

Technical White Paper: JIS Z 2801: 2000

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The Japanese Industrial Standard JIS Z 2801: 2000 (Ref 1) was developed to measure the antibacterial activity in hydrophobic materials, originally resulting from the incorporation of silver ions into rigid polymers. The method was developed by a consortium of workers comprised of manufacturers of silver-based antimicrobial agents, government-based research organisations and universities under the organisation of the SIAA. The method has been validated by ring tests within Japan (Ref. 2) and now forms the basis of a draft ISO standard which is being validated by the SIAA in collaboration with the International Biodeterioration Research Group (IBRG - see Ref 3). The method described in JIS Z 2801

- 1 generates fully quantitative data.
- 2 can be modified to accommodate both biocidal and biostatic effects
- 3 can be modified to accommodate a wide range of microbial species (including certain fungi, algae and protozoa as well as a wide range of bacterial species and viruses - Ref 4).
- 4 can be modified to allow a wide range of contact times and temperatures to be examined (Ref 5).

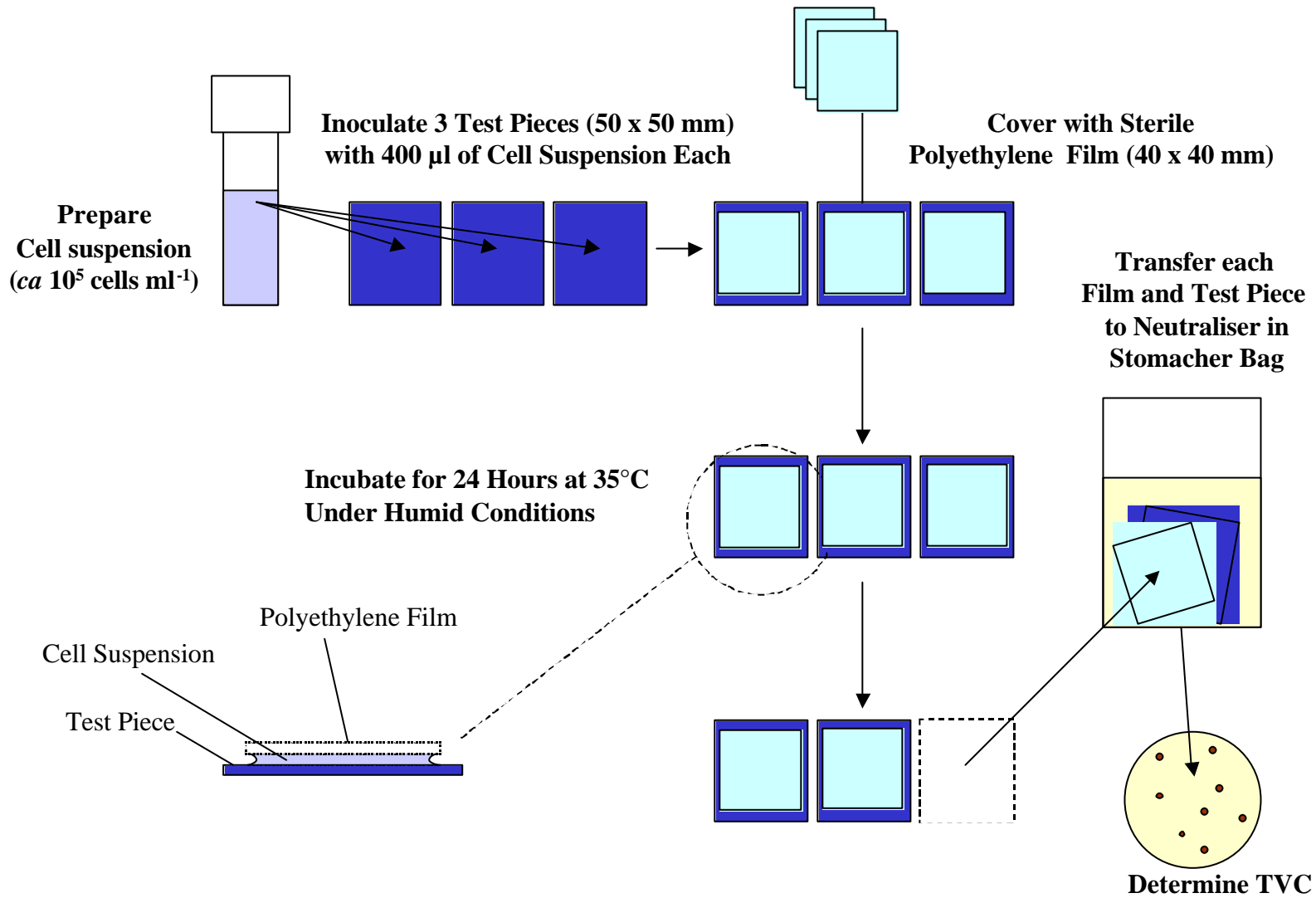
Method Outline

Antibacterial activity is measured by quantifying the survival of bacterial cells which have been held in intimate contact for 24 hours at 35°C with a surface that contains an antibacterial agent. The antibacterial effect is measured by comparing the survival of bacteria on a treated material with that achieved on an untreated material.

Determination of Antibacterial Activity using JIS Z 2801

A cell suspension of either *Escherichia coli* (2.5×10^5 - 1.0×10^6 cells ml⁻¹; NCIMB 8545) or *Staphylococcus aureus* (2.5×10^5 - 1.0×10^6 cells ml⁻¹; ATCC 6538p) is prepared in $1/_{500}$ nutrient broth. An aliquot (400 µl 16cm²) is then placed onto at least 3 replicate sub-samples per species of the treated surface under test and 6 replicate sub-samples per species of the untreated surface and held in intimate contact using a sterile polyethylene film (typically 40 x 40 mm on a test piece measuring 50 x 50 mm). The 3 replicate sub-samples of the treated material and 3 of the 6 replicate sub-samples of the untreated material are then incubated for 24 hours at 35°C at saturation humidity. After incubation, the samples are transferred to individual containers containing an aliquot (typically 10 ml) of a neutraliser validated for the biocide used in the treated material. The film is separated from the surface and the suspension remaining on the surface homogenised with the neutraliser. Three replicate sub-samples of the untreated material are also processed in this manner prior to incubation to provide base-line data. In some instances, where samples are either non-regular in shape, are overly large or have properties which make immersion in a neutraliser solution impractical, the cell suspension remaining on the surface is recovered by transferring the cover film only to the neutraliser solution and then recovering the remaining inoculum using sterile swabs. The number of colony forming units within the resulting suspensions are then enumerated using an appropriate microbiological technique (*eg* pour plate, spiral dilution *etc*). The method is described schematically in Figure 1 below.

Figure 1: JIS Z 2801: 2000 - Schematic Representation



The data from JIS Z 2801: 2000 is usually expressed as an antimicrobial value calculated from the difference between the Log₁₀ number of colony forming units (CFU) on the treated surface with that measured on the untreated surface although in some cases the actual data as CFU cm² is presented. A number of checks are imposed by JIS Z 2801 to validate the results. For example, the variability of the data for the number of CFU recovered from an untreated surface prior to incubation should be within a specified range. The population present on the untreated material after incubation should not be greater than 2 orders of magnitude lower than that recovered prior to incubation. Where an increase in the population on the untreated material is observed, any antimicrobial effect must be calculated using the population recovered from the untreated material prior to incubation. Failure to satisfy these criteria invalidates the formal test and compliance with JIS Z 2801 cannot be claimed however, data derived from the method can still be employed to describe the antimicrobial properties of a material provided a sound scientific interpretation is applied.

References

- 1 Japanese Industrial Standard JIS Z 2801: 2000 (E)
- 2 Suzuki S, Imai S and Korai H, (In Press) Background of JIS Standard Establishment for Antibacterial Products, Biocontrol Science.
- 3 Imai S (2005), The Development of an ISO Standard for Measuring the Antibacterial Activity of Surfaces, Hygienic Coatings and Surfaces, March 2005, Paris, France.
- 4 Askew PD, Iredale G (2002), Methods to Evaluate the Anti-Viral Activity of Coated Surfaces, PRA International Symposium on Hygienic Coatings, Brussels, Belgium, July 2002.
- 5 Askew PD (2005) Hygienic Surfaces: Defining the Terms and Supporting the Claims, Hygienic Coatings and Surfaces, March 2005, Paris, France.