

Insights into laboratory test methods: bringing validity to *in vitro* debridement models

KEY WORDS

- » Biofilm
- » Debridement
- » Infection
- » *In vitro* model
- » Wound healing

Abstract: In this article the importance of laboratory test method validation and accreditation is reviewed, in particular the validity of *in vitro* test methods for assessing wound debridement methodologies. Validated test methods predict pre-clinical outcomes, and robust laboratory data can effectively be used to compare the performance of different wound debridement technologies. The report highlights a recent study of a wound debridement gel, and the importance of test method validation for comparative performance testing of such a product.

Chronic (hard-to-heal) wounds represent a worldwide health issue, and it is acknowledged that biofilms colonise most hard-to-heal wounds (Malone et al, 2017). Biofilm protects associated microorganisms from antimicrobial agents, and the targeted treatment of biofilms has a beneficial impact on wound healing (Bianchi et al, 2016; Schultz et al, 2017). Consequently, wound care products, and protocols such as Wound Hygiene (Murphy et al, 2019), are becoming increasingly targeted towards antibiofilm activity. This is key in wound debridement, where strategies are required to remove devitalised tissue and disrupt and remove biofilm.

Modes of action of debridement procedures guide selection of test methods

The modes of action of an ideal wound debridement procedure are typically (i) softens or removes devitalised tissue and (ii) disrupts biofilm, kills microorganisms and then removes the debris. These actions aim to remove barriers to healing in the wound such that healing can proceed. In the literature, there are various laboratory methods that could be used to assess these factors. However, currently there are no standard test methods for wound debridement devices or procedures.

When considering a laboratory method, there is often a trade-off between clinical relevance and the type of test method available. If standard test methods do not exist, then standard methods can be adapted, or new methods can be developed by laboratories. In both cases, these methods should be validated (Bowler and Westgate, 2022). Data from standard or validated test methods allow benchmarking of new and existing antibiofilm technologies, which enables healthcare professionals in the selection of the most appropriate treatment options. Laboratories can also be accredited in their ability to perform such standard or validated test methods, e.g., by the UK Accreditation Service (UKAS), who can award International Organisation for Standardisation (ISO) 17025 accreditation.

This report explains the importance of using validated test methods while striving for clinical relevance, so that laboratory data can be used to compare the performance of different wound debridement technologies.

The importance of test method validation and accreditation

When test methods gain accreditation, such as UKAS accreditation for ISO 17025, it provides confidence that the method is reproducible

JENNIE RITCHIE
BA, Marketing Executive,
Perfectus Biomed Group
Now Part of NAMSA

SAMANTHA WESTGATE
PhD, Executive Director,
Perfectus Biomed Group
Now Part of NAMSA

Table 1. A comparative assessment of laboratory-based methods that can be used to assess debridement tools. Throughput: High > 24 samples; Medium < 24 samples; Low < 6 samples. Clinical relevance: Low – solid surface biofilm, does not model a hard-to-heal wound; Medium – mimics some elements of a hard-to-heal wound; High – custom designed to better mimic a hard-to-heal, infected wound. *Available at Perfectus Biomed, now part of NAMSA.

Test method	Throughput	Accreditation status	Clinical debridement relevance
Minimum Biofilm Eradication Concentration (MBEC) biofilm assay (ASTM E2799)	High	ISO 17025*	Low
Centers for Disease Control and Prevention (CDC) biofilm reactor (ASTM E2562)	Medium	ISO 17025*	Low
(Colony) Drip Flow Reactor biofilm model (ASTM E2647)	Low	ISO 17025*	Medium
Porcine skin burn wound model	Medium	ISO 17025*	Medium/high
Porcine skin biofilm model	Medium	None (Peterson et al, 2021)	High
Customised debridement model - gauze biofilm model (Figure 2)	Medium	Independently validated	Medium/high

between laboratories and scientists. The key stage of accreditation is the validation of the test method. The test method must be demonstrated to be robust with appropriate precision (repeatability, reproducibility), via test method validation. This provides confidence that the conclusions drawn are valid and comparable.

Table 1 compares some commonly used biofilm test methods that are standard test methods, or adapted and validated test methods, that may be considered for the assessment of the antibiofilm performance of debridement procedures. Microbiology experiments are widely expected as labour intensive, therefore Table 1 also shows the throughput of the test methods (defining how many samples can be tested concurrently) along with their accreditation statuses and relevance to clinical debridement.

A study to demonstrate the importance of test method validation

ChloraSolv® Wound Debridement Gel (RLS Global, Sweden; distributed by Convatec, UK), is a 0.45% hypochlorite gel of high pH. It aids debridement of wounds by softening devitalised tissue and providing antimicrobial activity. The antibiofilm activities of ChloraSolv were examined to provide comparative data to assess performance against other debridement

techniques (Metcalf et al, 2023). When tested in an adapted standard test method (MBEC; Table 1), ChloraSolv showed notably greater antibiofilm activity compared to an antimicrobial wound irrigation solution against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm. However, such a test lacks clinical relevance (Table 1).

Research methods can be developed that challenge products against clinically relevant parameters such as resistant pathogens, extended durations and more complex culture media or biofilm growth substrate, which can result in complex, mature biofilm. The methods can also consider how the product will be used clinically, therefore additional steps that mimic clinical use can be added, such as application of solutions in soak form, irrigation rinse steps, or use with a physical debridement tools. These were some of the considerations that the Convatec's Research & Development, Microbiology Laboratory team considered when adapting an original in-house test method, which historically had been used for assessing antibiofilm performance of wound dressings (Bowler & Parsons, 2016), to further challenge debridement procedures. This test method was then validated by Perfectus Biomed Group, now part of NAMSA, an independent laboratory (Metcalf et al, 2023).

In brief, methicillin resistant *Staphylococcus*

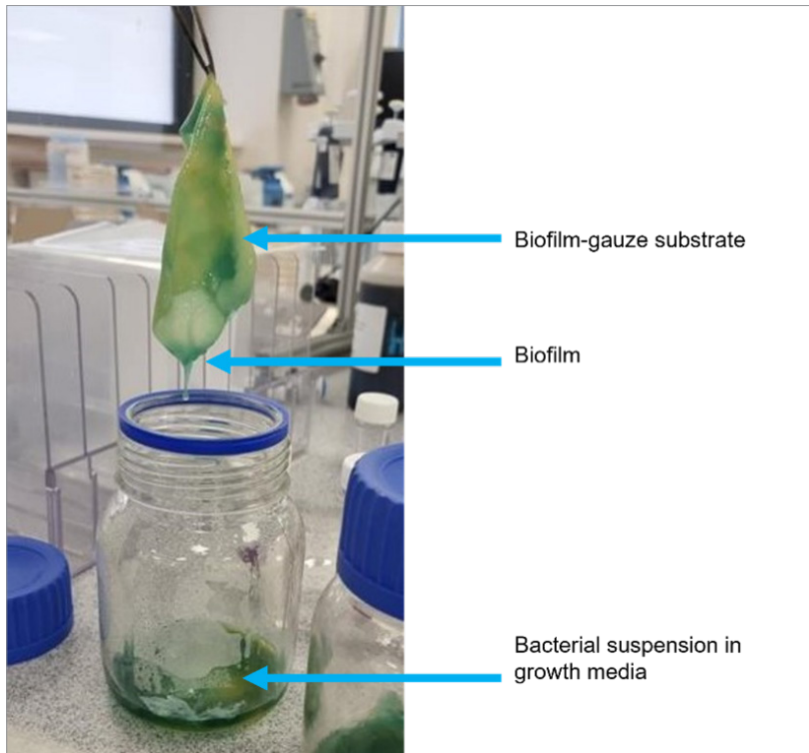


Figure 1. Biofilm cultured on gauze substrate. Loosely adhered/planktonic bacteria were removed by rinsing and the gauze biofilm was transferred to a sterile contact plate lid



Figure 2. Example of gauze-biofilm treated with an antimicrobial solution soak

aureus (MRSA) or multidrug-resistant *Pseudomonas aeruginosa* biofilm was cultured on gauze pieces in shaking flasks at $35\pm 3^{\circ}\text{C}$ for 48 hours to allow mature biofilm to develop (Figure 1). The biofilm-colonised gauze was processed to remove loosely adhered bacteria then transferred to the lid of a sterile contact plate for the treatment (Figure 2). The method was validated by challenging the following variables:

- ▶▶ Ensuring minimum differences are observed in results by testing performed (n=3 replicates) on three separate occasions by an operator (repeatability – within test)
- ▶▶ Comparative assessment when the above testing is performed by multiple operators (robustness)
- ▶▶ Comparative assessment between operators and occasions, when the use of different batches of media, equipment and prepared inoculums of the challenge organisms were used (repeatably precision and ruggedness).

In addition, as some of the products under test contained active antimicrobials, neutralisation validation was included in the test method validation. This part of the validation ensures that once a treatment timepoint has finished, a neutralisation fluid can stop any further antimicrobial activity.

This test method validation approach means that the products and procedures used in the validated test method have been challenged in a complex model, that can provide robust and reproducible data generated by a laboratory.

SUMMARY

In vitro biofilm test methods are used in wound care product development to meet commercial and regulatory requirements. Historically, standard microbiological testing focused almost exclusively on planktonic bacteria (free-floating, as opposed to biofilm), and as such these test methods may underestimate the quantity of active agent required to effectively reduce microbial biofilm. Standard test method adaptations are effective when looking to mimic a wound scenario more closely, however, any adaptation to a standard method means that validation is required to ensure that the method will continue to produce reproducible and robust results (Bowler and Westgate, 2022).

Method validation is an important requirement for any scientific experiment/test and the confidence in the validation and subsequent data generation can be increased using accredited testing laboratory. Using standard, validated and accredited test methods can collectively provide robust, reproducible and clinically

relevant data that can be used with confidence to support product development or use in-market. It can also be used to submit data to regulatory bodies for additional claims or to have meaningful discussions with clinicians about the mode of action and efficacy wound care products, such as debridement tools. **WUK**

Acknowledgements

This commentary on the significance of adapted and validated models summarises the study of the anti-biofilm performance of ChloroSolv® Wound Debridement Gel (RLS Global, Sweden, distributed by Convatec, UK) (Metcalf et al 2023). Development of the gauze biofilm model was by Convatec Research & Development Microbiology Laboratory, Convatec Global Development Centre, Deeside, UK.

Perfectus Biomed Group, now part of NASMA, is leading the market with UKAS ISO 17025 accredited biofilm methods and customised assays that support product development and marketed products.

REFERENCES

- Bianchi T, Wolcott RD, Peghetti A et al (2016) Recommendations for the management of biofilm: a consensus document. *J Wound Care* 25(6):305–17. <https://doi.org/10.12968/jowc.2016.25.6.305>
- Bowler PG, Parsons D (2016) Combatting wound biofilm and recalcitrance with a novel anti-biofilm Hydrofiber® wound dressing. *Wound Med* 4:6–11. <http://dx.doi.org/10.1016/j.wndm.2016.05.005>
- Bowler PG, Westgate SJ (2022) An insight into in vitro microbiological testing of wound dressings. *Wounds UK* 18(1):79–80. <https://tinyurl.com/2dfw7x8a> (accessed 23 February 2023)
- Malone M, Bjarnsholt T, McBain AJ et al (2017) The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data. *J Wound Care* 26(1):20–5. <https://doi.org/10.12968/jowc.2017.26.1.20>
- Metcalf DG, Meredith K, Olausson S (2023) The in vitro anti-biofilm performance of ChloroSolv® Wound Debridement Gel. *Wounds UK* 19(1):34–44
- Murphy C, Atkin L, Dissemont J et al (2019) Defying hard-to-heal wounds with an early antibiofilm intervention strategy: 'wound hygiene'. *J Wound Care* 28(12):818–22. <https://doi.org/10.12968/jowc.2020.29.sup3b.s1>
- Schultz G, Bjarnsholt T, James GA et al (2017) Global Wound Biofilm Expert Panel. Consensus guidelines for the identification and treatment of biofilms in chronic nonhealing wounds. *Wound Repair Regen* 25(5):744–57. <https://doi.org/10.1111/wrr.12590>
- Peterson M, Siller Z, Wang H et al (2021) An ex vivo model of medical device-mediated bacterial skin translocation. *Sci Rep* 11(1): 5746. <https://doi.org/10.1038/s41598-021-84826-1>




Leg Club Favourites

Order your copy now!

The perfect gift for a loved one or a treat for yourself!

Discover the joy of cooking *and* help out the Leg Club Foundation.
Order your copy now.

Visit: www.legclub.org/leg-club-recipe-book or email: info@legclub.org