

# Protocol for Validation of Laundry Disinfection Processes and Infection Control Monitoring of Processed Textiles

## Introduction

This document sets out a method to assess the disinfection of textiles in a laundry process. Currently, the methods of validation for laundry services vary depending on the setting and the type of process. This test protocol and the method were developed with the aim of providing a standard test protocol for all types of laundry processes (Owen et al, 2024). It takes a two-fold approach based on 1) the periodic validation of a specified wash process to achieve disinfection including the setting of minimum process parameters which will then be monitored to ensure that a specific load has been disinfected, and 2) monitoring infection control of the remaining laundering process by determining the microbial bioburden of finished processed textiles. Such monitoring and process validation should be undertaken upon implementation of a new process and at periodic intervals defined by the operator's infection control risk assessment procedures, e.g. BS EN 14065:2016 - Textiles - Laundry processed textiles - Biocontamination control system.

For approach 1, the test procedure for qualification of laundry processes is based on the use of semi-permeable dosing strips (bioindicators). These bioindicators comprise of textile samples inoculated with the thermotolerant microorganism *Enterococcus faecium*, enclosed in a membrane that retains the bacteria, but is permeable to laundry chemistries, allowing the evaluation of the microbial kill of the whole wash process. The *E. faecium* bioindicators used in this test may be purchased from a commercial supplier, or produced in-house using any suitable membrane that passes the validation procedure outlined in Annex II: *Preparation and Validation of Bioindicator Enclosures for Use in Industrial Laundry Disinfection Process Test* according to the outlined specifications. At the date of publication, commercially available DES laundry bioindicators are only suitable for thermal wash processes of 60°C and above but it is anticipated that the publication of this test method will encourage the future commercial production of bioindicators that work at lower temperatures.

For approach 2, bioburden of processed linen involves the destructive sampling of textiles after completion of the full laundering and finishing process and determining the quantity of microorganisms present. This method enables the routine monitoring of infection control procedures of the laundering process to assess microbial recontamination after the validated disinfection step.

## Test Method: Validation of Wash Processes

### 1. Introduction

This protocol concerns the validation of wash processes and consists of two parts. Part one determines the disinfection activity of the whole wash process against microbially contaminated textiles enclosed within a semi-permeable membrane (*bioindicators*). The microbicidal activity test is intended to qualify the specific wash process tested and sets minimum values for specified process parameters which must be exceeded to parametrically release a batch of work in an actual laundry process. Part two is used for determination of the

microbial bioburden of finished processed textiles, for monitoring the reinfection of textiles after the disinfection step of the qualified wash process.

## 2. Test One: Microbicidal Activity of Wash Process – Validation using Bioindicators

### 2.1. Scope

This method determines the microbicidal activity of wash processes using bioindicators. A disinfection level will need to be set by the laundry with regard to the end use of the textile. This will be a minimum of a  $\geq 5 \log_{10}$  CFU reduction per  $1\text{cm}^2$  textile sample, but will often need to be higher (6  $\log_{10}$  for example) for more critical end uses such as surgical textiles or for cleanroom garments used in the manufacture of aseptic drugs. This test covers only the specific conditions and machine tested. It should be used for initial qualification of the specific wash process and for periodic validation during use according to the establishment's risk assessment protocol, e.g. BS EN 14065:2016 (British Standards Institution, 2016). The validation process must identify the parameters that will potentially effect the disinfection efficacy of the wash process (typically temperature, time and chemical concentration through the disinfection step of the cycle). These must be measured and stated as part of the validation process and they will then form the minimum value for process parameters that must be exceeded (via in-process measurement) in an actual wash process to allow parametric release of a disinfected wash load.

### 2.2. Principle

This test relies on the use of bioindicators, either prepared in house or obtained from a commercial supplier. Cotton textile samples ( $1\text{cm}^2$ ) are to be contaminated with *Enterococcus faecium* NCIMB 2699 to achieve the required disinfection level as determined in 2.1. for example 5  $\log_{10}$  or 6  $\log_{10}$  CFU per textile sample. *E. faecium* NCIMB 2699 has been selected due to being a surrogate microorganism used in place of pathogens for validation of thermal processing technologies and systems. This organism also lacks, or contains nonfunctional copies of enterococcal virulence genes and lacks antibiotic resistance genes (Kopit et al. 2014). Enclosed contaminated textile samples should only be processed in BS EN 14065:2016 approved laundries with appropriate risk assessments. Each textile sample is sealed within a separate compartment of a semi-permeable membrane to form the bioindicator. The bioindicator is then washed using the test process to be validated and the reduction of *E. faecium* by the wash process is then inferred using a semi-quantitative method. In this semi-quantitative method, the presence or absence of *E. faecium* on the cotton samples is assessed by incubating them in a liquid culture medium. In order to pass the test the textile sample inoculated at the test concentration should show no growth in the liquid medium.

**Note: *Enterococcus faecium* is a biosafety level 2 microorganism and bioindicators should only be opened and cultured by trained personnel in a biosafety level 2 laboratory.**

## 2.3. Materials

### 2.3.1. Culture Media

- a) Tryptone soya broth (TSB), prepared according to manufacturer’s instructions and sterilised by autoclaving.
- b) Slanetz and Bartley agar (*Enterococcus* selective agar), prepared and sterilised according to manufacturer’s instructions and dispensed into sterile petri dishes.

### 2.3.2. Bioindicators

2.3.2.1 Commercially available bioindicators: PES membrane bioindicators enclosing *Enterococcus faecium* NCIMB 2699 (Annex II, Supplementary Material Section 5) or DES Controller bioindicators (**note DES controllers are currently only suitable for wash processes at 60°C or above and it must be ensured that the non-virulent *E. faecium* NCIMB 2699 is the test microorganism used**)

OR

2.3.2.2 In-house prepared bioindicator, validated according to Annex II: Tests 1 and 2 and prepared according to Annex II: Supplementary Material Section 5.

### 2.3.3 Defined laundering process to be validated.

2.3.3.1 Important parameters that will effect the disinfection process must be identified. Typically these will be temperature, time and chemical concentration but other parameters such as load weight or soiling must also be considered (Table 1). It may not be necessary to state each of these, but for any that are not stated, a “worst case” must be assumed for non-stated parameters.

Table 1: Example of minimum requirements for validation of disinfection parameters.

Process	“Worst Case” Parameters Tested
Thermal disinfection, no chemical concentration stated	Zero chemistry
Chemical disinfection	Ambient water temperature
Thermo-chemical process	Temperature and chemical concentration stated for a specified time

- 2.3.3.2 A disinfection test cycle is run on the washing machine for validation. Bioindicators are included in the wash as described in 2.4
- 2.3.3.3 For each disinfection parameter that is identified in 2.3.3.1, the value of the parameter that is used by the test process must be measured against time during the validation test. This will produce a figure similar to Figure 1 showing temperature against time and Figure 2 showing chemical activity against time (Supplementary Material).
- 2.3.3.4 The maximum value of the parameter should be recorded.
- 2.3.3.5 The value of the parameter at which there is known to be no disinfection activity should be noted. For temperature, this is 40°C. For a specific chemical, if the minimum value is not known, it must be assumed to be a zero concentration.
- 2.3.3.6 The time elapsed between the parameter first exceeding the value in 2.3.3.5 and returning to this value should be recorded.
- 2.3.3.7 The bioindicator is assessed after the disinfection cycle according to 2.4 to confirm that the required log<sub>10</sub> reduction per textile sample has been achieved.
- 2.3.3.8 Provided that the required level of disinfection has been achieved, the parametric release value for the process can be expressed as  $\geq \log_{10}$  reduction per textile sample (2.3.3.7) for the parameter  $\geq$  the maximum value (2.3.3.4) and a time  $\geq$  the elapsed time (2.3.3.6). For example if a thermal disinfection test for Figure 1 (Supplementary Material) produces no growth on a 10<sup>6</sup> bioindicator then a disinfection of 6 log<sub>10</sub> can be assumed provided that a temperature of  $\geq 70^{\circ}\text{C}$  for  $\geq 495$  seconds is achieved

#### 2.3.4 Parametric release of a laundry process

- 2.3.4.1 Section 2.3.3 obtained the values of wash process parameters (temperature or chemical concentration or both) against time that obtained a measured log<sub>10</sub> reduction in microbial contamination. The disinfection of an actual laundry batch can not be microbiologically determined for every process for commercial reasons and because of the time taken to incubate bioindicators. These batches can be parametrically released by measuring the parameters validated in section 2.3.3 to confirm that they exceed the test value for at least the test time. Parametric release examples are shown in Table 2.

Table 2: Example of minimum requirements for parametric release of actual wash loads based on validated parameters.

Parameter Validated	Minimum Requirement for Actual Wash Process
Water temperature 65°C for 10 minutes	Water temperature must be measured as $\geq 65^{\circ}\text{C}$ for all of a 10 minute period for the load to be parametrically released as disinfected
Chemical disinfectant (minimum concentration for a specified time)	Volume of each chemical disinfectant must be at least the specified minimum concentration for all of the specified time period for the load to be parametrically released as disinfected.

## 2.4 Test Procedure

### 2.4.2 Wash Test Procedure

- a) Place duplicate bioindicators (2.3.2) in a suitable cloth bag such as a pillowcase and seal with a zip tie or attach to a ballast sheet using a plastic tag.
- b) Launder using the test process (2.3.3) with a typical laundry load, stating all process parameter values measured during the test and whether these are minimum or maximum values and confirming that these are maintained for the stated time period.
- c) In addition to the bioindicators that have been laundered, two unlaundered bioindicator samples should be processed as a positive control and two autoclaved bioindicators as a negative control. These control bioindicator samples should be transported, stored and handled identically to the laundered samples, but remain unlaundered.

**Upon completion of the wash cycle, all following steps must only be completed by trained personnel in an appropriate biosafety level 2 laboratory.**

- d) Aseptically remove the cotton samples from the laundered and unlaundered bioindicators and place each in 10 ml TSB. Incubate at  $37^{\circ}\text{C}$  ( $\pm 2^{\circ}\text{C}$ ) for 48 hours ( $\pm 2$  hours).
- e) Inspect each sample for growth, indicated by turbidity of the TSB medium.
- f) Subculture by streaking each sample onto *Enterococcus* selective agar and incubate at  $37^{\circ}\text{C}$  ( $\pm 2^{\circ}\text{C}$ ) for 48 hours ( $\pm 2$  hours). To confirm growth of *E. faecium*.
- g) Inspect each sample for growth of *E. faecium*.

### 2.4.3 Interpretation

- a) All untreated positive control samples should produce positive growth of *E. faecium* for the test to be valid.
- b) For test samples, determine the log<sub>10</sub> reduction of *E. faecium* as follows:
  - No *E. faecium*. detected: Disinfection is  $\geq$  the concentration on the test sample as determined in section 2.1
  - *E. faecium* detected in test samples: Disinfection is  $<$  the concentration on the test sample as determined in section 2.1
- c) The test process passes the test where a  $\geq$ log<sub>10</sub> reduction than that set in section 2.1 is achieved.

## 3 Test Two: Microbial Bioburden of Finished Processed Textiles

### 3.3 Scope

This protocol determines the microbial load of textiles that have undergone laundering and finishing according to the wash process validated in Test One. It is intended as an infection control monitoring procedure, to monitor recontamination during handling and finishing after the disinfection step of the wash process. The sampled textiles, frequency and target/action levels of specified microorganisms should be ascertained by the operator according to their risk assessment policy e.g. BS EN 14065:2016 (British Standards Institution, 2016).

### 3.4 Principle

Samples are cut from finished textiles. The surviving microorganisms are recovered by shaking in buffer and quantified by the spread plate technique.

### 3.5 Materials

#### 3.5.2 Test textile sample

Laundered /processed test textile sample

#### 3.5.3 Diluent

Phosphate buffered saline (PBS) solution, prepared with distilled water and sterilised by autoclaving.

#### 3.5.4 PBS with polysorbate 80 (PBS-T)

PBS supplemented with 2 g/L polysorbate 80, prepared with distilled water and sterilised by autoclaving.

#### 3.5.5 Culture media

- a) Non selective agar: Nutrient or tryptone soya agar (TSA) in 90 mm agar plates.
- b) Selective agar, specific types determined according to establishment's risk assessment for indicator microorganisms, prepared and sterilised according to manufacturer's instructions and dispensed into sterile petri dishes. Examples include Baird Parker agar for *Staphylococcus aureus* and Mannitol Egg Yolk Polymyxin agar for *Bacillus cereus*.

#### 3.5.6 Membrane filters

Cellulose acetate, 0.45 µm, sterile, and vacuum filtration unit. Other filters may be used if validated to have the same recovery performance as cellulose acetate.

### 3.6 Test Procedure

- a) After completion of the wash cycle, cut 25 cm<sup>2</sup> samples of the test textile. Tests should be performed in duplicate for each type of agar to be tested.
- b) Place in 30 ml PBS-T and shake by hand 30 times.
- c) Dilute as required in PBS and plate dilution series on non-selective agar and required selective agars. Membrane filter the remaining PBS-T.
- d) Incubate non-selective agar for 48 hours at 37°C. Incubate selective agars according to manufacturer's instructions. PBS-T only should also be plated and incubated to ensure sterility alongside a blank agar plate.

### 3.7 Interpretation

- a) Enumerate the surviving colonies on non-selective agar to determine total microbial load and selective agars to determine the load of specified indicator microorganisms.
- b) Interpret pass/fail according to the action levels/specifications outlined in the establishment's risk assessment for both total microbial load (non-selective agar) and indicator microorganisms (selective agar).

### Supplementary Material

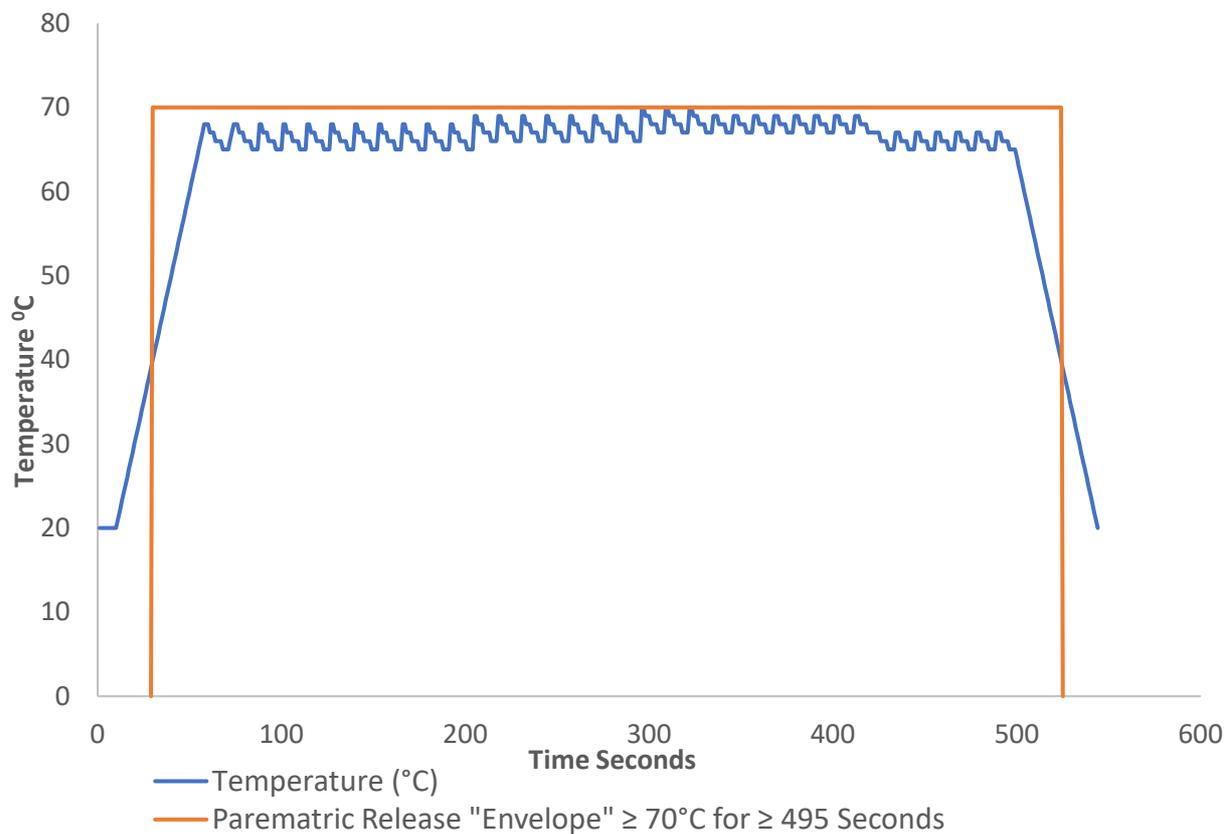


Figure 1 – Temperature Time Trace for a Thermal Disinfection Validation

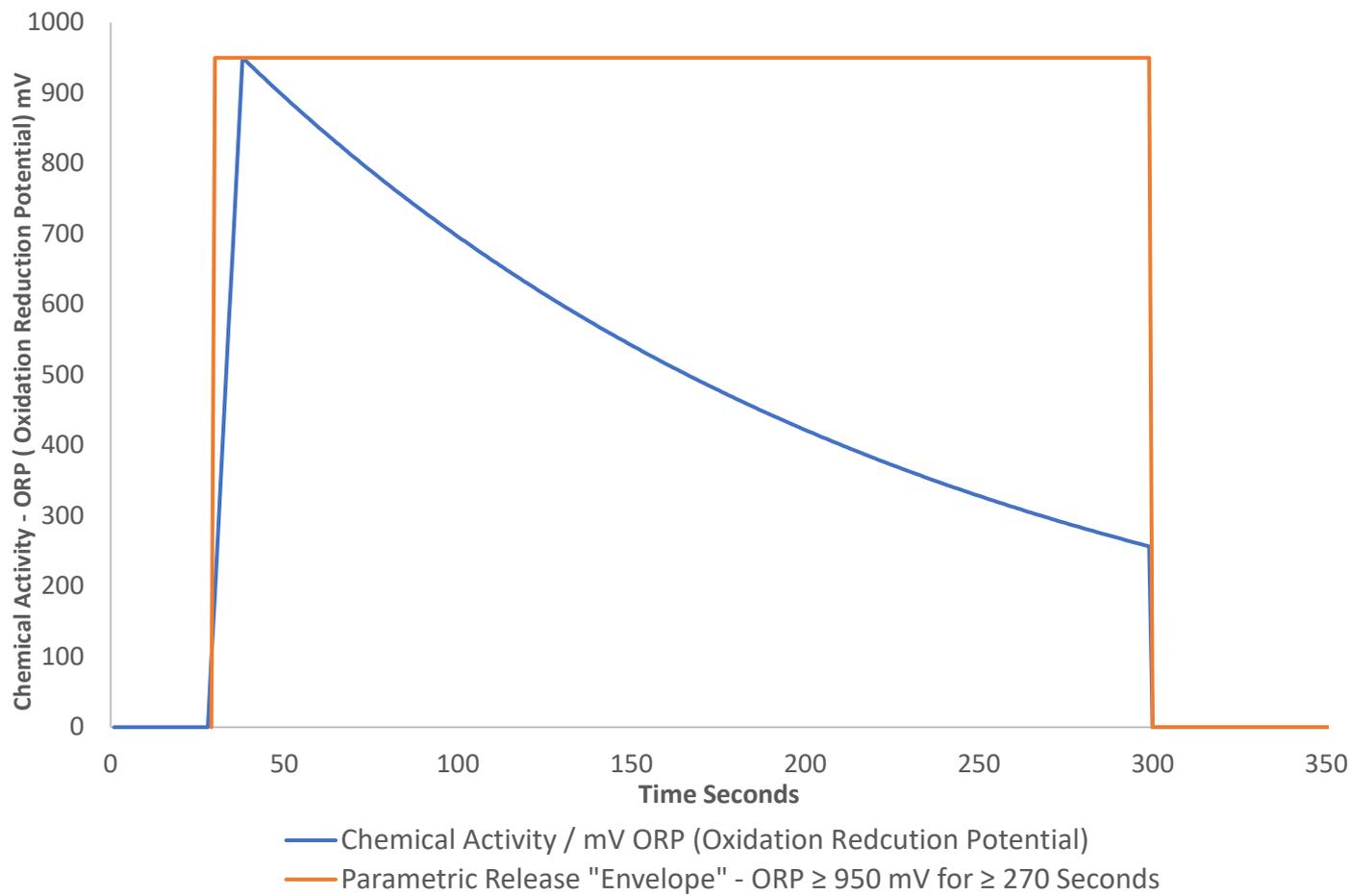


Figure 2 –Chemical Activity Time Trace for a Chemical Disinfection Validation

**References**

- 1) Owen, Lucy, Caroline Cayrou, Georgina Page, Martin Grootveld, and Katie Laird. (2024). "Development of a Standardised International Protocol for Evaluation of the Disinfection Efficacy of Healthcare Laundry Wash Processes" *Applied Microbiology* 4, no. 1: 194-214. <https://doi.org/10.3390/applmicrobiol4010014>
- 2) BS EN 14065:2016: Textiles. Laundry processed textiles. Biocontamination control system
- 3) Kopit LM, Kim EB, Siezen RJ, Harris LJ, Marco ML (2014). Safety of the surrogate microorganism *Enterococcus faecium* NRRL B-2354 for use in thermal process validation. *Applied and Environmental Microbiology*.;80(6):1899-909. doi: 10.1128/AEM.03859-13. Epub 2014 Jan 10. PMID: 24413604; PMCID: PMC3957640