Commentary

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# Microbial contamination in industrial animal cell culture operations

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Industrial animal cell culture is used to make many life-saving biopharmaceutical proteins, vaccines and cell therapies. Contamination of an industrial animal cell culture with a microorganism, such as a bacteria or virus, may occur through many means, for example, human error, inadequate aseptic protocols within biosafety level 2 (BSL-2) cabinets, failure of a processing step such as steam sterilization, loss of equipment integrity such as a crack in a disposable bioreactor, and/or introduction of a new adventitious agent not susceptible to current removal or inactivation procedures. The probability of having one such problem, anywhere along a linked sequence of operations (such as a batch), typically increases with the number of operations per sequence (or batch). As such, the probability of microbial (including viral) contamination typically increases with scale, as well as culture duration and/or complexity.

As defined in the Oxford dictionary, a microorganism is any noncellular or unicellar (including colonial) organism, most of which are too small to be seen by the unaided eye [1]. Microorganisms comprise bacteria (including cyanobacteria), lichens, microfungi, protozoa, rickettsiae, virinos, viroids and viruses, and also some algae; all prokaryotes are included. The term microorganism is synonymous with the common term microbe (adjective microbial).

A Biosafety Level 2 (BSL-2) cabinet, also called a Class ll biological safety cabinet, provides an enclosed workspace, ventilated with vertical downward flow of sterile-filtered air. The air flow is ideally laminar, and with proper aseptic technique, can provide a sterile workplace for open culture operations. A glass shield, along with the air flow path, protects the worker from exposure to cultures and/or contaminating agents rated at Biosafety Level 2 or lower. For drawings and photos, see figures 6.2, 6.3, and 6.7 in [2].

At various times in their history, many firms have suffered periods of unacceptably high contamination rates (20% or higher). For the reasons mentioned above, this has often occurred during initial scale-up, wherein many firms first painfully discovered that their operating protocols were not sufficiently robust. It has also occurred many times during initial plant start-ups. For viral contamination, it has occurred upon introduction of a new cell line to a large-scale plant, wherein the line is susceptible to an occasional viral contaminant that may have gone undetected when the plant held only a non-susceptible cell line(s).

In response to such contamination crises, many firms simultaneously implemented a large number of changes, in emergency mode, without first identifying the source of the problem or thus understanding the likely effectiveness of any given change. Over time, one key change or two typically solved the problem. Sometimes the source of the problem, as well as the key change(s) that actually solved the problem, were identified. Other times, no such clear identifications were made. In nearly all cases, the whole slew of changes were carried forward, even though some were likely ineffective, as well as a waste of time, money and focus.

For the mutual benefit of both the industry and patients, there is an on-going need

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for industry consortia, benchmarking, and published case studies, as well as targeted experimental studies, to identify best practices and new approaches to avoid microbial contamination. Some publications, such as the authors' recent one in this journal [3] and others by our industry colleagues [4–10], have partly addressed this need. Nonetheless, the majority of the case histories and outcomes are not published, nor included in benchmarking studies. Croughan has been involved with solving dozens of large-scale contamination problems for nearly 30 years and knows of only a handful of published case studies. Even the ones that are published are not well known nor often discussed among smaller firms.

Some specific observations and needs that have been identified, for example, are as follows:

» Many firms have implemented changes in their small-scale operations, wherein cultures are grown in shaker flasks or spinners and handled in BSL-2 cabinets. These changes have included improved staff training and protocols for open operations within BSL-2 cabinets, as well as changes in gowning, room access, segregation, air locks and/or pressure differentials adjacent to such cabinets. In many circumstances, these changes have substantially reduced contamination problems. These changes often had a sound scientific basis, usually centered on reducing the risk of a culture being contaminated during open handling within BSL-2 cabinets. Nonetheless, they are not well documented in the published literature and remain largely unknown among many smaller biopharmaceutical firms and most of the emerging cell therapy industry, where they would seem particularly valuable, as discussed further below;

» At substantial expense in terms of cost and employee time and comfort, many firms have also substantially increased the gowning requirements for large-scale operations, wherein cultures are maintained in closed bioreactors. There is no direct evidence to our knowledge that these changes have had any actual impact on contamination rates. In fact, many plants were successfully operated for years at low contamination rates with gowning requirements that consisted of a simple laboratory coat and safety glasses. Typically during a crisis 10–20 years ago, some firms implemented 'bunny suits', head covers, shoe covers, gloves, and so forth, as part of a slew of changes. The increased gowning requirements were subsequently carried forward, along with all the other changes, and have now become industry standard. If studies were done, we believe they would likely show that such gowning changes have resulted in no change in ongoing contamination rates over many subsequent years, separate from other more effective and often concurrent changes. The scientific basis for such gowning changes, for areas where cultures are maintained in closed bioreactors, has never been adequately investigated or legitimately defended to our knowledge and is certainly questionable. Unless the gowning changes bring an entire processing area to the same level of cleanliness as within a BSL-2 cabinet (a highly unlikely outcome), loss of culture vessel integrity would still very likely result in a contamination. Furthermore, if integrity is lost, isn't it best to know?

» For biopharmaceutical proteins, more and more firms have implemented high-temperature, short-time (HTST) systems for medium treatment and/other upstream viral barriers [3]. Broader implementation years ago would have likely eliminated many of the contamination crises that have occurred over the last decade or so. At least one crisis resulted in a shortage of a life-saving drug. For years, Genentech, Millipore, Amgen and other leading firms, including consulting firms, have openly shared information regarding the design and value of HTST systems and other viral barriers [4–10]. Nonetheless, to our knowledge, the value of such systems is not widely known nor often discussed among the smaller biopharmaceutical firms, as well as many vaccine and cell therapy firms;

» For biopharmaceutical proteins, no contamination crisis to our knowledge has resulted in a batch of final purified drug substance that was contaminated with an adventitious agent. Contaminated batches were typically identified pre-harvest and not processed through purification. Even if a low level and obscure contamination is not identified and the run still processed, the contaminating agent would still very likely be adequately removed and/or inactivated during downstream processing steps. For example, typical downstream processes were recently shown to adequately remove and/or inactivate an obscure *Leptospira licerasiae* contaminant [4]. Nonetheless, all affected batches were discarded [4]. For biopharmaceutical proteins, many people view implementation of upstream viral barriers as a business decision rather than a safety decision, due to the efficacy of downstream processing steps in removing and inactivating any

undetected microbial contaminants. This view may well be valid but certainly deserves further investigation and validation;

» For vaccines, few if any firms to our knowledge have implemented HTST systems and/or upstream viral barriers. Downstream processes for certain live-virus vaccines generally remove and/or inactivate many adventitious agents, such as a foreign virus, at a much lower rate than downstream processes for biopharmaceutical proteins. There is thus a higher probability that undetected microbial contaminants will make it into the purified drug substance. Previously undetected porcine circovirus contaminations were found in two licensed vaccines [11]. Such contaminations would likely have been avoided if upstream viral barriers were employed to remove and/or inactivate virus in raw materials, such as porcine trypsin;

» For cell therapies, few if any firms to our knowledge have implemented HTST systems and/or upstream viral barriers. Downstream processing of cell therapies often results in little, if any, removal or inactivation of many adventitious agents, such as a foreign virus. Furthermore, as mentioned above, many cell therapy operations are neither aware of, nor follow, industry leading protocols for avoiding contamination in small-scale operations. Finally, contamination of human cell cultures with human virus could pose a hazard to operations staff. Much of the emerging cell therapy industry has safety risks around contamination that, to our knowledge, are rarely if ever discussed or acknowledged.

### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

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