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Can we win the war against Mycoplasma contamination?

Biopharmaceutical manufacturers can suffer significant financial losses as a result of a Mycoplasma contamination in a cell culture. Preventing this, though, is easier said than done. Andy Kelly reveals more about the structure of these troublesome microorganisms and detail a study to determine the link between filtration pressure and Mycoplasma retention rates.

Mycoplasma maybe the smallest class of free-living microorganism, but the consequences of a contamination in a cell batch during bioprocessing can be huge.

The presence of Mycoplasma can result in a lost product batch and a reduced yield. More serious, in large scale manufacturing, is a resulting lack of potentially life-saving drugs for patients and, in the most extreme cases, the future of the manufacturing company is put at risk.

The acquisition of Genzyme by Sanofi in early 2011 can be directly attributed to the consequences of a contamination by Vesivirus 2117, detected at their Allston (Massachusetts) manufacturing facility in 2009.

Decreased production titres had been observed since 2008, but the root was never established until the virus contamination came to light. A court-appointed independent consultant acting on behalf of the FDA were given a significant level of oversight and

control over manufacturing in late 2009, with production halted on drugs with sales in excess of \$1bn. The resultant lawsuits and weakened stock value were significant factors in the subsequent takeover.

The Genzyme case dramatically highlights the importance of tackling Mycoplasma contamination. But this is not an easy task. Mycoplasma, which vary in size and shape from 0.2 micron upwards, have no peptidoglycan cell wall and

exhibit pleomorphism, the ability to alter size and shape in relation to environmental conditions. This means that they are capable of penetrating sterilizing grade filtration.

Mycoplasma are also typically slow-growing and, depending upon the specific organism in question, may be difficult or impossible to culture using standard microbiological techniques, often requiring very specific growth media and culture conditions.

Mycoplasma also possess the ability to infect mammalian cell cultures through adhesion and subsequent fusion to cell membranes, allowing them to exploit the conditions and synthesized molecules provided by the host cell. This means that suitable detection and quarantine procedures must also be implemented around any incoming cell lines.

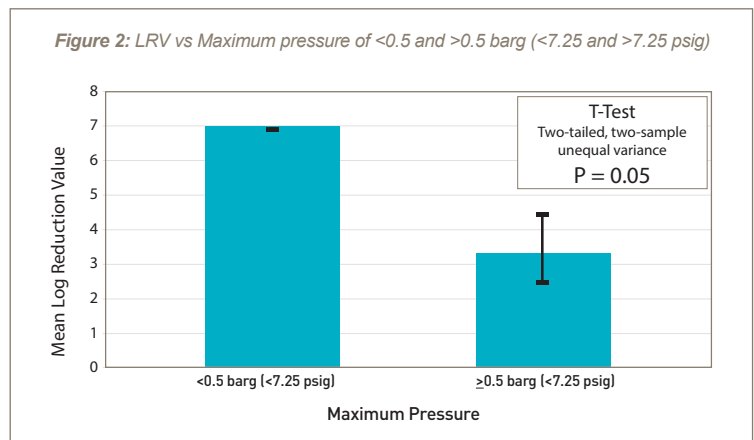
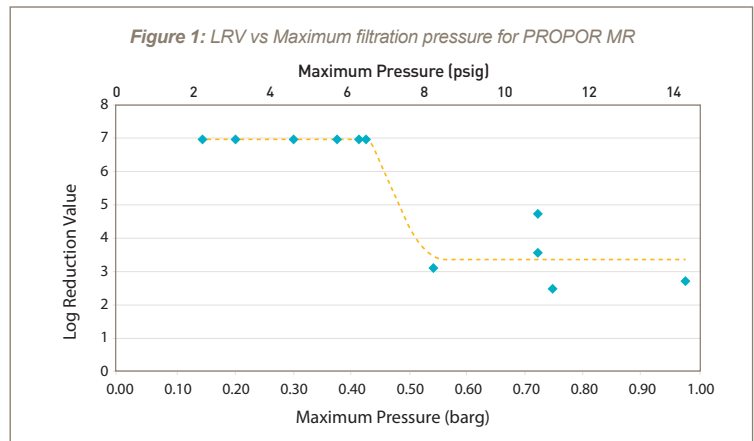
Strategies to guard against Mycoplasma contamination can include gamma irradiation or heat inactivation to eliminate Mycoplasma present on gamma or heat-stable incoming raw materials. Although there is a chance that the raw material is damaged as well.

Another technique is to filter out and retain Mycoplasma during a biopharmaceutical process.

Experimental design

Parker domnick hunter's PROPOR MR is a leading Mycoplasma filter for faster, more efficient and cost effective Mycoplasma-free cell culture media.

A study was performed on behalf of one of our customers to investigate the relationship between the differential pressure across the PROPOR MR filter and



retention of the organism *Mycoplasma faucium*. This *Mycoplasma* species has been identified as an occasional part of the normal human commensal flora, isolated from the oropharynx and had been identified as a contaminating species of the customer's cell cultures.

100 mLs of *M. faucium* challenge suspension was filtered through 47 mm PROPOR MR membrane discs. The membrane challenges were performed at a range of pressure between 0 and 1 barg with continuous system control and pressure monitoring performed using the SciLog[®] FilterTec[™] filtration system and SciPres[®] single-use pressure sensors.

The filtrate was collected and

the number of viable organisms determined by use of dilution plate counts and capture filters, enumerated following incubation on organism specific solid growth media. Filter challenge culture concentration was determined using the same method.

Influence of pressure on filter retention of *M. faucium*

The log retention values of *Mycoplasma* for filtration challenges conducted at increasing maximum differential pressure were recorded (Figure 1). It shows that below a threshold value of approximately 0.5 barg (7.25 psig), under the conditions tested, the *M. faucium* organism was retained to a mean log reduction value (LRV) of 7. Above this threshold value, the LRV decreased to an average of approximately 3.3. ▶▶

A horizontal straight line was drawn through the data points above 0.5 barg. Other relationships between pressure and retention may be considered, especially from 0.5 barg (7.25 psig) to 1 barg (14.5 psig), however, we were unable to find evidence for these with any statistical significance.

A comparison was made of the mean log reduction values of *M. faucium* at differential pressure below and above the 0.5 barg threshold value (Figure 2). A two-tailed, two-sample unequal variance T-test was performed and confirmed that the two means were significantly different ($p=0.05$).

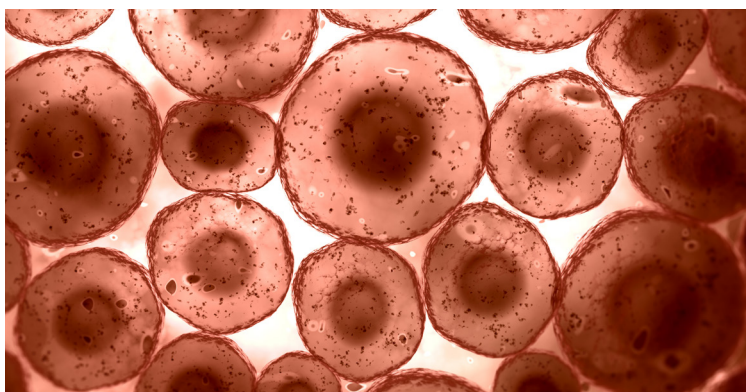
The retention data generated demonstrates a clear relationship between filtration pressure and organism retention.

We speculate that, at the threshold pressure, the mechanical strength of cells present on the membrane is overcome deforming them and causing them to be forced through filter pores. If the hypothesis is correct, then processing at the highest flow rate below the threshold filtration pressure will provide maximum Mycoplasma retention with the fastest processing times.

Automation offers maximum confidence of Mycoplasma retention

Maximum pressure peaks appear to be a critical influence upon retention. If these events only occur sporadically then they may be difficult to detect without continuous pressure monitoring.

Our SciFlex® NFF platform with single-use flow-path can be used to control differential pressure and maintain this below proposed threshold values at which Mycoplasma retention is not significantly diminished. The



system allows safe, walk-away processing and documented evidence that pressure limits are not exceeded. Pre-assembled single-use manifolds can be gamma-irradiated as an additional risk-mitigation step to prevent Mycoplasma contaminations.

Of course, to fully characterize the media filtration step, further study would be required to investigate the relationship between filtration pressure and other variables such as process conditions, fluid properties and media components. These parameters would be most effectively studied using a multivariate experimental design.

What is clear is that, if undetected and uncontrolled, a Mycoplasma contamination event can have potentially wide-ranging and costly implications for cell culture based manufacturing processes. To understand the true risk to a manufacturing process and avoid the potential for increased contamination from raw materials, it is imperative that suitably validated detection techniques are implemented. Any new cell lines should also be appropriately screened and quarantined prior to use.

In addition to techniques used to detect and prevent Mycoplasma from entering the facility, an appropriate filtration system

should be utilized for any media sterilization steps.

Our study demonstrates that a maximum threshold pressure will exist for each combination of specific Mycoplasma organism and process conditions, above which the retention of the organism through normal flow filtration will be significantly diminished.

Therefore, qualification of media filtration stages should be performed using process specific fluids, parameters, and contaminant organisms isolated from the manufacturing process to establish whether the desired level of retention can be achieved at a specific filtration pressure. This qualification should also ideally provide further understanding of filter retention were the system to move outside of the desired operational parameters.

Implementation of automation and control technologies can also be used to ensure that process parameters do not move outside of this qualified operating range - all methods that can be employed to prevent or minimize the threat of Mycoplasma. ■



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