificity of 22.2% and an accuracy of 26.3% (Table 2).

**Table 1. Patients exhibiting various numbers of clinical signs and symptoms (CSS) based on the NERDS and STONEES checklists**

| Number of signs/symptoms present | NERDS | STONEES |
|----------------------------------|-------|---------|
| 0                                | 26%   | 37%     |
| 1                                | 21%   | 32%     |
| 2                                | 32%   | 32%     |
| $\geq 3^*$                       | 21%   | 0       |

\*Wounds were considered CSS+ when patients exhibited  $\geq 3$  NERDS and/or STONEES checklist

**Table 2. Diagnostic accuracy measures of clinical signs and symptoms (CSS) with and without the addition of FL-imaging for detecting wounds with moderate-to-heavy bacterial loads**

|             | CSS alone <sup>*</sup> | FL added to CSS checklist <sup>†</sup> | CSS + FL <sup>‡</sup> |
|-------------|------------------------|----------------------------------------|-----------------------|
| PPV         | 100                    | 100                                    | 100                   |
| NPV         | 6.7                    | 11.1                                   | 16.7                  |
| Accuracy    | 26.3                   | 57.9                                   | 73.7 <sup>§</sup>     |
| Sensitivity | 22.2                   | 55.5                                   | 72.2 <sup>§</sup>     |

\*Wounds were considered CSS+ when patients exhibited  $\geq 3$  NERDS and/or STONEES checklist;  
<sup>†</sup>FL-positive status considered as an additional item on the NERDS and/or STONEES checklist;  
<sup>‡</sup>FL-positive status and CSS positive status were equally weighted in the determination of whether a wound had moderate to heavy bacterial loads; <sup>§</sup>p-value = 0.002

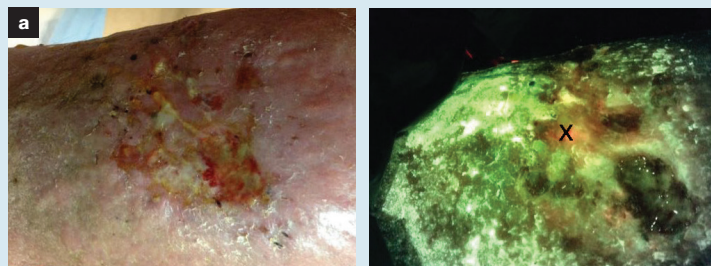
### Bacterial fluorescence imaging

Bacterial fluorescence imaging in combination with CSS assessment led to 2.5–3.2-fold improvements in reported diagnostic accuracy measures, compared with CSS assessment alone (Table 2). Example fluorescence images and their corresponding microbiology, from wounds that were CSS negative, can be seen in Fig 3. As an addition to the NERDS and STONEES checklist, information on bacterial presence from the real-time fluorescence images increased sensitivity to 55.5% (for detecting moderate-to-heavy loads) and accuracy to 57.9%. However, the large percentage of patients exhibiting only one or zero additional signs and symptoms hindered the ability of FL+ images to declare the wound positive for moderate-to-heavy growth when used in this capacity. In contrast, when bacterial fluorescence imaging (FL+ status) was given equal weighting to CSS+ status in its ability to call a wound positive for moderate-to-heavy bacterial loads, this significantly increased sensitivity to 72.2% (p=0.002) and accuracy to 73.7% (p=0.002), vastly superior to CSS alone (Table 2). Regions of bacterial fluorescence, whether red or cyan (Fig 2), resulted in positive microbiology results in all cases, i.e. no false positives were detected. Microbiology (qPCR) from bacterial fluorescence positive (FL+) biopsies yielded total bacterial loads ranging from  $10^7$  to  $10^{10}$ CFU/g. The low number of true negative wounds in this patient population ( $<10^4$ CFU/g, 1/19) prevented statistical analysis of specificity and limited ability to properly test negative predictive value. NPV results should be interpreted with caution.

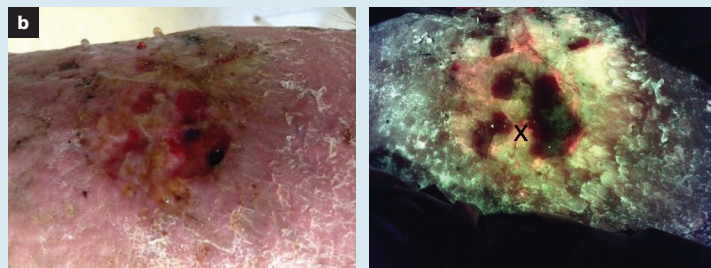
### Use of bacterial fluorescence information

Based on questionnaire data, clinicians reported that overall patient care was improved by fluorescence imaging assessment in 18/19 cases (95%). Assessment with fluorescence imaging led to identification of wounds with moderate-to-heavy bacterial loads that were missed by CSS evaluation alone (47% of study

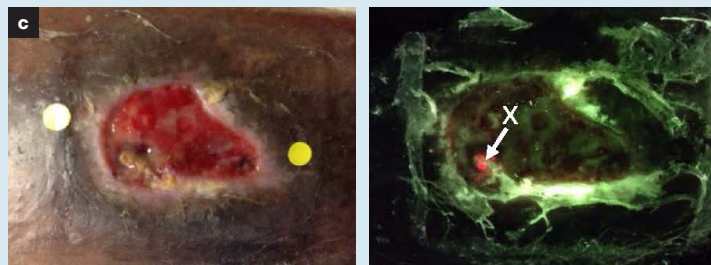
**Fig 3.** Examples of study wounds missed by CSS assessment, but were identified by fluorescence imaging as having moderate-to-heavy bacterial burden. Standard images taken during CSS assessment (left), corresponding fluorescence images (right). X denotes regions of identified bacterial fluorescence from which a biopsy was taken. Total bacterial load (qPCR) and the number of NERDS or STONEES observed are also shown.



Bacterial load:  $3.1 \times 10^7$  colony forming units (CFU)/g (*Staphylococcus aureus*, *Fingoldia* spp.); no NERDS or STONEES detected



Bacterial load:  $4.4 \times 10^8$  CFU/g (*Campylobacter* spp., *Clostridium* spp., *Porphyromonas somerae*); two NERDS detected



Bacterial load:  $1.5 \times 10^8$  CFU/g (*Streptococcus* spp., *Klebsiella* spp., *Fingoldia* spp.); three NERDS and two STONEES detected



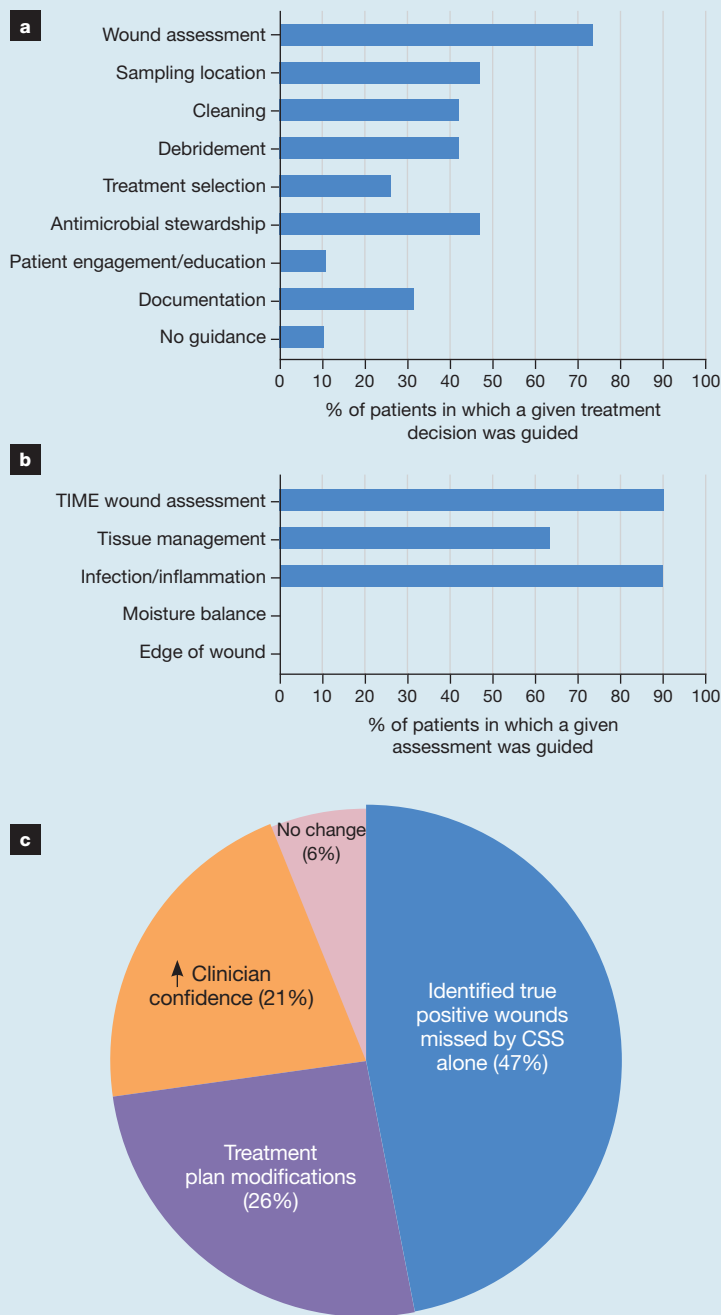
Bacterial load:  $2.8 \times 10^8$  CFU/g (*Pseudomonas aeruginosa*); no NERDS or STONEES detected

wounds), modified treatment plans for 73% of wounds, and improved clinician confidence in the original CSS (alone) assessments in 21% of wounds (Fig 4). Overall, some clinician treatment decisions for a given study participant were directly and immediately altered after seeing wound fluorescence images in 14/19 cases (73%). The specific procedures of wound care that were influenced by fluorescence image-guidance in this study are summarised in Fig 4, and include wound assessment (74%), more targeted sampling location (47%), and cleaning (42%). Antimicrobial stewardship decisions were guided by fluorescence imaging in 47% of cases; treatment plans under fluorescence guidance increased prescription of topical antibiotics in 26%, but also decreased topical antibiotic usage in 10% of study wounds. This may indicate more effective and evidence-based antimicrobial resource use and should be investigated in a larger patient population. Fluorescence imaging influenced and guided wound debridement decisions, such as method, extent, location, in 42% of cases. Fluorescence imaging prompted the use of image-guided debridement in 26% of wounds that would not otherwise be debrided based on CSS assessment alone. Moreover, fluorescence imaging prompted a change of decision from mechanical to sharp debridement (5%) and avoidance of otherwise planned debridement procedures when fluorescence images suggested it was unwarranted (10%). Based on clinician survey data, wound bed preparation (T.I.M.E. framework) was aided in 90% of wounds, primarily by tissue management (63%) and infection/inflammation (90%; Fig 4).

### Discussion

Wound bacterial management is critical, therefore evaluation of CSS to determine a wound's status along the accepted bacterial contamination-to-infection continuum is important to any wound assessment. Infection is a highly variable host response to high bacterial loads, governed largely by the innate immune response to this bacterial burden.<sup>3,10,18,19,53</sup> Many patients with infected wounds are asymptomatic,<sup>15,17,18,22</sup> and non-infected, heavily colonised wounds are supposed to be asymptomatic by definition,<sup>4,22</sup> yet they still require identification to guide appropriate treatment and prevent escalation to local infection.<sup>2-4,53,54</sup> We hypothesised that the addition of bacterial fluorescence imaging<sup>30,33</sup> to standard wound assessments using CSS checklists (NERDS and STONEES) could improve identification of wounds with moderate-to-heavy bacterial loads, identifying those wounds that would otherwise have received improper treatment in the absence of real-time bacterial visualisation. Study data revealed a threefold increase in sensitivity when bacterial fluorescence imaging was added to the clinician's traditional assessment process. This resulted in the identification of wounds with moderate-to-heavy bacterial loads that were missed by CSS alone (47% of study wounds), treatment plan modifications for 73% of study wounds, increased clinician confidence, and

**Fig 4.** Clinician-reported clinical use questionnaire. Clinicians reported for each patient whether bacterial fluorescence images guided various care decisions during wound assessment, treatment, and overall care (a). Clinicians reported whether bacterial fluorescence images guided the T.I.M.E. wound assessment framework for wound bed preparation (b). Pie chart showing how bacterial fluorescence images influenced wound assessment and treatment plans in the 19 patients (c)



questionnaire-reported usefulness in 90% of study wounds for procedures including assessment, wound bed preparation, treatment selection, debridement and antimicrobial stewardship. Results suggest that

handheld, point-of-care bacterial fluorescence imaging offers unique, real-time, bacteria-specific information (otherwise invisible to the unaided eye).

The data in this pilot study suggest that current standard of care assessment for wounds fails to identify many wounds with moderate-to-heavy bacterial loads, leaving patients with undetected, untreated bacterial burden due to delayed and often inappropriate treatment regimens. This finding agrees with numerous studies describing the inherent limitations of current algorithms for diagnosis of infection in chronic wounds.<sup>17,18,22</sup> A meta-analysis of CSS effectiveness in >1000 wounds across 15 studies found that most classic signs did not predict the presence of infection.<sup>17</sup> Moreover, no individual signs of infection are a significant predictor of bacterial loads of  $\geq 10^6$  CFU/g,<sup>16</sup> nor do any individual signs have significant discriminatory capability for detection of these heavy bacterial loads.<sup>16</sup> Indeed, many of these signs fall below the line of chance in terms of discriminatory capability.<sup>16</sup> Still, the sensitivity of CSS (22%) in this study was lower than some previous reports<sup>9</sup> and, perhaps more pertinent, is much lower than many clinicians surveyed believe it to be. Clinician confidence in CSS effectiveness, despite evidence to the contrary,<sup>15,17,18,22,23,55</sup> prevents progress on the unsolved wound assessment problem.<sup>16,56</sup> Clinician confidence in CSS assessment may stem from the tendency for CSS sensitivity values to be exaggerated by the 'clinical trial' setting, in which patients receive a level of care typically above and beyond that of a standard clinical setting.<sup>55</sup> The effectiveness of CSS alone reported in this study is more in line with its effectiveness in standard clinical practice.<sup>17,18,55</sup> Some studies have reported CSS sensitivities as low as 3% in chronic wound populations<sup>18</sup> and meta-analysis revealed that sensitivity of CSS in chronic wounds is often less than 20%.<sup>17</sup> It should be noted that the microbiological sampling and advanced microbiological analysis metrics (qPCR and DNA pyrosequencing) used in this study may have contributed to a low CSS sensitivity. Swab-based sampling and culture-based analysis, used in most wound care studies, are more prone to false negatives (poor bacterial detection on culture) than the gold-standard biopsies and qPCR microbiological analysis used in this study.<sup>48,49</sup> Poorer bacterial detection abilities such as culture analysis would artificially inflate CSS effectiveness in a heavily contaminated population. The single-visit nature of this study may have also impacted CSS effectiveness. Study clinicians assessed patients for CSS, rather than their regular clinician who would have known more about the patient's symptoms over time. Several of the CSS checklist items are more effectively assessed by studying their relative change over time.<sup>18</sup> However, given the agreement with previous meta-analysis,<sup>17</sup> the reported sensitivity of CSS in this study's heavily bioburdened population (at study visit) appears to

correctly depict a single-visit assessment under the current standard of care.

The addition of bacterial fluorescence information by way of real-time imaging, improved sensitivity and accuracy of assessments for detecting moderate-to-heavy bacterial loads. There is a growing body of evidence to support the use of point-of-care bacterial fluorescence imaging to identify chronic wounds,<sup>31,57-59,30,32,60</sup> burns,<sup>27,29,61,62</sup> surgical<sup>27</sup> and traumatic wounds.<sup>27,28,62</sup> These studies, representing more than 200 imaged wounds, largely concluded that fluorescence information improved detection of clinically-concerning levels of bacteria, thus empowering evidence-based treatment decisions including treatment selection<sup>28,30,57,62-64</sup> and antimicrobial stewardship practices.<sup>62,63</sup> In addition to earlier detection, bacterial fluorescence images provide information on the location and extent of bacterial loads within or around a wound. This information enabled treatment to be targeted to specific regions of the wound, for example debridement of colonised tissues while leaving non- or lesser-colonised tissues intact. In the present study, fluorescence information guided debridement-based treatment decisions in 63% of wounds. Targeted treatment to areas of bacterial fluorescence could include expanding the region over which the wound is cleaned or over which a topical antimicrobial is applied. This could target periwound regions of bacterial fluorescence which otherwise may have gone untreated, for example the bacterial fluorescence surrounding the wound in Fig 1.

The health economic implications of earlier and more accurate detection of high bacterial burden will surely be a topic of active research in the coming years. A pilot study demonstrated potential for faster wound area reduction when fluorescence guided care was used over a seven-week period, compared with a period of standard of care alone,<sup>30</sup> which would greatly reduce total wound care expenditure. Preliminary health economic modelling of the data in the current study predicted progression of each of the 19 patients within the IWII bacterial load continuum<sup>1</sup> based on Medicare chronic wound progression and expenditure data (from 2252 chronic wound patients).<sup>6</sup> This work reported that earlier identification of concerning bacterial loads, and the resulting earlier and more appropriate interventions, would likely prevent long-term expenditures associated with spreading and systemic infections (e.g. hospitalisation).<sup>65</sup> Indeed, a recent article reported cost savings of £3500 in a single patient when fluorescence images found an otherwise undetected region of pus and contamination in an amputated stump about to be grafted.<sup>62</sup> Future studies should be planned to directly determine the health economic potential of this fluorescence device.

### Limitations

The interpretation of findings of this single centre study need to be considered in the context of study limitations. Due to the pilot, exploratory nature of this study, the sample size was small and the study was not statistically

powered. The single visit nature of the study prevented follow-up to determine whether fluorescence-guided decisions had an effect on longer-term clinical outcomes. Moreover, the percentage of enrolled patients that were true negatives based on microbiology (bacterial load  $<10^4$  CFU/g, 1/19 patients) was surprisingly low, preventing statistical testing of specificity and challenging the ability to determine NPV in this patient cohort (though these have been reported in other studies; recent reports of NPV have ranged from 90 to 100%).<sup>27,60,61</sup> This may have been due, at least in part, to the study's exclusion of patients on antibiotics. Patients actively taking systemic or oral antibiotics would be anticipated to have low bacterial loads. Future, larger sample size studies should consider including these patients to achieve the true (microbiology) negatives required for more comprehensive diagnostic accuracy testing. However, the lack of true negatives may also have been due to the study being conducted at a single site and the fact that many of the study participants did not receive regular wound care. This is likely to have selected for patients in the study population who were microbiologically positive patients for heavy bacterial burden. However, it does not explain the ineffectiveness of CSS for detecting bacterial loads in this population. Future studies should evaluate relative effectiveness of CSS in combination with fluorescence imaging across multiple sites and geographic locations, to increase heterogeneity of the study clinicians and patient populations and therefore reduce the possibility of any single-site bias.

Recognising the new knowledge about the patient population and study design learned from this pilot study, a larger, multi-centre clinical trial is currently underway to address the current study's limitations. We anticipate the results of that larger study to be reported in late 2019.

### Conclusions

In summary, data from this pilot trial demonstrate that point-of-care bacterial fluorescence imaging using the handheld imaging device, when used in combination with standard of care CSS, can improve sensitivity and accuracy of wound assessments in detecting moderate-to-heavy bacterial loads in real-time. This simple to use imaging tool was found to be useful by study clinicians for assessment and treatment of a wide variety of chronic wounds, including image-guided debridement, sampling, cleaning, treatment selection and antimicrobial administration. Fluorescence image-interpretation was rapid and simple and the device integrated into the clinical workflow well. Evidence from this study demonstrates that it is possible to produce patient-centric treatment plans in real-time using bacterial fluorescence. By enabling clinicians to visualise bacterial burden in wounds during routine assessment and treatment procedures, this novel technology helps them to make more objective decisions which can be documented, thus helping to address important limitations in conventional wound care. **JWC**

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#### Reflective questions

- How do you currently determine whether a wound has moderate-to-heavy bacterial loads and is that method effective?
- What are the current challenges with standard of care wound assessment and how can these be tackled?
- How would you apply real-time fluorescence information on bacterial presence and location into your clinical wound assessment and treatment?

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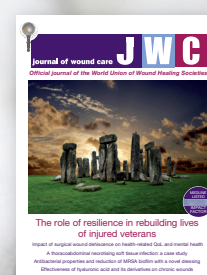


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