

Phage Testing of Bacterial Cultures

Regulatory agencies require companies using microbial cell lines that produce products for human use to regularly complete biosafety testing to ensure that cell banks are free of phage contamination.

Bacteriophage (phage) is a virus that infects and replicates within bacteria¹, basically bacterial predators. One hundred times smaller than a bacterium, phage are found wherever bacteria thrive and often exceed bacterial densities by an order of magnitude². In fact, bacteriophage are the most abundant organisms in the biosphere and are thought to be the vectors of horizontal gene transfer that drives bacterial evolution³.

Phage have genetic material (DNA or RNA, single or double stranded) that is encapsulated by a protein coat and play a key role in bacterial biology by infecting bacteria and propagating by one of two methods⁴:

The lytic life cycle, when virulent phage infect a bacterium after attachment to a host cell receptor, inject nucleic acid, replicate and synthesize the capsid proteins. These de novo components are assembled to form a new viral particle. The lytic phages rapidly multiply causing cell lysis and on-going infection of the host bacteria.

In the lysogenic life cycle, phage either inserts its DNA into the host genome or remains as plasmid DNA in the host cell. In either case, the phage infection causes the host cell machinery to express phage genes by a process known as lysogenic conversion. Phage integrated into the genome of the bacterium are latent and called prophage until they revert to the lytic cycle.

The phage virus acts as a parasite and uses the metabolism of the bacterial cell to replicate and multiply. Phages also are able to mobilize genetic material by transduction, or phage-mediated transfer of bacterial DNA between two bacterial cells.

Phage contamination of bacterial cultures is a common complication for the biotech and pharmaceutical industries. The presence of phage in cultures can bias results by interfering with bacterial identification and isolation. Phage are not susceptible to antibiotic treatments and therefore difficult to remove from

infected cultures of bacteria⁵. Being ubiquitous and abundant, phage can become a serious problem for companies using microbial cell lines to produce recombinant products.

Regulatory agencies require companies using microbial cell lines that produce products for human use to regularly complete biosafety testing to ensure that cell banks are free of phage contamination. (US FDA (21 CFR Part 58 and 210/211) Both master cell banks (MCB), and end of production cell banks (EOP) must be tested regularly for phage contamination. The testing frequency varies between products and is regulated on a case by case basis by the applicable regulatory agency.

Phage testing includes both lytic and lysogenic testing with positive and negative controls in each type of testing. It is customary to contract a specialty lab to perform phage testing to avoid introducing positive phage controls into your manufacturing environment. GMP lot and/or batch release testing services for biologic drug substances or drug products are the core of Avance Biosciences' business. We offer both lytic and lysogenic phage testing using several positive and negative controls for each type of test as well as a variety of additional biosafety testing services. Because we perform phage testing on a regular basis, our expertise in this area enables us to recognize any testing problems.

If you have a new bacterial cell line, a recently modified cell line, or are ready to bank your developed line, we offer mitomycin C induction followed by lysogenic testing to insure your cell bank is free of chromosome-integrated phage DNA. If detected, next generation sequencing is used for de novo construction of the contaminating phage sequence and blasted against the NCBI database to identify the contaminating phage. Additionally, we offer titer testing to determine the concentration of phage contamination, if any, in your bacterial cell bank.

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Avance Biosciences offers comprehensive services for bacterial cell lines for all stages of production under cGMP/GLP regulatory compliance. Our team has more than two decades of experience and provides high quality cell bank characterization services. We can help you with fast and accurate testing results!

References

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