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Why it's time to strengthen and widen the microbial test panel

Tim Sandle **Head of Microbiology, BPL, UK**

Corresponding Author: **Tim Sandle, Bio Products Laboratory,**
Bio Products Laboratory
England

Email: timsandle@btinternet.com

Introduction

The testing of culture media, and conducting microbiological method suitability studies, disinfectant efficacy studies, Antimicrobial Effectiveness Test (or Preservative Efficacy Test) and associated activities, requires the use of a test panel of microorganisms. These organisms need to be representative of the intended application and be of a suitable range in order to demonstrate that a low level of viable cells can be recovered without any indication of the inhibition of growth or excessive growth-promotion (typically defined as the recovery of between 50 to 200% of the challenge count). This activity provides the basis for the release of culture media or for verifying that a method is suitable for recovering any microorganisms that might be present in a product sample.

This approach has been established for several decades and in many cases the types of organisms selected for the panel are drawn from guidance presented in the major pharmacopeia. The test panels of organisms recommended by compendia along with specific standards are in place to allow for reproducibility between laboratories. In the case of most standards (such as the disinfectant efficacy norms), these are designed to be multi-industry. Hence the presented organisms may or may not be suitable for the intended application.

In addition, with the compendia, all too often the organisms recommended for, say, the Microbial Limit Test method verification, are applied to the release of all culture media used for all activities, be that the test for sterility or for the recovery of organisms from water systems.

Not only is this inappropriate – for the microbiologist should be reviewing the panel and deciding upon the appropriate organisms – the recommended panels have not kept pace with improvements with our understanding of the types of microorganisms likely to cause contamination. Take, for example, the inclusion of Salmonella in the panel for the recovery of so-called 'objectionable microorganisms' for use with the Microbial Limits Test (1). To my knowledge and based on discussions with microbiologists over a couple of decades, no Salmonella has ever been recovered from a raw material.

Equally the selection, especially in relation to the testing of culture media used for cleanroom environmental monitoring, has not moved forwards with the findings from the human microbiome project and the depth of species richness found on the human skin microbiome.

The argument that the test panel quoted in the compendia is representative and thus if culture media release testing can recover such organisms then it can reasonably be assumed that any other similar organisms can be recovered does not really hold up. Unless, that is, the only concern is with the recovery of non-fastidious mesophilic organisms.

Instead, I maintain that in terms of the appropriate panel, this should be based on:

- What is currently being recovered, and
- What should theoretically be recovered.

Representing what is in the environment

Addressing the former, the aim would be to widen the test panel, to make the challenge organisms used more representative of what is being recovered from the environment or samples. With this many of the organisms recommended by compendia are less suitable. For example, for cleanroom monitoring if Cladosporium species are the most commonly isolated fungi, why not include these organisms? Consequently, why continue to include alternative organisms isolated many decades ago? Such as the isolate from a North Carolina blueberry bush (*Aspergillus brasiliensis* ATCC 16404). In addition, if *Ralstonia pickettii* is the most common isolate recovered from a purified water system, why not use this organism in place of the isolate from an outer ear infection (*Pseudomonas aeruginosa* ATCC 9027), as described in the compendia. Similar arguments can be made with *Staphylococcus aureus*, carried on the nares of around 25% of the population. Why use this organism rather than organisms found in greater abundance on microbial carrying particles released from the outer epithelial layers of the skin, such as *Micrococcus luteus* or *Staphylococcus epidermidis*? (2)

Another consideration is with Acinetobacter, which the is the genus of Gram-negative organisms most commonly associated with the moist areas of the outer layers of the human body (such as between the toes or under the arms) (3). Where anaerobic spore formers are of a concern, metagenomic analysis has identified a higher abundance of *Cutibacterium acnes* associated with human hair follicles, meaning that this organism is far more representative than the compendia prescribed *Clostridium sporogenes* (4).

In terms of what to include, microbiologists should regularly screen and trend the microorganisms recovered according to area monitored and sample type. Studying the range, types and patterns of microorganisms also assists with contamination control.

Using environmental isolates?

A related matter is whether replacement organisms or panel extension should be made up from cultures purchased from a culture collection (and hence which permit inter-laboratory reproducibility, should it be necessary) or made up of environmental isolates (synonyms are 'wildtype' and 'plant isolate') drawn from the manufacturing site. Arguments in favour of environmental isolates is that they will not have been sub-cultured innumerable times; they are representative of the facility microbiota; and they are sometimes more challenging to recover, given the adaption they have undergone when subjected to environmental stressors within the cleanroom (such as nutrient depletion, exposure to ultraviolet light, or osmotic shock) (5).

Whereas cultures derived from culture collections will not be in the same physiological state. Organisms that have resided in culture collections for decades have been grown on nutritive rich media over many, many generations and hence they have 'adapted' to be recovered on such media). The sole use of these organisms may not be sufficient to provide confidence that the medium can recover sublethally damaged organisms, exposed to a disinfectant, for example (6).

Some microbiologists, who favour an expanded panel, have a preference for type cultures and maintain that environmental isolates are not able to be standardized and therefore the test methods developed and used across different laboratories will not be comparable since different organisms have been used. Others are of the opinion organisms may well have been recovered in a stressed condition, but the stress adaptive response is not permanent and, after subculturing, the organism from the environment is not significantly different from any other laboratory strain.

Regulatory trends

Microbiologists should also pay attention to regulatory concerns in relation to certain products or their route of administration. With inhalation products, simply screening using the compendial test panel does not address the serious contamination potential posed by the thirty or so serovars that constitute the Burkholderia cepacia complex (7).

What theoretically should be recovered?

With the second point, the human microbiome studies indicate that the organisms deposited into the environment are more diverse than perhaps environmental monitoring data suggests. While it is impossible to test every potential organism, the desire to ensure that the test panel is representative should lead microbiologists to reconsider what is included.

An example is with the inclusion of Streptococcus. Not only is this organism difficult to recover from some formulations or preparations of tryptone soya agar its recovery signifies a contamination control concern (such as an ill-fitting face mask). In aseptic operations in particular, pharmaceutical microbiologists should wish to know whether or not their agars and incubation regime can recover organisms associated with the nasopharynx contamination vector (8).

Summary

The arguments made here are for a review of the panel and the likely replacement of some or all of the test set, with the test varying according to application (media used for water testing will have a different panel to organisms used for sterility testing, for example) (9, 10). There can be no specific guidance given in terms of the size of the panel, as this needs to be based on risk assessment, balancing practicalities and resources against the desire to be suitably representative.

In outlining the argument for a more scientific approach for the microbial test panel, it should also be noted that there is some regulatory shift in this direction. While this is less so with method qualification there are indications, from FDA warning letters for instance, for requiring periodic challenges of prepared media with low levels of organisms beyond compendia indicator organisms, such as those which represent normal flora.

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