Neutralizers to avoid false negatives in microbial monitoring

Regina Petrasch

Senior Application Development Manager BioMonitoring
Merck KGaA Darmstadt | Germany

Dr. Anne-Grit Klees

Global Product Manager Environmental Monitoring
Merck KGaA Darmstadt | Germany

Most applications for monitoring microbial contamination are based on general-purpose, nonselective culture media, such as Tryptic Soy Agar. However, aseptic processing environments require the routine use of disinfectants, so during surface sampling the swab or contact plate is likely to take up disinfectant residues alongside the contaminants, which could suppress microbial growth and lead to false negative results. This is why the culture media for such purposes must be supplemented with substances known as neutralizers that inactivate the disinfectants being used. USP <1116>, the FDA Aseptic Guide (2004) and ISO 14698-1 specify this requirement.

The right match

To neutralize antimicrobial activity and avoid false negative results, culture media have been developed that contain inactivating agents. Many different disinfectants are being used to prevent biocontamination at manufacturing facilities, therefore it is important to select the appropriate neutralizer formulation (Table 1).

Experiments performed by Merck have shown that volatile disinfectant components like pure alcohols and oxidative disinfectants, such as hypochlorite or hydrogen peroxide, may not leave enough residue on a dried surface to inhibit the growth of microorganisms.

The challenging ones

The stable ingredients of disinfectants, such as aldehydes, biguanides, quaternary ammonium compounds (QACs) and phenolics, remain on dried surfaces after sanitizing. To assess the neutralization efficiency of a medium the "direct plating method", simulating a worst case situation during sampling, is used. An amount of disinfectant is spread directly onto the agar surface of a neutralizer-containing agar plate. After a 20-minute exposure period, the plates are inoculated with 10 to 100 colony forming units (CFUs) of contaminant strains. After incubation, the colony count is compared with that of control plates not treated with disinfectant. Merck used this method to test its neutralizer-supplemented culture media in the presence of several commercially available disinfectants containing either phenol or QACs. All plates were shown to be effective at inactivating the tested disinfectants. Although lecithin and polysorbate 80 can neutralize most products efficiently,

Table 1: Widely used disinfectants and suitable neutralizers

Disinfectant	Suitable Neutralizer
Alcohol (e.g. IPA, ethanol)	Polysorbate 80 or dilution
Aldehydes	Sodium hydrogen sulfite, sodium thiosulfate, glycine, histidine
Sodium hypochlorite	Sodium thiosulfate
Biguanides (e.g. chlorhexidine)	Lecithin
Quaternary ammonium compounds (QACs)	Polysorbate 80
Phenolics	Polysorbate 80, lecithin
Peracetic acid	Buffer (e.g. phosphate buffer)
Hydrogen peroxide (VHP)	Pyruvate, catalase

also adding histidine (against aldehydes) and thiosulfate (against aldehydes and sodium hypochlorite) can expand the scope. Some disinfectants, however—especially those based on higher concentrations of QACs and polyhexamethylene biguanides, but also mixtures of aldehydes and QACs—are not sufficiently inactivated by lecithin, polysorbate 80, histidine and thiosulfate. For many of these challenging products, Merck has demonstrated its proprietary "Neutralizer A" mixture to possess sufficient inactivation efficiency.

It's similar for swabs

While agar-based contact plates are designed for monitoring of flat surfaces, swabs are more practical and often the only option for testing irregular or difficult to access surfaces, such as filling needles, cavities, tubing and Neoprene® gloves in cleanrooms and isolators. An "all-in-one" swab requires opening only once for sampling. All subsequent steps, such as the addition of the broth, incubation and detection, are performed within the closed system. Unlike contact plates, which allow colony counts, swabs are presence/absence tests, with turbidity indicating the presence of contaminants.

Swabs require neutralizers in their culture medium like contact plates do, but the procedure for testing neutralization efficiency is different. One set-up is that 5 x 5 cm stainless steel coupons are immersed in disinfectant and left to dry for three hours. Then the surfaces are swabbed, the growth medium added, and each swab tip inoculated with less than 30 CFU of the test strains. After incubation, the swabs are checked for media turbidity and the results compared with those of control swabs without prior disinfectant take-up. To test the neutralizing efficiency of Merck's ICR Swabs, which contain a general growth medium supplemented with lecithin and polysorbate 80, eight commercially available disinfectants were tested on five common contaminant strains, and six swabs processed for each combination. While the controls showed no growth in some or all swabs for disinfectants containing phenolics, aldehyde or QAC, all neutralizer-containing swab media displayed turbidity, except for *Staphylococcus aureus* against a high-concentration QAC disinfectant. But even this combination led to growth in all but two swabs, and spraying the disinfectant (as the manufacturer recommends) rather than immersion yielded a 100% growth response.

In-house testing required

The tests Merck conducts cover only a few disinfectants and the microbial strains stipulated in the regulations. Manufacturers must go beyond this and also test the products they use on strains that they have previously detected in the areas and isolators used for aseptic filling, as well as in adjacent cleanrooms.

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