Role of in-house isolates in pharmaceutical quality control

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MICROBIAL contamination of pharmaceutical drugs adversely impacts their efficacy and quality presenting a significant patient risk. To ensure potency and safety of sterile drugs, it is imperative to monitor the quality of the aseptic processing environment by deploying an effective and robust environmental monitoring (EM) programme. This involves evaluating key sources of contamination during the aseptic process such as personnel, facility, utilities, materials, equipment and production processes. Continuous tracking and trending of EM data not only helps determine the effectiveness of cleaning and disinfection procedures, but also allows changes in local microbiota to be closely monitored, identifying any early warning signs of a breakdown in control measures.[1,2]

In-house isolates are the culture of microorganisms recovered from the manufacturing environment, including cleanrooms, water systems, raw materials, contamination incidents and equipment. These strains are representatives of the local microflora and are also known by other names such as environmental isolates, plant isolates, wild-type isolates, factory isolates or facility isolates.[3,4]

Relevance of in-house isolates in microbiological QC testing

Over the years, the importance of in-house isolates in microbiological QC testing has been a topic of discussion within the industry community. This is primarily due to multiple regulatory guidance documents published in past couple of decades, emphasising the incorporation of in-house isolates in growth promotion testing (GPT) of culture media and validation studies.[3-6] More recently, EU GMP Annex 1 "Manufacture of Sterile Medicinal Products" recommends use of representative local isolates for the GPT of media fill material.[7,8] Moreover, there are several Food and Drug Administration (FDA) Form 483 observations and warning letters reported for non-inclusion of in-house isolates in microbiological assays, which confirms regulatory expectation around it. Interestingly, the warning letters for this expectation have dwindled in recent years, which perhaps indicates that most companies have accepted this as a regulatory requirement and hence have extended the panel of their compendial test strains to include in-house isolates for growth promotion challenge of culture media, disinfectant efficacy study, microbial assay validations, and antimicrobial effectiveness testing.[3-5]

Since in-house isolates are considered to be true representatives of facility microbiota and have not been subcultured numerous times, it can be argued that they are a good choice to bring greater confidence in microbiological quality control. Moreover, microflora within cleanroom and water systems are adapted to environmental stressors (such as nutrient deprivation, disinfectants, osmotic stress, UV light) resulting in phenotypic variations within same species and hence behaving differently. Therefore, stressed and acclimatised local microorganisms can well complement the small number of standard test strains for culture media challenge testing and other validation studies, as well as enhance robustness of test methods.[4,9,10] However, local isolates are difficult to standardize, making it problematic to compare test methods across different laboratories. In addition, the stability of their "wild" attributes upon subculturing on laboratory culture media is often challenged.[6,7,11] Serial subculturing on nutritive-rich media almost certainly drives strain evolution causing loss of stress adaptive response; however, how quickly these unique phenotypes are lost is not well understood. One of the common arguments for reducing the risk of losing wild-type characteristics is by minimising the number of subcultures of local isolates.[3,4,6]

Selection and implementation of in-house isolates

It is vital to formalise the selection approach of local strains in a procedure, as failing to do so presents compliance risk. Although compendia and regulatory guidance documents have made recommendations about the use of in-house isolates, they mostly lack specific guidance regarding how to select. Nevertheless, one common approach is to understand the intended use of a culture media before determining the relevant local isolates to include. For example, inclusion of isolates associated with EM and utility systems in a GPT challenge panel of EM culture media. Likewise, it is worth considering the inclusion of isolates from sterility test and media fill failures for GPT of sterility test media and media used for aseptic process simulations.[5]

Continuous preparation and maintenance of microbial isolates is challenging, putting

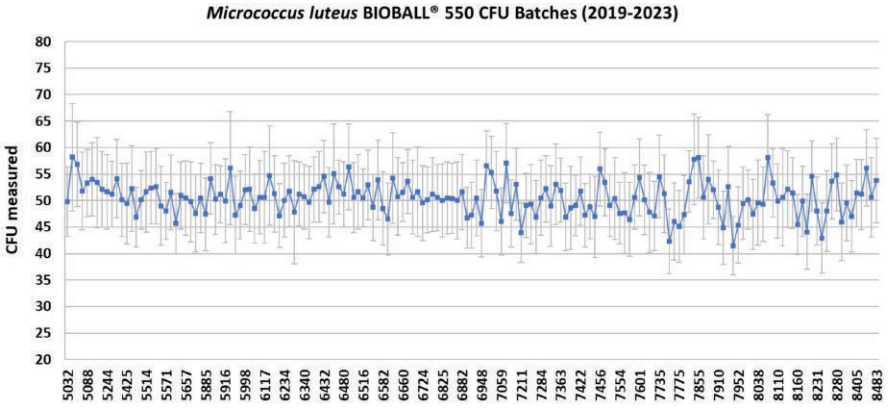

Figure 1: Quality control results of the released Custom BIOBALL® 550 batches over five years (2019 to 2023), manufactured from Micrococcus luteus isolates received from different geographies. Error bars represent standard deviation based on the repeated aliquots.

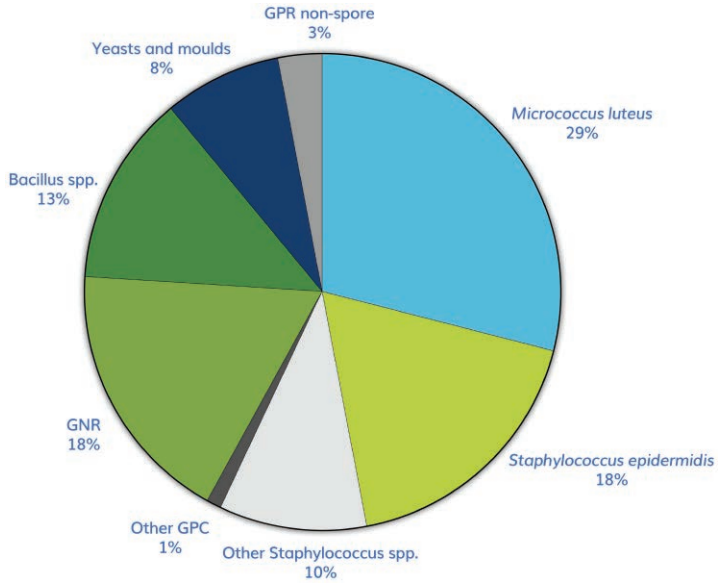

Figure 2: Distribution of in-house isolates converted into BIOBALL® format as part of bioMérieux's BIOBALL® Custom Services (2019-2023). GPC: Gram-positive cocci; GPR: Gram-positive rods; GNR: Gram-negative rods.

additional pressure on laboratory resources and increasing overall operational cost. Additionally, in-house preparation of microbial suspensions presents a high risk of inoculum variability. Therefore, experts suggest performing stability studies on microbial suspension during storage and assigning an expiry date to a standardised suspension, further increasing complexity and cost of the process.[5]

BIOBALL® Custom Services helps to simplify the implementation of in-house microbial strains and bring greater confidence in routine microbiological quality control by preserving and manufacturing received in-house isolates in ready-to-use BIOBALL® format. It utilises proprietary cytometry technology to produce an accurate and precise quantitative reference material demonstrating consistent CFU counts batch after batch (**Figure 1**). Every year, BIOBALL® Custom Services works with a vast number of in-house isolates from different geographies, customising them into an accurate and precise BIOBALL® meeting specific QC testing needs of pharmaceutical manufacturers.

Common microorganisms isolated in pharma labs

Over the last decade, the in-house isolates developed into BIOBALL® have remained extraordinarily consistent. The following is a list of some common isolates frequently requested by pharma labs to be converted into BIOBALL® format as part of bioMérieux's BIOBALL® Custom Services (**Figure 2**).

1. *Micrococcus luteus* and *Staphylococcus epidermidis*, gram-positive cocci, are the most common skin commensals observed in pharmaceutical cleanrooms attributed to the operators within the environment.
2. *Bacillus cereus*, gram-positive spore-forming rod, is notably one of the most commonly isolated species of *Bacillus* in pharmaceutical cleanrooms. Due to its ubiquitous nature, spore-forming *Bacillus cereus* presents a considerable risk to product quality and patient safety, thus requiring an effective cleaning and disinfectant regime, efficient air handling systems and a robust EM programme to continuously monitor contamination concerns.
3. *Ralstonia pickettii*, gram-negative oxidase-positive rod, is one of the most common isolates found in pharmaceutical water systems due to its ability to proliferate and form biofilms in low-nutrient conditions.
4. Mould such as *Penicillium chrysogenum*, is a fungal contaminant found in cleanrooms due to poor design, ageing facilities, poor control of incoming materials and ineffective air handling systems.

Conclusion

Based on generally accepted opinion, in-house isolates indeed have a place in pharmaceutical microbiology bringing greater confidence in efficiency of culture media and validation studies. It is recommended to establish the selection rationale of in-house isolates and document in a procedure before implementation in microbiological assays. Considering the challenges associated with their internal handling, BIOBALL® Custom Services helps to simplify in-house isolates management by manufacturing them into ready-to-use BIOBALL® format, thereby improving operational efficiency and minimising QC failure risks.

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Megha is Global Solutions Manager for Pharma Quality Control in bioMérieux Industrial Applications Unit. With over eight years of experience in product management and commercialisation, Megha currently manages global growth strategies, market research, scientific marketing and business activities for BIOBALL® portfolio. Megha holds a PhD in Biomolecular Chemistry and Microbiology from The University of Queensland, Australia, and has authored several peer-reviewed articles in microbiology and chemical biology.

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