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A CRITICAL EVALUATION OF SAMPLING METHODS USED FOR ASSESSING MICROORGANISMS ON SURFACES

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ABSTRACT

Methods used to evaluate the effectiveness of cleaning and disinfection regimes, or of putative antimicrobial surfaces, rely on an estimate of the amount of viable microbial cells remaining on a surface after treatment. In essence, microbial cells are applied to the surface, and the number remaining after a specified time/treatment is assessed by a variety of methods.

The simplest of these methods relies on removal of the cells from the surface, plating onto culture media, and counting the number of colonies obtained – since each colony is deemed to derive from one original cell/colony forming unit. Cells are removed by swabbing the surface, or by placing the surface in a diluent and applying force to remove the cells (eg vortex mixing, glass beads). However, there are significant problems associated with interpretation of results if the surface is not subsequently examined for residual cells: low numbers of colonies are deemed indicative of effective cleaning (*i.e*. few cells on the surface), but they may conversely indicate that the cells have not been removed from the surface. For transparent/translucent surfaces, light microscopy provides a relatively simple screen. For opaque surfaces, epifluorescence microscopy and a fluorescent stain to label cells is commonly used. Limitations of this method include non-specific staining of the surface, and the small area sampled using microscopy.

Contact plates utilise agar plates that are pressed against the inoculated test surface. The premise is that attached cells are transferred to the nutrient medium, and colonies are indicative of viable cells on the surface. The strength of attachment has been evaluated by repeated use of contact plates, until no cells are removed. The problem of strongly retained cells and non-flat surfaces are immediately apparent, and the number of cells on the surface can affect the accuracy of results obtained: too many cells, and counts are not possible; too few cells are not easy to find, by any method.

Agar overlays can obviate some of these problems. Molten agar is applied to the surface plus attached viable cells, which multiply to produce colonies. A vital stain can be applied to the top of the agar after incubation, to improve visibility of colonies. However, if an antimicrobial agent is able to diffuse out of the surface, then inhibition can be achieved under these conditions, when it might not be apparent *in situ* at a solid-air interface.

The diffusion of antimicrobial agents from surfaces is easily demonstrated via zones of inhibition of lawns of cells on agar plates. However, if surfaces are claimed to kill or inhibit cells by contact, then a different method is required. The LiveDead stain is applied to the surface: live cells stain green, and dead cells stain red, thus providing an indication of the proportion of viable cells on the surface. However the method is not entirely infallible.

Clearly it is important to be aware of the limitations of a given method for assessing the presence of microorganisms on a surface, as well as of the intended antimicrobial property of the surface or agent applied to the surface.