How Environmental Monitoring Equipment can Reduce the Likelihood of Contaminations

Environmental monitoring of cleanrooms and isolators has been subject to a significant rise in regulatory requirements in recent years, as contaminations can spoil pharmaceutical products, slow their time to market and even result in recalls. The resulting need for sound and effective environmental monitoring processes has made the selection and handling of monitoring equipment used for monitoring increasingly important. Pharmaceutical companies and their suppliers have therefore been working to assess and improve the products used to test surfaces and ambient air in critical environments.

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A key concern of decision-makers in pharmaceutical production is that the instruments and disposables they use are validated according to established standards, in particular those of the FDA. As a matter of course, reputable suppliers that maintain a comprehensive range of environmental monitoring products make sure they fully validate their instruments for all relevant markets the world over, knowing that this gives them a competitive edge by saving their customers considerable time and costs. Many providers of culture media disposables perform studies to support the in-house validation that end-users need to perform.

Disposables which are intended for use in critical environments should be produced under at least equally controlled environmental conditions. Settle plates, contact plates and swabs should thus be filled in cleanrooms that meet high microbiological standards. They are usually double or triple packaged, depending on the class of cleanroom for which they are intended. This allows the outermost bag to be removed on advancing into the next higher class of cleanroom. For class A (ISO 5) cleanrooms and isolators, transparent triple bagging is perfectly suited. To neutralise a certain amount of H_2O_2 which may accumulate on the culture media (e.g. as a consequence of active air sampling), Merck Millipore supplements all culture media designed for use in isolators.

Dosing the radiation

Because the final packaged product cannot be sterilised outright, it is gamma-irradiated. However, the radiation dose must be finely tuned to ensure a contaminant-free product while also guaranteeing that the media does not degenerate and thus affect its capacity to grow microorganisms. In addition, the plastic packaging material must be qualified to withstand the radiation so that it does not become damaged easily when handled or shed particles that would compromise cleanroom safety. Over the years, Merck Millipore has found doses of between 9 and 20 kGy to provide just the right balance. Because aseptic filling is not a terminal sterilisation process, a Sterility Assurance Level (SAL) cannot be defined. However, supplier introduced colonies are only very rarely detected on gamma-irradiated media. When contaminants are indeed encountered these are usually not viable. In 2011, Merck Millipore irradiated some 14 million contact and settle plates. Not a single one was found to be contaminated by viable microorganisms as a consequence of the production process. Even so, as a precautionary measure, such culture media should be visually checked before use.

Minimising moisture loss

Passive air sampling generally involves the use of solid media settle plates. These usually contain Tryptic Soy Agar (TSA), a general purpose medium, or Sabouraud Dextrose Agar (SDA), which is ideally suited for growing yeasts and molds. The plates are placed at certain locations within the test area. After removal of their lids they are exposed to the air for a pre-determined period of time. Following exposition, the plates are closed and incubated, whereupon any colonies found are counted and the organisms identified.

A critical issue with settle plates is that, while exposed, they lose water due to evaporation, leading to an increasingly dry skin on the agar surface. This can lead to poor growth of certain microbes on the media and thus to an underestimation of the proportion of these organisms in the air. Over a typical four-hour exposure period in a unidirectional airflow cabinet, TSA plates were found to lose up to 16% of their original weight. However, when these plates were inoculated with typical contaminants and incubated, all recovery rates exceeded 70%. [1] To enable prolonged exposure and incubation periods while ensuring that the plates continue to deliver reliable results, settle plates should be poured to a particularly high filling level. However, the maximum length of exposure must be validated for each production line, taking into consideration air flow, temperature, relative air humidity and turbulences.

Dealing with residues

Surface sampling helps to determine the presence of viable microorganisms on surfaces in critical environments and on personnel. Typical surfaces that require such bioburden testing are laminar airflow workbenches, floors, gloved hands and difficult to reach areas such as the interior of tubing or filling needles.

To sample flat or convex surfaces contact plates are used. These plates are filled so that the solid media, usually TSA or SDA, protrudes. The agar surface is pressed against the surface to be tested, and any media residues are subsequently removed from the sampling site. After incubation the number of resulting colonies is determined.

By coming into contact with test surfaces, contact plates may pick up residues of sanitising agents used for cleaning or disinfecting cleanrooms. These substances, of which hundreds are used in pharmaceutical facilities around the world, may inhibit the growth of contaminants taken up on the same plate. To counteract the growth-inhibiting properties of such sanitisers, so-called neutralisers are added to the culture media. Appropriate combinations of lecithin, polysorbate 80, histidine and sodium thiosulphate can neutralise up to 80% of the sanitisers typically used. However, some of the remaining substances, such as polyhexamethylene biguanides, have proved very tricky. To cover a larger proportion of the sanitisers, Merck Millipore recently introduced a new neutralising mixture, designated Neutraliser A, in TSA contact plates. This has proved successful in neutralising all sanitisers tested so far, with recovery rates all above 50% compared to control plate counts. [2]

[1] *Sandle, T. (2011): Microbial recovery on settle plates in unidirectional airflow cabinets. Clean Air and Containment Review 6/2011.*

[2] *Hedderich, R. and Klees, A. (2012): Neutralization of Disinfectants by Culture Media used in Environmental Monitoring in Environmental Monitoring – A comprehensive Handbook, Volume 6: 159-180*

BSE/TSE-free vegetable media

Because TSA contains animal ingredients that may possess the potential to transmit BSE/TSE, contact plate media have become available that contain no substances of animal origin at all. The growth promoting properties of one such solid vegetable contact medium was tested for various strains originating from culture collections (see Fig. 1). The results indicate culturability roughly comparable to that of TSA.

Material of non-animal origin, and thus free of BSE/TSE, is also used for media fill trials, which are conducted at regular intervals to simulate the actual filling process as closely as possible. In these trials, media is used instead of the pharmaceutical ingredients. Like animal-based peptone media, these vegetable media allow growth of a similarly broad range of microorganisms. In quantitative tests of 20 Gram positive and 8 Gram negative bacterial strains as well as 8 fungal strains, the growth-promoting properties of this vegetable peptone broth were found to be similar to those of Tryptic Soy Broth (TSB). [3]

All-in-one swabs

For testing irregular surfaces, such as equipment recesses, nooks, crevices, tubing and filling needles, where contact plates are difficult to cope with, pre-moistened swabs may be used to validate cleaning and sanitation procedures and to verify that a required level of cleanliness has been reached.

For maximum recovery the swab is carefully removed from its tube and rubbed across a surface area using a twisted motion. Conventional swabs are then resuspended in a specified amount of rinse solution and agitated to transfer any microorganisms present on the swab head into the solution. The collection medium is then tested, usually by either direct plating or membrane filtration and incubation of filters on culture media.

A drawback of conventional swabs is that the recommended procedure involves a number of steps, each of which carries the potential for handling errors and thus adventitious contaminations. To undertake without having to open the tube several times, an all-in-one swab system has been developed which requires opening only once because it already carries with it a reservoir containing the culture broth. After returning the swab to the tube, the reservoir at the top is snapped and squeezed until the broth floods the swab head. After incubation the broth is monitored for turbidity, which would indicate contamination. This ICR swab is gamma-sterilised in its final packaging at a relatively high dose of 25-35 kGy. Despite this, both the moistened swab tip and the medium were shown to be non-inhibitory against the challenges of eight microbial species that are more commonly isolated from aseptic processing environments. [4] This swab is also suited for use in isolators because it is triple-packaged, with the inner bag possessing a pre-punched hole to hang it up.

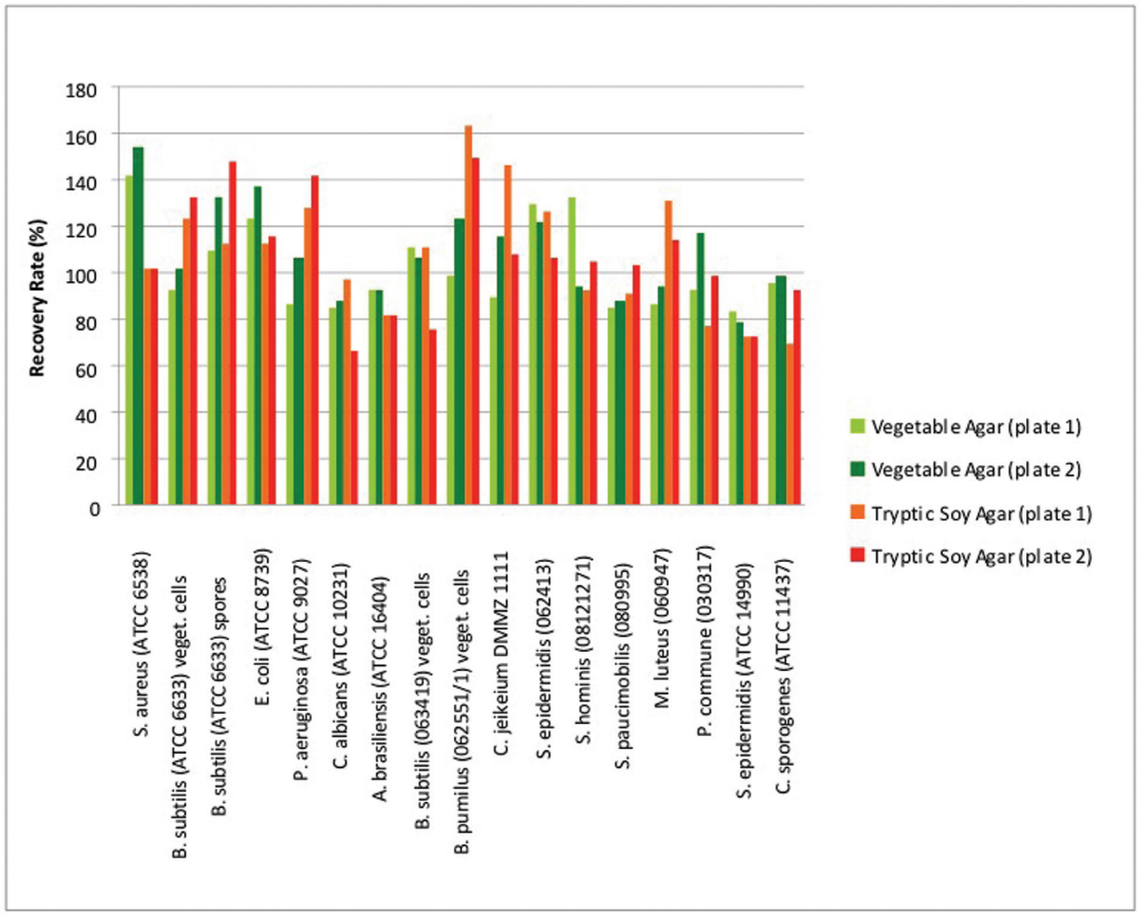

Figure 1: Recovery rates of microbial strains on a solid vegetable contact medium and TSA (test in duplicate)

Instrumentation properties for reduced risks

Instruments used to actively sample air for microbes or particles draw a pre-determined volume of ambient air. Microbial air samplers direct the air stream at an agar plate or strip for collection. It is important that the air flow is not disrupted by the instrument's operation, placement or removal, as contaminants then find it easier to attach to its housing. The MAS-100® and RCS High Flow Touch air samplers (Merck Millipore) are specifically designed with rounded edges to minimise air flow disruption. To minimise disruption in isolators and other confined areas and to save space, manufacturers have developed variants of their instruments. Their air ducting is directed from the sampling points outside the controlled area where all electronic and moving parts remain. The internal pumps of the instruments enable easy decontamination of the sample heads and aspiration tubes.

Additionally, particle monitoring of ambient air is regularly conducted to determine the air quality in controlled environments. In many pharmaceutical production facilities, the particle counters are pre-installed. However, if the need arises to modify the particle counting system at a later date, this can prove disruptive. Many pharmaceutical companies thus opt for the flexibility of portable particle counters. For example, Merck Millipore's APC SmartTouch can be programmed from a touchscreen and is able to display a visual representation of a cleanroom's sampling locations, thus reducing the likelihood of human errors in sampling. Because it is equipped with two high-capacity batteries, it can sample continuously without the need for downtime during recharging. With all the pros and cons to consider, it is usually a strategic decision whether to choose pre-installed or portable particle counters.

[3] *Hedderich, R., Klees, A., Eiermann, K., Greulich, Y. and Müller, R. (2009): Growth promoting properties of a vegetable peptone broth (VPB) in comparison to tryptic soy broth. Poster at PDA Annual Global Conference on Pharmaceutical Microbiology.*

[4] *Sandle, T. (2011): A study of a new type of swab for the environmental monitoring of isolators and cleanrooms (the heipha ICR-Swab). European Journal of Parenteral and Pharmaceutical Sciences, Vol. 16, No.2, pp42-48.*