Title: A single-centre, prospective cohort study assessing the proprietary Glycologic test kit to detect bacterial infection in diabetic foot ulcers; prospects for point-of-care testing..

Running title: Point-of-care infection testing for DFU

Authors: Leon Jonker, Danielle Smith, Emma Mark, Jose Schutter, Sarah Thornthwaite, Shona Johnston**.**

Abstract

Aims. To appraise the performance of a new point-of-care wound infection detection kit in diabetic foot ulcers (DFU), using clinician opinion as the primary comparator. The proprietary swab-based chromatic Glycologic detection kit is designed to detect host-response to pathogenic levels of bacteria in wounds.

Methods. In high-risk podiatry clinics, 136 DFU patients were recruited and Glycologic test result compared to initial clinician opinion. Chi-squared test, principal component analysis (PCA) and multiple regression analysis were performed to determine which variables are possibly associated with infection. Variables were patients' wound parameters, wider vascular co-morbidity, and demographics.

Results: Total agreement in terms of DFU wound assessment for infection – between podiatrists' clinical opinion and Glycologic kit test result - was observed in 79% of cases (301 out of 383 wound assessments), whereas podiatrists identified more (possible) infections than the Glycologic kit (55 [15%] vs 14 [4%] swabs respectively). Regression analysis and PCA showed that clinical signs of wound infection, namely erythema, purulence, and odour, are all significantly associated with both a positive clinical opinion and Glycologic test result.

However, in case of the Glycologic kit a patient's number of lesions and vascular comorbidities are also significantly correlated with a change in colour.

Conclusion: A host-response to critical pathological levels of bioburden in a wound – as detected with the Glycologic test kit – may partly be determined by an individual patient's (vascular) health and therefore be person-specific. Further research is indicated to determine the relationship between a Glycologic test result and the microbiological status of the wound.

Acknowledgements:

We are grateful to the North, East and West Cumbria podiatry teams at Cumbria Partnership NHS Foundation Trust for accommodating and supporting the study.

Conflicts of interest: None of the authors have any conflict of interest to declare**.**

Sources of funding: A non-restricted research grant, through provision of the Glycologic test kit, was received from Glycologic Limited to conduct this study.

Background

Diabetic foot ulcers (DFU) are a common complication of diabetes and have enormous cost implications, totalling £650 million per year once associated morbidity is taken into account.[1] Bacterial infection of wounds carries the risk of further degenerative complications including cellulitis, necrotising fasciitis, and sepsis; it may lead to amputation if osteomyelitis develops.[2] An additional undesirable effect of infection of wounds is that it delays – or stops altogether – the wound healing process.[3]

Detection of DFU infection remains predominantly reliant on clinical judgement. O'Meara and colleagues[4] concluded from a systematic review on clinical examination, sample acquisition and sample analysis in DFUs that there is a lack of evidence regarding what samples should be taken and how they should be analysed. They also suggested that semi-quantitative sample analysis could be a useful alternative to quantitative analysis. Quantitative detection of infection is relatively costly and labour intensive[5]; it is still predominantly undertaken by swabbing the wound, rather than arguably superior tissue sampling[6], and then culturing the pathogens in a microbiology laboratory. Obtaining these results generally takes days, and even molecular profiling does not give an instant result, which hampers clinicians in making in an instant informed decision on wound management; instead, recommended first line antibiotic therapy may be initiated without knowing the sensitivity results. Microbiological counts and species identification – though used for sensitivity testing if there is indeed an infection present - do not necessarily reflect infection as defined by other assessments, as demonstrated by Gardner et al.[7]

Clinical guidelines stipulate that the only available laboratory-based diagnostic option, microbiological testing, should only be used to identify the pathogen strain in clinically

confirmed infection. Therefore, clinical opinion is the mainstay of the initial diagnosis of infection.[8,9] The lack of a simple cost-effective and repeatable point-of-care testing method may have three consequences: 1) potential for lack of uniformity in diagnosis, due to differences in clinical judgement, which in turn may result in 2) over-diagnosis of infection with inappropriate prescription of antibiotics or antimicrobial dressings, or 3) under-diagnosis and subsequent late presentation of patients with systemic signs and spreading cellulitis or osteomyelitis requiring hospital admission and treatment with intravenous antibiotics or emergency surgery. The provision of a rapid, reliable, accurate and relatively low-cost point-of-care infection detection test kit could potentially provide cost savings as well as improve clinical outcomes for patients.

Glycologic Ltd has developed a proprietary swab-based chromatic point-of-care test, in which a clear solution changes to either yellow or orange within a 10 minute incubation of the wound swab at room temperature. Although the company is not in a position to disclose the exact mechanism of action at this point, the reaction involves a patient-derived molecule implicated in host-response to a pathological infection and not the microorganisms present in the wound. This therefore does not necessarily mean the quantity/reactivity of this molecule is linearly correlated with the quantity of bioburden in a wound. In this study we appraise the performance of the Glycologic infection detection kit in terms of comparison of outcomes when compared to clinicians' opinion of DFU infection and results obtained with the kit.

Patients & methods

Study design and patients

This concerns a controlled, non-randomised, single-blinded, prospective comparative study of the Glycologic detection kit versus clinical judgement of DFU infection status, carried out between July 2017 and August 2018. The study was conducted within a single NHS Trust and podiatry team, but across seven different community care locations. The podiatry team consists of more than 20 members of staff, each of whom were involved in the clinical care and study's infection appraisal process; the vast majority of staff have a minimum of ten years of experience as a podiatrist managing DFUs. Full approval was obtained from the national research ethics service (ref 17/LO/0703), health research authority, and hosting NHS Trust. Written informed consent was obtained from all participating patients, in accordance with the Declaration of Helsinki. Those eligible were diabetic patients aged 18 years or older, having mental capacity to provide informed consent, with a clinical diagnosis of DFU that was being cared for by the podiatry service.

A pragmatic approach was taken to allow the appraisal of the Glycologic detection kit in parallel to standard podiatry care of the DFU. As such, a cohort of 136 patients was seen at baseline and at a second visit approximately one week after the first baseline visit. The first 37 patients were seen for a further three samplings/visits, each one circa one week apart from one another; this follow-up number was reduced to allow recruitment and sampling of a larger cross-section of patients in view of planned multiple regression analysis. If an infection was identified whilst any participant was enrolled, through either clinician opinion or Glycologic swab result, they were followed up for up to ten visits maximum or less if the wound was no longer infected (as determined by clinical opinion), had healed or the patient

had to be referred to a specialist such as vascular surgery. Infected wounds were managed according to local clinical guidelines, with flucloxacillin prescribed as first line antibiotic therapy where possible and referral to tertiary services if required.

Study dataset

At each clinic visit, the podiatrist recorded whether they felt the wound was not infected, possibly infected, or infected. A clear definition of these three options was not given (i.e. they were not operationalised), since we wanted podiatrists to draw their own conclusions based on their assessment of the DFU wound; this is similar to standard clinical practice where predefined criteria are also not present. The podiatrist recorded this opinion *prior* to conducting the Glycologic test and was therefore essentially blinded to the test result. The order of events was therefore: a) podiatrist observes DFU and forms opinion on infection status, b) podiatrist takes wound swab for Glycologic test, c) researcher adds swab to Glycologic test tube and takes photos at 0 and 10 minutes, d) researcher and podiatrist agree on colour of Glycologic test result at 10 minutes. The following wound characteristics were recorded individually for each patient, as determined by the podiatrist: erythema around DFU (-/+/++, indicating absence, mild-moderate presence, and moderate-severe presence), purulence (-/+/++, grading as for erythema), odour (none, low, moderate, high degree of odour), patient perception of DFU pain (linear visual display score between 0 and 10, with supporting emoticon faces). Patient demographics such as age, sex and BMI, and wound data such as chronicity, number of lesions, ulcer location, ulcer recurrence (same location, at least four weeks after being defined as healed), ulcer-related pain and use of prophylactic antibiotics (not necessarily prescribed for the index wound, and always instigated prior to enrolment into this study) were also recorded. Other variables that were

collated included smoking status, alcohol consumption, and presence of neuropathy (evidenced by monofilament test). Furthermore, vasculature-related co-morbidities were recorded and graded, with the most severe co-morbidity defining the grading. Their presence was based on confirmatory medical notes by GPs and hospital specialists. Classed as mild-moderate were type I diabetes, hypertension, chronic kidney disease (CKD) up to and including stage 3, retinopathy, varicose veins, atrial fibrillation, history of deep venous thrombosis, heart failure. Moderate-severe co-morbidities were history of myocardial infarction, stroke or transient ischaemic attack, CKD stage 4 or higher, peripheral vascular disease, history of amputation. This practical approach was taken to minimise the total number of variables to be included in inferential statistical analyses; a comprehensive published comorbidity score was not applied since the focus is on vascular health and not all non-vascular comorbidities were known for all patients.

Since this study sought to appraise the performance of the Glycologic detection kit versus standard clinical judgement, overall management of the DFU, including microbiology testing of samples, use of off-loading, dressings, bandaging and antibiotic use was decided by the treating podiatrist and not influenced by the additional Glycologic test.

Glycologic detection kit

At present, the Glycologic infection detection kit is very similar in size and design to a standard CE-marked sterile swab used to obtain e.g. a wound exudate sample. The device contains two separate reagents, one in the clear plastic vial end and the other in a foil-sealed compartment (seen in blue in Figure 1). Although the kit is stored between 2 – 8°C, which was monitored throughout this study, shortly before use it is brought up to room temperature to allow any reaction to take place at ambient temperature. In this study, this

meant 10 minutes at room temperature. The sterile swab with the wound exudate sample is pushed into the device, breaking the foil-sealed compartment (which has foil at the top and bottom and therefore both solutions are sterile before used for diagnostics) and allowing the reagents to mix with the sample. The Glycologic diagnostic kit contains a substrate that is acted by a host factor contained in the patient's wound exudate through a biochemical reaction. The 10 minute cut-off incubation period is designed to distinguish by a developing or diminishing state of infection (yellow result), and an active infection (orange-red result). If the test was to be incubated for longer then: a) a yellow result will eventually turn orange-red, and b) the point-of-care approach would be lost due to slower turnaround time. In clinic, the Glycologic detection kit is applied as follows: first, for comparison, a baseline 0 minute photo is taken of the detection kit. Then, following initial debridement of the wound (including removal of any biofilm, and use of saline) a sterile swab is used to sample the wound according to the method described by Levine and colleagues.[10] The middle of the wound is targeted and the sampling should be from surface of the wound, rather than exudate only. Care needs to be taken to avoid sampling (fresh) blood. The swab is then pierced through the two foil layers to mix the reagents; the swab is gently swirled for five seconds and then left to incubate for 10 minutes at room temperature, after which a photo is immediately taken. Depending on the degree of host response to infection, the solution can turn from colourless to either yellow or orange.

Figure 1. Glycologic detection kit

The Glycologic detection kit (top) is very similar in size and design to a standard swab kit (bottom).

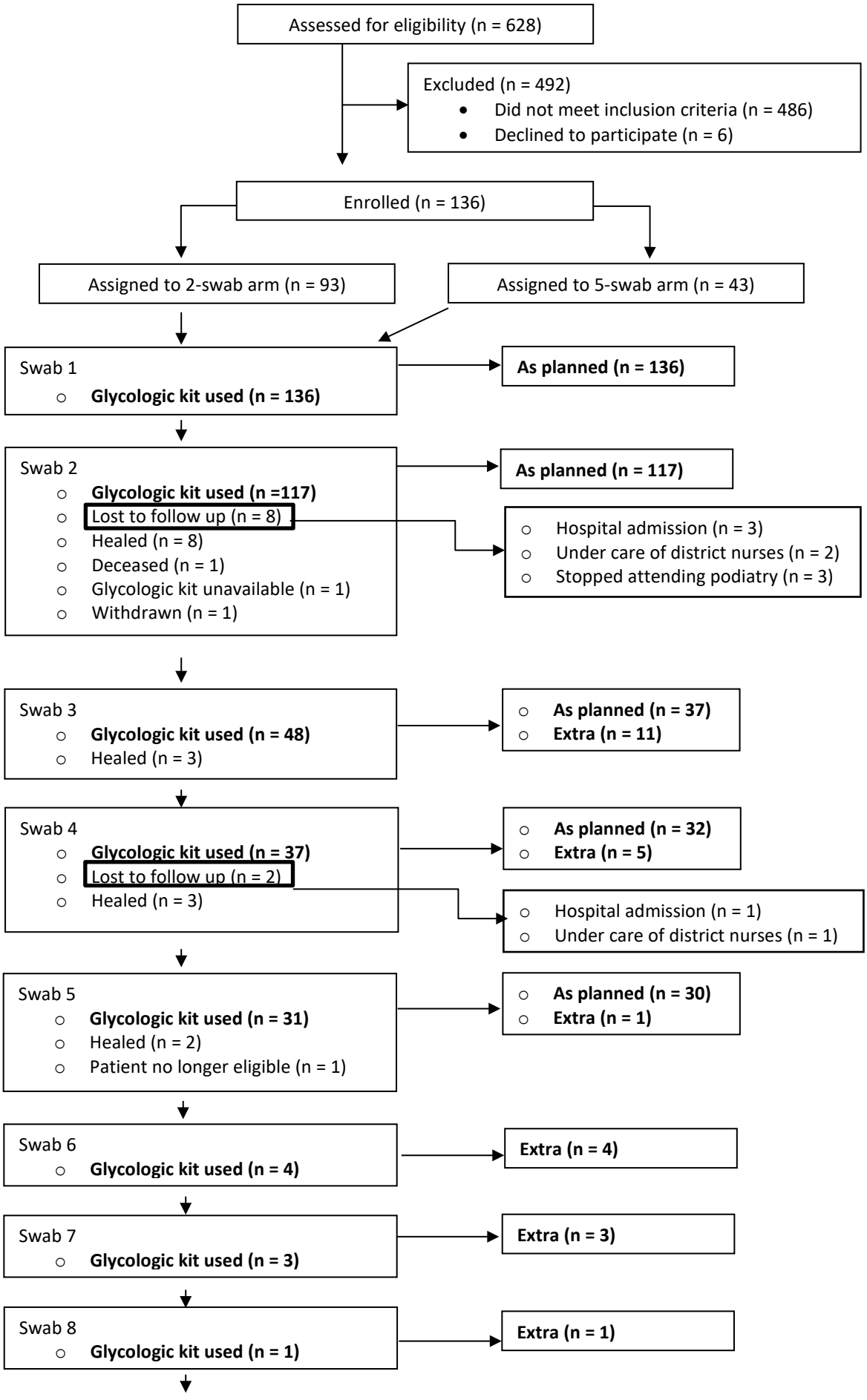
Statistical analysis

The primary outcome, presence and distribution of (potential) bacterial infection was used for sample size calculation. Based on one publication, incidence of infection is 9% over a two-year period.[11] Therefore an approximate infection incidence of between 5% ('opinion 1') to 9% ('opinion 2') – with a possibly infection rate for both of 10% - was assumed. A power calculation to achieve 80% power and 5% significance, based on two-sided Chi-squared test, results in a sample size of 380 samples. Descriptive statistics were used to parameters collated at baseline, such as wound characteristics and demographics. Four inferential tests were deployed to explore the relationship in outcomes between clinical judgement, Glycologic kit detection testing, and wound infection status: Chi-squared test (cross-tabulation of clinical opinion and kit test results); logistic regression analysis and Cox regression analysis – using swab number as time variable for the latter – when the dependents were either clinical opinion (no infection vs possible/definite infection) or Glycologic test result (negative results vs yellow/orange results); and principal component analysis (PCA). To make the outcome the dependent outcome binary, possible and definite infection, and also yellow and orange colour change, were pooled and compared to a negative result. All data was first collated in Microsoft Excel before analyses were conducted using SPSS v20. For inferential statistical analyses, a p-value < 0.05 was considered significant.

Results

The 136 consented patients provided a total of 383 DFU wound swabs. The number of participants per number of eventual swabs was as follows: one swab (n = 19); two swabs (70); three swabs (11); four swabs (4); five swabs (28); six swabs (1); seven swabs (2) and ten swabs (1). Figure 2 shows a flowchart overview of how many patients were considered for the study, consented, and how many swabs were taken. Deviation from the planned two or five swabs happened due to loss to follow-up of patients and a positive clinical opinion or Glycologic kit result triggering a longer follow-up. In total, 628 patients who attended the clinics were considered for the study, of which 486 patients were not eligible (reasons include non-diabetic, no ulcer) and 6 eligible patients did not agree to take part. For enrolled patients who had multiple wounds, the largest wound was considered the index wound and the same wound was swabbed whilst the patient was in the study. Incorporating the Glycologic swab test proved relatively straightforward, since the DFU can be cleaned and re-dressed whilst the Glycologic incubation takes place. To allow the Glycologic tubes to stand upright and be photographed, a bespoke 3D-printed stand was produced. Table 1 summarises the patient demographics for the study participants, whereas Table 2 focuses on the wound-related characteristics for each participant.

Figure 1, Flowchart overview depicting number of patients, participants and swab sampling.


Assessed for eligibility (n = 628)
Excluded (n = 492)
Did not meet inclusion criteria (n = 486)
Declined to participate (n = 6)
Enrolled (n = 136)
Assigned to 2-swab arm (n = 93)
Assigned to 5-swab arm (n = 43)
Swab 1
Glycologic kit used (n = 136)
As planned (n = 136)
Swab 2
Glycologic kit used (n =117)
Lost to follow up (n = 8)
Healed (n = 8)
Deceased (n = 1)
Glycologic kit unavailable (n = 1)
Withdrawn (n = 1)
As planned (n = 117)
Hospital admission (n = 3)
Under care of district nurses (n = 2)
Stopped attending podiatry (n = 3)
Swab 3
Glycologic kit used (n = 48)
Healed (n = 3)
As planned (n = 37)
Extra (n = 11)
Swab 4
Glycologic kit used (n = 37)
Lost to follow up (n = 2)
Healed (n = 3)
As planned (n = 32)
Extra (n = 5)
Hospital admission (n = 1)
Under care of district nurses (n = 1)
Swab 5
Glycologic kit used (n = 31)
Healed (n = 2)
Patient no longer eligible (n = 1)
As planned (n = 30)
Extra (n = 1)
Swab 6
Glycologic kit used (n = 4)
Extra (n = 4)
Swab 7
Glycologic kit used (n = 3)
Extra (n = 3)
Swab 8
Glycologic kit used (n = 1)
Extra (n = 1)

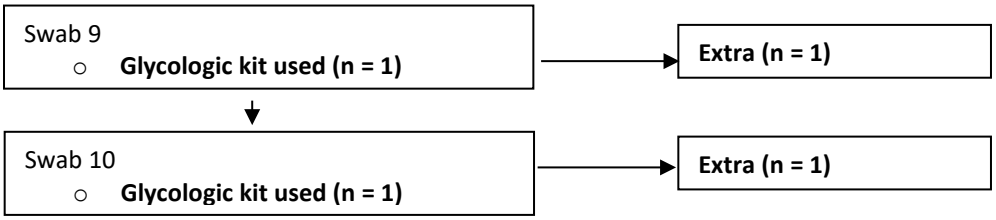


Table 1, Patient demographics

variable	**Mean; 95% CI (SD)**
Age, years	70; 67-72 (14)
Height, m	1.72; 1.70-174 (0.10)
Weight, kg	93; 89-98 (25)
BMI	31; 30-32 (7)
Alcohol consumption, U/week	4.2; 2.5-59 (9.9)
	n [%]
Smoking status	Never: 63 [46%] Ex-smoker: 52 [38%] Current: 21 [15%]
Sex	Male: 92 [68%] Female: 44 [32%]

Table 2, Ulcer characteristics and vascular health status

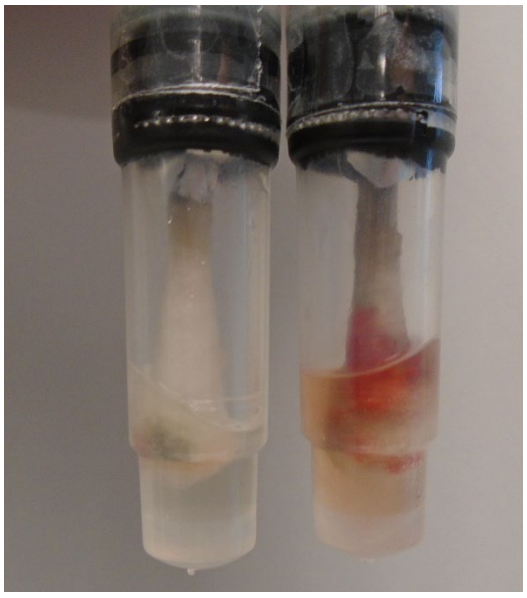
Item	**n [%]**
Ulcer location (calcaneus, digital, lateral, plantar, dorsal, n [%])	Calcaneus: 19 [14%] Digital: 70 [51%] Lateral: 14 [10%] Plantar: 25 [18%] Dorsal: 8 [6%]
Ulcer duration	16; 8-24 (47)*
Ulcer recurrence	Yes: 44 [32%]
Number of lesions	One: 102 [75%] 2-3: 32 [24%] >3: 2 [1.5%]
Systematic prophylactic antibiotics use	No: 109 [80%] Yes: 27 [20%]
Neuropathy	No: 79 [58%] Yes: 57 [42%]
Vascular co-morbidities	None: 8 [6%] Mild-moderate: 68 [50%] Moderate-severe: 60 [44%]

*mean weeks; 95% CI (SD)

The distribution of clinical opinion versus Glycologic test outcome was compared and analysed with the Chi-squared test. Table 3 shows that there is a significant difference in distribution of the two outcome measures. Overall agreement was seen in 301 (78.6%) of wound swab cases. An outcome of either possible infection or definite infection was concluded more often by podiatrists when the corresponding Glycologic result was negative, when compared to vice versa. Numerically, this meant that 56 [14%] of clinical opinion samples were (possible) infections and 14 [3.7%] of the Glycologic test result were positive through chromatic colour change. For the clinical opinion sample this is higher than some published rates[11,12] and similar to a more recent publication[13] but repeat swabs on the same patients contribute to an increased rate in positive results. Figure 3 show a representative example of a yellow and orange outcome with the Glycologic test kit after incubation for 10 minutes.

Figure 3, representative results with Glycologic test kit

No chromatic change (left) and chromatic change to orange after incubation (right)



Cox multiple regression, using the swab number as the time reference, and multiple logistic regression analyses were performed for both clinical opinion and Glycologic result as the dependent. Backwards likelihood ratio elimination was applied to determine which variables – listed in the Methods section - were most-significantly associated with a change in clinical opinion or Glycololologic test result. An increase in the number of lesions (p-value and severity of vascular co-morbidities were positively significantly associated with a positive Glycologic result, but not with the clinical opinion result. Conversely, females, smokers and high DFU pain scores showed a negative association with a positive Glycologic result. The variable 'patient height' produced aberrant results – an odds ratio of virtually zero - and was excluded. Clinical signs such as erythema, purulence and odour are significantly associated with both outcome measures, as outlined in Tables 4 and 5. Due to the applied backward elimination process, non-significant associations are not shown. Prophylactic antibiotic use was not associated with a positive Glycologic result (HR 1.66 , 95% CI 0.57-4.80, p-value 0.35) nor clinical opinion for the presence of infection (HR 1.59 , 95% CI 0.55-4.66, p-value 0.40) , and was therefore eliminated in the Cox multiple regression analysis presented in Table 4.

Prior to performing PCA, the factorability of the dataset was assessed. The Kaiser-Meyer-Olkin measure of sampling adequacy indicated that the strength of the relationships among variables was moderate (KMO = 0.51), and Bartlett's test of sphericity was significant (χ^2 (153) = 2518.59, $p<0.001$), indicating that the data were suitable for PCA. Parallel analysis recommended that six components be extracted from the data.[14] A PCA was performed using varimax rotation, with the six components explaining 59.84% of the variance. Given the fairly large sample size, items were considered to load onto a component if their loading

was ≥ 0.32; all items had primary loadings over this value. [15] One item had a cross-loading above 0.32 ("glycologic colour change"). The loading matrix is presented in Table 6. Labels have been provided for the 6 components, based on an interpretation of the items that constitute them. One item in particular had strong cross-loading between factors ("glycologic colour change"). The first component was termed "Signs of infection", the second "Lifestyle", the third "Physical patient profile", the fourth "Lifestyle and wound profile", the fifth "Patient's vascular health" and the sixth "Ulcer duration and antibiotics use".

Table 3, Clinical opinion and Glycologic test outcome; comparison of respective results

Clinical opinion	**Glycologic test result**		
	Clear, n [%]	Yellow, n [%]	Orange, n [%]
Not infected, n [%]	293 [77%]	13 [3.4%]	1 [0.3%]
Possibly infected, n [%]	33 [8.6%]	5 [1.3%]	3 [0.8%]
Infected, n [%]	23 [6.0%]	9 [2.3%]	3 [0.8%]
p-value < 0.001 (Chi-squared test)			

Table 4, Cox regression analysis, with Glycologic test result or clinical opinion as the dependent and swab number as time reference

Variable	**Hazard ratio (HR)**	**95% CI**	**p-value**
Dependent: Glycologic test result			
Sex	0.063	0.01 – 0.41	0.004
Weight	1.019	1.00 – 1.04	0.046
Smoking status	0.46	0.25 – 0.87	0.016
Number of lesions	4.18	2.06 – 8.48	<0.001
Vascular co-morbidity	2.70	1.12 – 6.54	0.028
Erythema	3.76	2.10 – 6.74	<0.001
Odour	1.92	1.25 – 2.96	0.003
DFU pain scale score	0.74	0.61 – 0.89	0.002
Dependent: clinical outcome			
Erythema	4.23	2.90 – 6.16	<0.001
Purulence	1.42	1.00 – 2.01	0.049

CI = Confidence interval. Backwards stepwise elimination of non-significant variables.

Table 5, Binary logistic regression analysis, with Glycologic test result or clinical opinion as the dependent

Variable	Odds ratio (OR)	95% CI	p-value
Dependent: Glycologic test result			
Number of swabs	1.58	1.22 – 2.05	<0.001
Patient age	0.94	0.90 – 0.99	0.023
Sex	0.025	0.003 – 0.22	0.001
Weight	1.02	1.00 – 1.05	0.047
Smoking status	0.35	0.15 - 0.79	0.011
Number of lesions	10.42	3.82 – 28.37	<0.001
Vascular co-morbidity	2.43	0.96 – 6.13	0.061
Erythema	4.32	1.92 – 9.69	<0.001
Purulence	2.96	1.16 – 7.57	0.023
Odour	2.20	1.20 – 4.02	0.010
DFU pain scale score	0.74	0.58 – 0.94	0.012
Dependent: clinical outcome			
Sex	4.23	1.29 – 13.86	0.017
Alcohol consumption	1.04	1.00 -1.07	0.039
Erythema	9.84	4.85 -19.96	<0.001
Purulence	4.55	2.21 -9.34	<0.001
Odour	2.83	1.28 -6.27	0.010
DFU pain scale score	1.22	1.07 -1.38	0.002
Glycologic result	3.32	1.28 -8.58	0.014

CI = Confidence interval. Backwards stepwise elimination of non-significant variables.

Table 6, Principal component analysis – rotated component matrix (0.32 as cut-off) showing the six identified components where factors showed an interaction with each other

	Component					
Variable	1 Signs of infection	2 Lifestyle	3 Physical patient profile	4 Lifestyle and wound profile	5 Patient's vascular health	6 Ulcer duration & antibiotics use
clinical opinion	0.84					
erythema	0.74					
purulence	0.67					

odour	0.63					
glycologic colour change	**0.46**				**0.44**	
BMI		0.92				
weight		0.89	0.36			
alcohol consumption		-0.38	0.35	0.32		
sex			-0.90			
height			0.85			
neuropathy				0.67		
patient age				-0.59		
pain scale score				-0.58		
number of lesions					0.73	
vascular co-morbidity					0.53	
number of swabs				0.39	-0.41	
ulcer duration						0.72
antibiotics use						-0.68

Discussion

In this study the performance of a novel point-of-care infection detection kit, which measures host-response to pathogenic levels of bioburden and displays its result through a chromatic colour change, was appraised in patients with DFU and compared to clinical opinion regarding the presence of wound infection. The podiatrists based their opinion on the presence of recognised clinical signs of infection. On the other hand, a positive result from the Glycologic kit is reliant on an active host response (i.e. internal cell signalling) to the presence of bacteria in a wound. Therefore, the clinical opinion utilised in this study works to the recommended diagnostic approach to detecting diabetic foot infection[16,17,18]. The Glycologic test correlates with clinical signs of infection too, but also to other factors (vascular comorbidity and number of DFU lesions). Pain, the single component associated with prediction of clinical infection of chronic wounds in a meta-analysis conducted by Reddy and colleagues[19], produced conflicting results in our study. This may be due to the

presence of neuropathy in some patients, making the DFU less painful compared to for those patients who do not have neuropathy - even if the wound is infected. The Glycologic kit substrate is not designed to detect or predict pain levels in patients.

The key differences between clinician opinion and the Glycologic test result are: clinicians identify an infection more often than the Glycologic kit; the Glycologic kit sometimes identifies an infection where the clinician does not, and vice versa; and a positive result for the Glycologic kit is associated with poorer vascular health, in addition to established clinical signs of infection. The results in this study indicate that it is not necessarily the recurrence or chronicity of a wound, or a patient's lifestyle, that increases the risk of wound infection, but rather a poorer (vascular) health status. This corroborates with previous literature suggesting that bioburden itself is not necessarily sufficient for true infection to occur in a wound; instead, another factor such as poor vascular supply may be required to trigger this.[20,21] It should be noted that our classification of vascular co-morbidity applied in this study is not a recognised scoring approach like that by Charlson or Elixhauser[22], but rather a pragmatic categorisation of different conditions affecting the vascular system. Ndosi and colleagues[23] also observed that multiple lesions are associated with increased infection and poorer healing. We found that a positive Glycologic test result was significantly associated with patients having more than one DFU, though this was not observed when clinical opinion of infection was the outcome measure. Due to intellectual property and commercial sensitivities, the molecular process that underpins the colour change in the test kit cannot be disclosed by Glycologic Ltd. Therefore, a detailed discussion on any relationships between bioburden and host-related inflammatory and wound healing processes are not possible. However, it is known that the host-produced molecule involved in the kit reaction

is produced in chronic wounds where the host deems the bioburden to be pathogenic. Apart from the Glycologic kit, various approaches to develop a point-of-care test for chronic wound infection are being developed and appraised, and this molecular approach is anticipated to be introduced in clinical practice in the near future.[24,25,26,27]

There are a few practical drawbacks to the study which should be taken into account. Firstly, at present, the Glycologic test kit does not include a negative or – perhaps more importantly - positive control. Therefore, if there is no colour change the clinician needs to have faith that there is genuinely no host-response to infection present. However, if the kit solution has expired or is not at room temperature then this may lead to false-negative outcomes. Another aspect around the colour indication is that those who perform the test need to be vigilant that a wound swab does not contain significant amounts of blood, since doing so will contaminate the Glycologic test solution and render it pink. In our experience a small degree of blood contamination did not hinder the test interpretation. Secondly, in this study a comparison of Glycologic test result versus microbiological results of a wound swab was not performed. The reason for this was pragmatic; as part of standard practice, podiatrists do not routinely send samples to the microbiology laboratory for testing. In fact, in only 15 out of 383 (4%) wound swabs was a microbiology sample processed. This therefore made the sample too small to be included in the analyses for this present study. Having a point-of-care test to hand may assist clinical staff in obtaining a chromatic result to determine if a chronic wound is infected. It may also provide physical evidence to patients and for medical notes. However, a microbiology sample would still need to be obtained from confirmed infected wounds for antibiotics sensitivity testing; the Glycologic diagnostic kit does not identify what type of bacteria may be causing the infection. In relation to how the study

sample represents the wider patient population, a few observations can be made. Although a mix of males and females with varying BMI, smoking status and co-morbidities were included in the study, the ethnicity of all patients was white British.

From the results obtained in this study, we cannot say if the Glycologic test kit challenges the paradigm around wound assessment in which quantification of bioburden is deemed the gold standard for determination of wound infection.[28] To ascertain if this is the case, more research is indicated where microbiological samples are compared to both clinical opinion and the Glycologic kit. It is important to stress, however, that the biochemical reaction between the substrate in the Glycologic test tube and the host factor in the patient's wound exudate is not dependent on presence of bacteria per se. It is the effect that bacteria have on the host's immune and inflammatory responses that drives the eventual reaction in the Glycologic diagnostic kit. This present study indicates that the Glycologic test result corresponds with a clinician's opinion of wound infection presence; it is not known if there is any correlation between a positive Glycologic test result and the degree of bacterial load in a wound. For further validation of the Glycologic kit, a comparison to microbiology swab or tissue sampling results is indicated. Finally, different cytokines and other biomarkers can be elevated and suppressed differently, depending on the type of chronic wound.[29] Thus, other wounds need to be assessed with the Glycologic kit, including chronic non-diabetic ulcers, pressure ulcers and non-healing post-surgical wounds, to determine if the results obtained for DFUs are also observed in other wound types and in patients with different (non-vascular) co-morbidities.

Conclusions

This study demonstrates that it is relatively straightforward to incorporate the Glycologic point-of-care test kit in a clinical care setting, albeit as part of a clinical research project. Results demonstrate that the Glycologic test results for DFU wound swabs mostly agree with clinical opinion, though the test kit appears to be more conservative in terms of the identification of infection. Furthermore, the Glycologic test kit's colour change is associated with the clinical signs and symptoms of infection – namely erythema, purulence and odour – and apparently also indicators of a patient's poor (vascular) health, such as the presence of numerous DFU lesions and/or co-morbidities such as peripheral vascular disease or severe chronic kidney disease. These outcomes suggest that a host-response to bioburden may be a patient-specific event rather than something where – for example - a generalised lower limit threshold can be applied. Further research appraising the performance of the Glycologic test kit in patients with other wounds, and a wider comparison of outcomes involving clinical opinion and microbiological assessment results will inform how the Glycologic test performs and how its results may be interpreted for clinical application.

References

1. NHS Diabetes report. https://www.networks.nhs.uk/news/new-foot-care-report-shows-ps650m-cost-of-ulcers-and-amputations (accessed 8 October 2018)
2. Grothier L, Stephenson J. An audit to determine the clinical effectiveness of a pathway for managing wound infection. Wounds UK, 2015; 11(2): 32-40.
3. Halbert AR, Stacey MC, Rohr JB, Jopp-Mckay A. The effect of bacterial colonization on venous ulcer healing. *Australasian journal of dermatology*, 1992; *33*(2): 75-80.

4. O'meara S, Nelson EA, Golder S, et al. Systematic review of methods to diagnose infection in foot ulcers in diabetes. Diabetic medicine. 2006 Apr 1;23(4):341-7.
5. Spichler A, Hurwitz BL, Armstrong DG, Lipsky BA. Microbiology of diabetic foot infections: from Louis Pasteur to 'crime scene investigation'. BMC medicine. 2015;13(1):2.
6. Nelson A, Wright-Hughes A, Backhouse MR, et al. CODIFI (Concordance in Diabetic Foot Ulcer Infection): a cross-sectional study of wound swab versus tissue sampling in infected diabetic foot ulcers in England. BMJ open. 2018 Jan 1;8(1):e019437.
7. Gardner SE, Haleem A, Jao YL, et al. Cultures of diabetic foot ulcers without clinical signs of infection do not predict outcomes. Diabetes Care. 2014; 37(10):2693-701.
8. NICE Clinical Guideline 15, Oct 2008, Surgical site infections: prevention and management, https://www.nice.org.uk/guidance/ng19 (accessed 8 October 2018).
9. NICE Guideline 15, Aug 2015, Diabetic foot problems: prevention and management, https://www.nice.org.uk/guidance/ng19 (accessed 8 October 2018).
10. Levine NS, Lindberg RB, Mason JA, Pruitt JB. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. The Journal of trauma. 1976;16(2):89-94.
11. Lavery LA, Armstrong DG, Wunderlich RP, et al. Risk factors for foot infections in individuals with diabetes. Diabetes care. 2006; 29(6):1288-93.
12. Boulton, A. J., P. Meneses, and W. J. Ennis. 1999. Diabetic foot ulcers: a framework for prevention and care. Wound Rep. Regen. 7:7–16.
13. Guest JF, Fuller GW, Vowden P. Diabetic foot ulcer management in clinical practice in the UK: costs and outcomes. International wound journal. 2018 Feb;15(1):43-52.

14. Horn JL. A rationale and test for the number of factors in factor analysis. Psychometrika. 1965 Jun 1;30(2):179-85.
15. Tabachnik BG, Fidell LS. Using multivariate statistics (6. Baskı). US: Pearson. 2012.
16. Cutting KF, White R. Defined and refined: criteria for identifying wound infection revisited. British journal of community nursing. 2004 Mar;9(Sup1):S6-15.
17. Richard JL, Sotto A, Lavigne JP. New insights in diabetic foot infection. World journal of diabetes. 2011 Feb 15;2(2):24.
18. Lipsky BA, Aragon-Sanchez J, Diggle M, *et al*. IWGDF guidance on the diagnosis and management of foot infections in persons with diabetes. *Diabetes Metab Res Rev* 2016;32(Suppl 1):45–74.
19. Reddy M, Gill SS, Wu W, Kalkar SR, Rochon PA. Does this patient have an infection of a chronic wound?. Jama. 2012 Feb 8;307(6):605-11.
20. Siddiqui AR, Bernstein JM. Chronic wound infection: facts and controversies. Clinics in dermatology. 2010 Sep 1;28(5):519-26
21. Tuttle MS. Association between microbial bioburden and healing outcomes in venous leg ulcers: a review of the evidence. Advances in wound care. 2015 Jan 1;4(1):1-1.
22. Sharabiani MT, Aylin P, Bottle A. Systematic review of comorbidity indices for administrative data. Medical care. 2012 Dec 1:1109-18.
23. Ndosi M, Wright-Hughes A, Brown S, Backhouse M, Lipsky BA, Bhogal M, Reynolds C, Vowden P, Jude EB, Nixon J, Nelson EA. Prognosis of the infected diabetic foot ulcer: a 12-month prospective observational study. Diabetic Medicine. 2018 Jan;35(1):78-88

24. Boykin JJ. The future of wound care diagnostics: biomarkers. Ostomy/wound management. 2009 Sep;55(9):20-1.
25. Heinzle A, Papen-Botterhuis NE, Schiffer D, et al. Novel protease-based diagnostic devices for detection of wound infection. Wound Repair and Regeneration. 2013 May;21(3):482-9.
26. Glucksman M, Philibert K, Shao X, et al. 'Ome is where the Wound is: Biomarkers of Healing in Chronic Diabetic Foot Ulcers. The FASEB Journal. 2017 Apr;31(1_supplement):780-14.
27. Ingram JR, Cawley S, Coulman E, Gregory C, Thomas-Jones E, et al. Levels of wound calprotectin and other inflammatory biomarkers aid in deciding which patients with a diabetic foot ulcer need antibiotic therapy (INDUCE study). Diabetic Medicine. 2018 Feb;35(2):255-61.
28. Gardner SE, Frantz RA, Doebbeling BN. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair and Regeneration. 2001 May;9(3):178-86
29. Clark R. Diagnostic markers of infection in chronic wounds. PhD thesis. 2007 https://orca.cf.ac.uk/55725/1/U584244.pdf (accessed 18 October 2018)