OMNIgene™•GUT

Inactivation of bacterial pathogens in the OMNIgene™•GUT collection device

Introduction

Biological samples can contain many bacteria and viruses, some with the potential to cause disease in humans. The presence of live pathogens in biological samples can pose a significant risk to laboratory personnel responsible for handling and processing the samples, unless the pathogens are known to be inactivated. Here, we conducted a research study to evaluate the ability of the DNA Genotek OMNIgene™•GUT collection device stabilization solution to inactivate common bacterial pathogens. As per the U.S. Centers for Disease Control and Prevention (CDC) guidelines[1], pathogen inactivation is demonstrated by generating a kill curve (Figure 1A) that shows the gradual death of the pathogen of interest following exposure to the inactivating agent (i.e., the stabilization solution) over time. For the test to be valid, viability (i.e., evidence of bacterial growth) must be observed for at least one of the time points. When treatment of the pathogen with the inactivating agent results in no detectable growth, it cannot be concluded that complete inactivation has occurred given the limit of detection (LoD) of standard plate count methods (i.e., 1 colony-forming unit [CFU]). Furthermore, to comply with CDC guidelines, one must show that the inactivating agent causes bacterial cell death over time (bactericidal) as opposed to merely inhibiting bacterial growth (bacteriostatic). This is typically achieved by adding a neutralizer (Figure 1B) to the inactivation reaction which quenches the inactivating agent and prevents it from inhibiting growth following plating. Without quenching, the inactivating agent can be carried over onto culture plates, potentially resulting in an overestimation of bacterial cell death. Establishing the assay LoD and demonstrating neutralization are crucial aspects of inactivation procedures that collectively ensure the bactericidal activity of the inactivating agent is not overestimated.

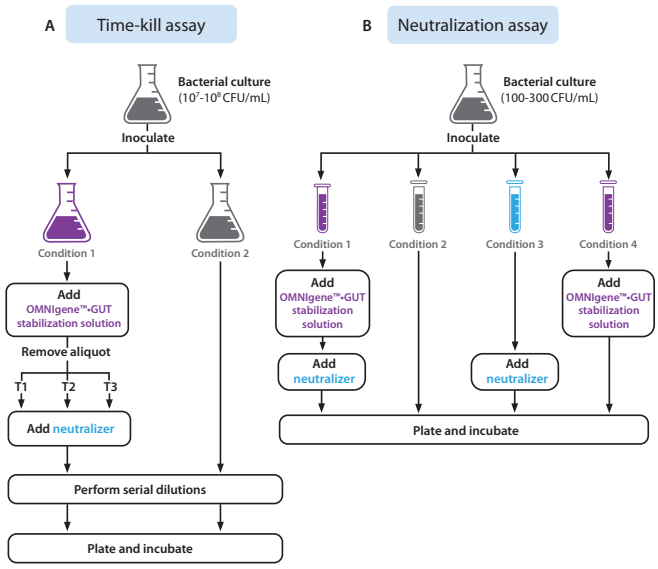

Figure 1. Overview of inactivation procedures developed in-house. *(A) During the time-kill assay, bacterial cell death caused by the OMNIgene™•GUT collection device stabilization solution was measured by removing aliquots at appropriate time points (T1, T2, T3) and neutralizing them (condition 1) prior to dilution and plating. For each time point, CFU/mL and $\log_{10}$ reduction were calculated and compared to the growth control (condition 2). (B) A neutralization assay was performed to ensure that the OMNIgene™•GUT collection device stabilization solution was effectively quenched by the neutralizer (condition 1) and that the neutralizer itself was non-toxic to bacterial cells (condition 3). For a valid neutralization assay, bacterial growth for conditions 1 and 3 must be equivalent to the growth control (condition 2). The negative control (condition 4) demonstrates that the OMNIgene™•GUT collection device stabilization solution is bactericidal and must be statistically different from the growth control (condition 2).*

Results

Using inactivation procedures developed in-house, we demonstrated that the OMNIgene™•GUT collection device stabilization solution inactivates *Escherichia coli, Pseudomonas aeruginosa* (both gram-negative species) and *Staphylococcus aureus* (gram-positive species) by a minimum of 6-$\log_{10}$ (Figure 2), equivalent to 99.9999%, which is on par with high-level disinfectants according to CDC standards.[2]

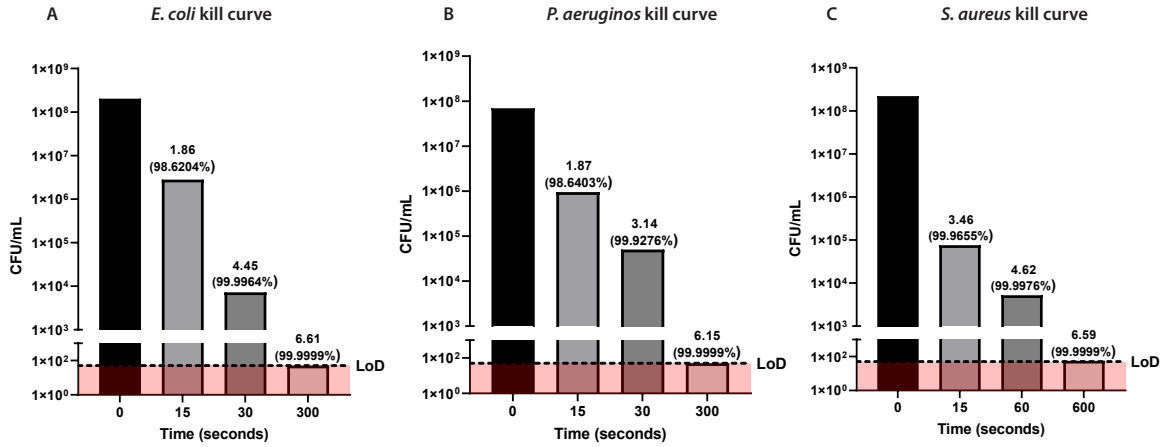

Figure 2. Representative time-kill curves for (A) E. coli, a gram-negative species, (B) P. aeruginosa, a gram-negative species and (C) S. aureus, a gram-positive species following exposure to the OMNIgene™•GUT collection device stabilization solution. *Log$_{10}$ reduction of > 6, equivalent to > 99.9999% reduction, was achieved after a 5-minute exposure for E. coli and P. aeruginosa, or a 10-minute exposure for S. aureus. Cell viability was seen at a minimum of 2 time points for each species tested, each time point having a minimum of 3 independent measurements across 2 independent assays*

The neutralization assay demonstrated that the neutralizer used in the inactivation procedure was non-toxic to bacterial cells and effectively quenched the OMNIgene™•GUT collection device stabilization solution (Figure 3). Therefore, it can be concluded that any cell death observed in the time-kill assay (Figure 2) was driven by exposure to the OMNIgene™•GUT collection device stabilization solution.

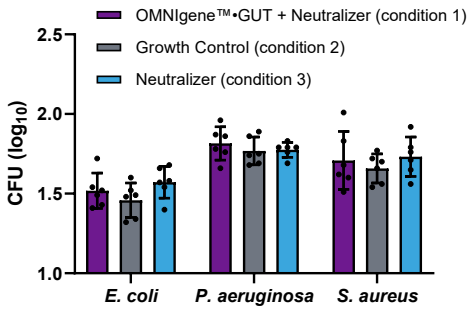

Figure 3. Representative neutralization assay results for E. coli, P. aeruginosa (gram-negative species) and S. aureus (gram-positive species) shown as means -/+ standard deviation. *No statistically significant differences were found between the group means of the OMNIgene™•GUT collection device stabilization solution + neutralizer (condition 1), growth control (condition 2) and neutralizer alone (condition 3) by one-way analysis of variance for E. coli (F(5,29) = [1.79], p = 0.145), P. aeruginosa (F(5,30) = [2.28], p = 0.072) or S. aureus (F(5,30) = [0.33], p = 0.891), indicating a valid neutralization assay for all species. In addition, no growth (0 CFU) was detected for the OMNIgene™•GUT collection device stabilization solution alone for all species (condition 4, data not shown).*

Conclusions

The OMNIgene™•GUT collection device stabilization solution inactivates common gram-positive and gram-negative pathogens such as *E. coli, S. aureus* and *P. aeruginosa* by > 6-log$_{10}$ or 99.9999% (Table 1). For each organism, we were able to demonstrate gradual death over time upon exposure to the stabilization solution and effective neutralization as required by CDC inactivation guidance.[1] Overall, this research study highlights the reduced risk of pathogen survival in samples collected in the OMNIgene™•GUT collection device, providing an extra layer of safety during downstream handling by laboratory personnel.

Table 1. Summary of bacterial inactivation in the OMNIgene™•GUT collection device stabilization solution.

Collection device	Assay	*E. coli*	*P. aeruginosa*	*S. aureus*
OMNIgene™•GUT (OMR-200, OM-200)	**Kill curve** Log$_{10}$ reduction (Percent reduction)	**> 6.61 logs** (> 99.9999%)	**> 6.15 logs** (> 99.9999%)	**> 6.59 logs** (> 99.9999%)
	Neutralization assay	Passed	Passed	Passed

Materials and methods

Bacterial species used for inactivation testing were *E. coli* (ATCC 10536), *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 15442). Cultures of each species were grown for 18-20 hours using conditions recommended by ATCC and bacterial cells were re-suspended in diluent [0.1% (w/v) tryptone, 25 mM

KH_2PO_4 pH 7.2 -/+ 0.2] to achieve an approximate cell density of 10^4 or 10^9 CFU/mL for the neutralization assay or time-kill assay, respectively.

The time-kill assay was performed using a procedure developed in-house based on the American Society for the Testing of Materials (ASTM) E2315-16: Standard Practices for Assessment of Antimicrobial Activity Using a Time-Kill Procedure.[3] This procedure compares a stabilization solution-treated culture to an untreated culture over time to assess survival. Bacterial inoculum was diluted in either diluent (initial growth control) or appropriate dilution of the OMNIgene™•GUT collection device stabilization solution (test condition) and incubated for 15 seconds to 10 minutes with continuous mixing. At each exposure time and at the end of the experiment (final growth control), an aliquot was diluted in neutralizer and/or serially diluted in diluent and plated in duplicate to obtain 20-200 CFU per plate. Each condition was tested in triplicate.

The neutralization assay was performed using a procedure developed in-house based on ASTM E1054-21: Standard Practices for Evaluation of Inactivators of Antimicrobial Agents, Neutralization Assay with Recovery on Semi-Solid Medium.[4] Composition of the neutralizer was 0.5% (w/v) Tween 80, 0.1% (w/v) tryptone, 10 mM $MgCl_2$ and 25 mM KH_2PO_4 pH 7.2 -/+ 0.2, while composition of the diluent was 0.1% (w/v) tryptone and 25 mM $KH2PO_4$ pH 7.2 -/+ 0.2. In triplicate for each condition, bacterial inoculum was diluted in either neutralizer (the OMNIgene™•GUT collection device stabilization solution + neutralizer and neutralizer alone), diluent (growth control) or stabilization solution (the OMNIgene™•GUT collection device stabilization solution alone) to achieve a final concentration of 100-300 CFU/mL. For the OMNIgene™•GUT collection device stabilization solution + neutralizer and neutralizer alone conditions, diluted chemistry and diluent, respectively, were added at the same ratio as for the time-kill procedure. For all conditions, aliquots were plated in duplicate after 1 minute and 15 minutes (data not shown) to ensure that bacterial growth did not occur while performing the experiment.

Plates from the neutralization assay and time-kill procedure were incubated under appropriate growth conditions alongside sterility controls for reagents used during both procedures. Resulting CFUs were then counted and recorded. As per ASTM recommendations, counts above 200 CFU were omitted while those below 20 CFU were reported as estimates.[5]

References:

1 U.S. Centers for Disease Control and Prevention, Division of Select Agents and Toxins. (2018). Guidance on the inactivation or removal of select agents and toxins for future use. https://www.selectagents.gov/compliance/guidance/inactivation/inactivation.htm. [webpage].

2 U.S. Centers for Disease Control and Prevention. (2019). Guideline for Disinfection and Sterilization in Healthcare Facilities. https://www.cdc.gov/infection-control/hcp/disinfection-and-sterilization/. [webpage].

3 ASTM International. ASTM E2315-16. (2016). Assessment of antimicrobial activity using a time-kill procedure. https://www.astm.org/e2315-16.html. [webpage].

4 ASTM International. ASTM E1054-21. (2021). Standard practices for evaluation of inactivators of antimicrobial agents. https://www.astm.org/e1054-21.html. [webpage].

5 Sutton S. (2011). Accuracy of plate counts. *Journal of validation technology.* 17(3), 42-46.