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Microbiological quality control procedures improved through use of modern image analysis

by Karen Capper

One of the main functions of microbiology laboratories in modern pharmaceutical companies is to carry out routine quality control testing. This involves testing various types of samples for the presence of contaminating bacteria. Although the initial screening tests are performed using relatively simple culture plates, the fact that large numbers of such plates have to be screened means that accurate archiving and transmission of the results can be a challenge. Modern image handling and archiving systems can dramatically improve the reliability of microbiology QC systems and ensure speedy transmission of potentially vital data to the appropriate site. The case study presented in this article describes how AstraZeneca have successfully incorporated the use of image handling systems into their microbiology QC procedures.

Based at Macclesfield in the UK, AstraZeneca is a major international manufacturing site of pharmaceuticals. Here pharmaceutical products are produced and converted into the most suitable format (tablet or injection). They are tested and packed in strict accordance with regulatory data and are dispatched to customers in the UK and over 100 export markets.

Pharmaceutical manufacturing plants have to be kept as sterile as possible because many of their products are administered to patients suffering from debilitating conditions, such as cancer. This means that patients who are being treated with a particular drug can often be immunocompromised. The accidental introduction of even a simple microorganism - which when infecting most healthy subjects would be harmless - could produce a serious, life threatening infection.

At AstraZeneca in Macclesfield, the microbiology department of 85 staff is responsible for quality testing of around 30,000 samples per month. These include not only pharmaceutical products themselves, but also water and environmental samples taken from a number of sterile environments at eight manufacturing sites.

To perform microbial testing for bacteria and fungi that may have accidentally contaminated any of these sterile areas, the microbiology department uses a variety of plating methods such as pour and contact plates containing all purpose culture media.

On average, out of the large number of samples tested, two to three positive plates are detected per day. These require further investigation. For quality control purposes a digital photograph of each positive plate has to be taken as documentary evidence of the contaminant.

Based upon this evidence, decisions are then taken as to whether to carry out corrective action. This can sometimes be as straightforward as excluding certain personnel from a plant area. In more serious cases it may be necessary to shut down a whole plant to allow a more detailed investigation of the source of the problem and to thoroughly decontaminate the area.

Since the department's microbiologists are not experts in digital photography, many found it difficult to set up the digital camera in such a way as to produce consistently good images of the plates. A small number of the department's personnel were therefore trained to use a digital camera and were assigned the responsibility for taking images in a batch fashion on a weekly basis.

This process could take around one to two hours per week. Depending on the availability of the assigned technicians, this system frequently resulted in positive plates not being photographed as soon as they were generated but having to be stored or refrigerated until a suitable staff member was available to record the plate image.

The department was concerned that this could result in manufacturing plant staff not being alerted to contamination as rapidly as they should be. This situation in turn had the potential of allowing the original, minor problems to escalate into something more serious.

To overcome all the difficulties of capturing and storing images of culture plates, the microbiology department at AstraZeneca chose the aCOLyte colony counting system (Figure 1) as a quick and simple method of generating plate images.

Microbiological testing of water

Samples of water are taken from filters that are used in the production of water for pharmaceutical products. The water samples are added to molten R2A agar, poured into petri dishes and are then incubated at 35°C for 48 hours. R2A agar is used because of its nutritional richness which allows the recovery of certain organisms, even though they may be stressed or chlorine tolerant. The result is a more realistic enumeration of bacteria in water.

Figure 1. The Synbiosis aCOLyte system used by the microbiology department in Astra Zeneca.

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Any positive plates are photographed using the aCOLyte system and the images are then emailed to the appropriate plant manager with recommendations of what action to take to prevent further contamination.

Quality control of environmental samples

To monitor the quality of air that is circulating in the plant, contact plates containing Tryptone Soya Agar (TSA) are placed open for 10 minutes around the plant area. Plates are then incubated at 37°C for 48 hours.

The protective gowns worn by the manufacturing staff, as well as their hand hygiene, can be monitored by pressing a section of the gown and their fingertips respectively, onto separate TSA contact plates. These plates are then incubated at 37°C for 48 hours. Any positive results are photographed using the aCOLyte and the images are emailed to the appropriate plant manager.

Figure 2. Image generated by the image analysis system showing a TSA plate with Staphylococcus spp. colonies around the finger dab areas.

TSA is used for the monitoring of environmental samples because it is a good all-purpose medium, which will support the growth of a wide variety of organisms. It is suitable for the cultivation of both aerobes and anaerobes.

Monitoring plate labelling

To keep a track of the hundreds of samples that pass through microbial quality inspection each day, every plate is fully labelled with details of where and when it was sampled. Any labels that are incorrectly filled out are photographed using the aCOLyte system and emailed through to the manufacturing department concerned.

Results

AstraZeneca operates an index system of acceptable levels of contamination based on established international quality standards. This index ranges from 1 colony forming unit (c.f.u.) per ml in critical samples such as the pharmaceuticals themselves to 100 c.f.u/ml in the water used in production.

The image analysis system helps in keeping records of contamination levels by generating, for example, images of bacteria from water used in the manufacture of pharmaceutical products. These results are used as a guide to decide whether or not to decontaminate the water filtration units. The system is also being used to produce images of fungi and bacteria, the most common being *Staphylococcus spp.* (Figure 2) isolated from environmental sources such as finger dabs and protective gowns. Any positive plates obtained result in the staff undertaking a more rigorous programme of good hygiene practices. These include more regular gown changing and hand washing, as well as the use of antiseptic hand sprays.

Images of common air borne contaminants such as mould or *Staphylococcus aureus* are also produced using the system. In the case of moulds, this often indicates a problem with the pressure in the laminar flow units leading to microorganisms not being filtered out quickly enough. If unacceptable levels of moulds, are seen, corrective action is initiated, involving checking the pressure in the air filtration units and carrying out a sporicidal clean-up process.

Staphylococcus aureus is, of course, a skin borne bacterium, so *Staph. aureus* contamination is often linked to specific personnel. The generation of positive plates is a stimulus for staff to carry out more rigorous hygiene practices, which include using antiseptic hand and nasal sprays. If these measures fail to solve the problem, then individuals are removed from critical aseptic areas.

The system also allows the microbiology department to highlight any incorrect labelling of quality control plates. The ability to quickly send email images of labels improves the overall quality process by helping to pinpoint where individuals require more training in administrative procedures.

Conclusions

The aCOLyte image analysis system in use at the microbiology department of AstraZeneca generates and archives a range of plate images, together with the time and date at which they were photographed. These GLP-compliant results are provided in a format which can be sent by email and can be presented with confidence to external regulatory auditors such as the Medicines Control Agency (MCA).

In practice, many of the images produced by the system are of such high quality that they can be used to train plant personnel, (most of whom are non-microbiologists) to distinguish between different types of common contaminants, such as, *Staphylococcus aureus, Bacillus spp.* and *E.coli.*

The use of the system has significantly reduced the time it takes each microbiologist at AstraZeneca to capture and email images of plates and labels. This is principally because the system is simple to set up and requires no camera skills or special training. The built-in LED illumination also offers optimised viewing of any plate type, making it easy to produce focused full-colour on-screen images. All of the microbiologists in the microbiology department are now using it to simultaneously produce and email results as soon as the plates come out of the incubator. This alerts manufacturing plant personnel to any potential microbial infections much more rapidly and prevents minor contamination from escalating into serious problems.

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