Pharmaceutical BIOPROCESSING

Microbial contamination: challenges and perspectives

Pharmaceutical Bioprocessing invited a selection of leading researchers to express their views on managing and eliminating the risks of microbial contamination during production of biopharmaceuticals. Topics discussed include emerging microbial detection technology, risk assessment strategies and insight into what the future holds for microbial elimination.

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There have been many developments in analytics in the last 5 years. What do you believe the future holds for microbial detection technology?

Next-generation sequencing (NGS) or massively parallel sequencing provides exciting possibilities for identifying viral contamination but the routine use of this type of technology is not yet with us and will require careful standardization and availability of reference materials to assure suitable and reliable sensitivity. It will also need establishment of genomic databases of greater reliability regarding presence of adventitious sequences from experimental contaminants.

Expanded PCR assays with focus primer/oligo mix of panels of microorganisms, viruses are more practical for the short term.

Looking to the future, what are the key challenges we are still facing in terms of microbial contamination? Where do you predict improvements will lie in the next 5–10 years?

Determination of contamination source is the challenge. Some contamination was caused by raw materials and some were assumed but without solid evidence. Reliable raw material management programs and more sensitive detection technology will help to minimize the contamination. In addition, development and implementation of microbial inactivation/removal technologies for medium treatment will be a very effective prevention approach. Culture medium treatment via heating, radiation, UV light and viral filtration (VF) have been presented lately. Choice of technology for use depends on cell culture scale, culture type (fed batch vs continuous perfusion) and medium composition. For large-scale culture vessels, the demonstrated technology is heating medium by high temperature short time (HTST). We still face great challenges to get other technologies like VF, UV and radiation into routine use. These challenges are scalability, operation complexity, incompatibility with some medium chemicals and not economic. For these reasons, it seems that only very few organizations are able to have the protection in their cell culture facilities.

A key consideration in ensuring safety is obtaining a thorough cell line history – what would you say are the key challenges in this respect?

For avoiding contamination via a cell line, the clear history of the cell line is not sufficient. Attention must be paid to a safety proof program in cell cultivation, passage, cell line development, characterization and testing. Raw materials of cell culture are also very important.

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In your opinion, what part does a robust risk assessment strategy play in preventing microbial contamination?

Reduction and elimination of bioprocess contamination is a comprehensive play. As I always mention at seminars, we have to have a risk management plan to assure host cells, cell banks free of pathogen contamination, reagents and medium components are free of animal or human sources. Careful monitoring process with sensitive methods to detect adventitious agent into bioreactors. The industry should continue to develop and implement more advanced technologies to contain and clear viruses from any feed stream of cell culture and facility. In addition, information and knowledge sharing will benefit us to eliminate potential contamination.

Considering recent developments in viral filtration technology, how do you envisage this process developing over the next 5–10 years?

Viral filtration has progressed significantly in retention of viruses. Some virus filters achieve complete removal of viruses such as parvoviruses, below detection level. In the next few years, viral filtration can remove viruses smaller than parvoviruses with higher capacity and reduced cost. Circoviruses and nanoviruses are smaller than parvoviruses. Circoviruses are commonly found in a broad range of animal species – that is, avian, bovine, canine, feline and porcine etc. Porcine circovirus (PCV) or its genetic substance contamination of cell bank, cell culture and final products have been reported lately [1]. PCV is the smallest virus which has two types, PCV1 and PCV2. It is nonenveloped with an unsegmented circular genome, a ssDNA of 1759 nucleotides (nt) for PCV1 and 1769 nt for PCV2. The viral capsid is icosahedral and approximately 17 nm in diameter. PCV would be an ideal model virus for viral filtration assessment as the worst case when the mechanism is based on size exclusion. Significant and robust removal of PCV will bring an elevated level of safety confidence if the biological manufacturing process involves animal-derived raw materials or cell lines. However, challenges are faced in the lack of simple, fast and sensitive infectivity assays, as well as in the production of high titer virus stock used for viral filtration studies.

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In your opinion, what has been the biggest technological advancement in terms of eliminating the risks of microbial contamination in the last 5 years?

In the last 5 years, the risk of microbial contamination in industrial animal cell culture has been substantially reduced through the ongoing development and implementation of high-temperature short-time (HTST) systems. This includes the implementation of at least 10 new HTST systems at leading firms over that time period [2]. Note that the term 'microorganism' (synonymous with microbe) refers to any noncellular or unicellular (including colonial) organism, most of which are too small to be seen by the unaided eye. Microorganisms comprise bacteria (including cyanobacteria), lichens, microfungi, protozoa, rickettsiae, virinos, viroids and virus and also some algae; all prokaryotes are included [3]. HTST is a method of inactivating virus and certain other microbes by treating cell culture medium with high heat (approximately 102°C) for a short period of time (typically 10 s). When used with animal cell culture, HTST treatment at 102°C for 10 s will typically kill all virus and many vegetative microbes, but is not adequate to kill certain heat-resistant bacterial spores. Such spores are typically removed from cell culture medium through filtration, following HTST treatment at 102°C. HTST systems with much higher temperatures than 102°C can be used to kill all microorganisms, including spores, and have been used for this purpose for years by industrial microbiologists. Approximately 40 years ago, these HTST systems were often called 'continuous sterilizers' and were implemented to reduce contamination rates and increase throughputs, while minimizing thermal destruction of nutrients, for industrial microbial cultures [4].

Looking to the future, what are the key challenges we are still facing in terms of microbial contamination? Where do you predict improvements will lie in the next 5–10 years?

One key challenge is how to more efficiently identify and handle 'false positives' from PCR assays. These occur when DNA is released from dead organisms but remains sufficiently intact to later be detected by PCR. This can lead to very time-consuming investigations as well as needless discard of uncontaminated batches. Well-accepted follow-up protocols to positive PCR readings, which discern false versus true positives, as well as ensure patient safety and satisfy the requirements of regulatory agencies, are hopefully in development. When complete, they

need to be widely communicated and adopted.

Another key challenge is how to drop certain activities or protocols that were implemented years ago in panic situations but are not actually effective in reducing contamination rates. For instance, gowning in full coverage (bunny) suits to enter areas that have closed bioreactors has no rational basis and has not reduced contamination rates versus simple lab coats and safety glasses [5].

There have been many developments recently in the field of NGS technology – what implications do these developments have for microbial detection?

NGS technologies will allow us to more rapidly sequence the genes in microbial contaminants, with more thorough coverage. By comparing these delineated sequences to those in published databases, as well as those determined for microbial samples from various possible contamination sources, 'smoking guns' (highly likely sources) will be found more quickly and more often, with greater reliability. NGS also offers the capability to identify unsuspected contaminants that might not be addressed in risk assessment thus will promote safer products in the long term.

Considering recent developments in viral filtration technology, how do you envisage this process developing over the next 5–10 years?

In the next 5–10 years, viral filtration technology will be increasingly used for 'point-of-use' barriers around industrial cell cultures. It will be used for smaller-volume liquid supplements that cannot withstand regular HTST treatment at 102°C for 10 s. It will be used for all gas streams, including air, oxygen and carbon dioxide. New filters will hopefully be implemented that provide for good viral removal (log reduction value >>4) from gas streams even when the filter is wet. For small volume applications, such as manufacturing of personalized medicines, including cell therapies, viral filtration will hopefully be implemented to reduce the risk of virus contamination in such products. At such scales, it may prove to be cheaper and faster to implement than HTST. For moderate scales, viral filtration will become increasingly adopted as new filters are developed which have both higher flux values for culture medium and higher retention of virus in the 20 nm range. Hopefully, viral filtration and/or HTST will be implemented for medium pretreatment and/or 'point-of-use' upstream barriers for viral vaccine processes; such processes often have little or no downstream clearance of potential viral contaminants.

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Looking to the future, what are the key challenges we are still facing in terms of microbial contamination? Where do you predict improvements will lie in the next 5–10 years?

One of the major challenges in progressing cell culture based therapies is the ability to deliver sufficient bulk cultures while assuring freedom from microbial contamination. Key elements to address this challenge include:

- The need to train lab workers in good cell culture practice to avoid common routes of lab contamination (e.g., [6,7]);
- Assuring appropriate risk assessment of raw materials from an increasingly diverse (such as raw materials of plant, insect and crustacean origin) and an international supply chain;
- Ability to discriminate between contamination with live organisms and their non-viable DNA alone;
- Standardization of NGS technology (e.g., qualified sample processing to assure detection of a broad range of organisms, provision of reference materials [see below]).

A key consideration in ensuring safety is obtaining a thorough cell line history – what would you say are the key challenges in this respect?

Use of cell lines established in research laboratories may represent a significant risk, where traceability of cell source, raw materials and storage environment may be difficult to establish. The development of a cell line history file con-

taining a record of derivation, preparation and testing of a candidate cell line for human application provides a central source of information on each cell line. Such a cell line history file can incorporate data from the various approaches to assuring cell safety of a cell line, from donor selection and traceability of raw materials to low risk sources, to records of cell bank preparation and testing. This presents a means to obtain regulatory approval for the cell line in numerous and independent applications (International Stem cell Banking Initiative, Submitted July 2014).

Demonstration of traceability to fully informed and appropriate consent for donor cells can be difficult and may vary between countries. The hESCreg project [8] has recently been funded by the European Commission to establish the means to check ethical and scientific suitability of pluripotent stem cell lines (both human embryonic cells and induced pluripotency stem cell lines cells) for research and clinical use. This registry will provide an important starting point for those seeking pluripotent stem cells for clinical application and hESCreg qualification will be a requirement for use of any pluripotent stem cell lines proposed for development in European Commission-funded programs.

There have been many developments recently in the field of NGS technology – what implications do these developments have for microbial detection?

NGS potentially could provide a single test for all microbial contaminants in cell cultures and reagents, which would be ultimately far cheaper and less complex than the multiple detection assays carried out today. This technology has been responsible for discoveries of new and unexpected contaminants, including genetic variants of an original virus missed by established PCR tests; agents not previously known to grow in cell culture and even previously unknown agents. Thus, there is clear evidence that NGS provides a screening method that can enhance product safety more comprehensively than competing microarray techniques. However, there are significant scientific issues relating to the use of NGS for safety testing that need to be addressed to provide data that can be used with confidence by manufacturers of cell-derived products, such as vaccines, biotherapeutics and cell-based medicines. It will be vital to be able to determine the veracity of a 'negative' result, in particular, optimizing sample preparation to assure detection of a wide range of different agents and monitoring sensitivity of detection for these agents. Appropriate responses to these challenges include the development of exogenous control samples for virus-like nucleic acids for the detection of different viral nucleic acid forms. Work recently initiated in the Pluripotent Stem Cell Platform project (Theme 1 of the UK Regenerative Medicine Platform [9]) will address detailed molecular characterization of pluripotent stem cell lines for clinical application and part of this program aims to generate optimized protocols to ensure minimal bias and maximum sensitivity in NGS for adventitious agent detection. The project also intends to produce prototype, publicly available, reference standards for NGS testing.

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