NCL Method STE-3

Detection of Mycoplasma Contamination in Nanoparticle Formulations

Nanotechnology Characterization Laboratory
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
Frederick, MD 21702
(301) 846-6939
ncl@mail.nih.gov
https://ncl.cancer.gov

https://ncl.cancer.gov

This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

Method Written By:
Timothy M. Potter
Barry W. Neun
Marina A. Dobrovolskaia*

Nanotechnology Characterization Lab, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, MD 21702

*- address corespondence to: marina@mail.nih.gov

Please cite this protocol as:
Potter TM, Neun BW, Dobrovolskaia MA, NCL Method STE-3: Detection of Mycoplasma Contamination in Nanoparticle Formulations. https://ncl.cancer.gov/resources/assay-cascade-protocols DOI: 10.17917/PHFP-8573

1. Introduction

Mycoplasma is a microorganism that lacks a nucleus and a cell wall which makes it unaffected by many antibiotics. Nanoparticles submitted to the NCL may be subjected to testing for mycoplasma when deemed necessary. The types of nanoparticle formulations generally tested for mycoplasma contamination include those that incorporate a component derived from an animal or hybridoma cultures.

2. Principle

The NCL does not perform mycoplasma testing in our laboratory. Rather, this test is outsourced to another department of the Frederick National Laboratory for Cancer Research, the Protein Expression Laboratory (PEL), part of the Cancer Research Technology Program (https://frederick.cancer.gov/science/Pel/Protocols.aspx).

Briefly, the NCL cultures nanoparticles with cells for the initial 24 hours, then passage these cells twenty-five (25) times. The supernatants from the final culture will then be transported to the PEL for mycoplasma testing via VenorGem Mycoplasma PCR-based Detection Kit from Minerva Biolabs, which is also available through Sigma at the link below:
http://www.sigmaaldrich.com/catalog/product/sigma/mp0025?lang=en&region=US.
For more details on the mycoplasma detection protocol, please contact the PEL (https://frederick.cancer.gov/science/Pel/Protocols.aspx).

3. Preparation of study samples

The assay requires 2.2 mL of the test nanomaterial final formulation. The concentration of nanoparticles in this formulation is case-specific. When such information is not available, for example when a test nanomaterial is received from a commercial supplier in a form not intended for biomedical applications, prepare stock solution at a concentration of 1mg/mL. The weight information can refer to either active pharmaceutical ingredient or total construct; it can also represent total metal content or other units. Such information is specific to each nanoparticle and should be recorded to aid result interpretation. Test nanoparticles should be reconstituted in sterile PBS, water or in appropriate vehicle. If vehicle is a buffer or media

other than water or PBS, the vehicle control should be included in the test. The samples are tested from stock. However, when nanoparticles carry cytotoxic drugs or are otherwise toxic to cells, select the highest non-toxic concentration. The pH of the study sample should be checked using a pH microelectrode and adjusted with either sterile NaOH or HCl as necessary to be within the pH range 6-8. If NaOH or HCl are not compatible with a given nanoparticle formulation, adjust pH using a procedure recommended by nanomaterial manufacturer. To avoid sample contamination from microelectrode, always remove a small aliquot of the sample for use in measuring the pH.

4. Reagents and Equipment

Note: The NCL does not endorse any of the suppliers listed below; these reagents were used in the development of the protocol and their inclusion is for informational purposes only. Equivalent supplies from alternate vendors can be substituted. Please note that suppliers may undergo a name change due to a variety of factors. Brands and part numbers typically remain consistent but may also change over time.

4.1 Reagents

4.1.1 Phosphate buffered saline (PBS) (GE Life Sciences, SH30256.01)

4.1.2 Fetal bovine serum (FBS) (GE Life Sciences, Hyclone, SH30070.03)

4.1.3 Penicillin streptomycin solution (GE Life Sciences, Hyclone, SV30010)

4.1.4 Trypan Blue solution (Gibco, 15250-061)

4.1.5 RPMI-1640 (GE Life Sciences, HyClone, SH30096.01)

4.2 Equipment and Materials

4.2.1 Pipettes, 0.05 to 10 mL

4.2.2 Polypropylene tubes, 50 and 15 mL

4.2.3 T25 culture flasks

4.2.4 Centrifuge

4.2.5 Refrigerator, 2-8ºC

4.2.6 Freezer, -20ºC

4.2.7 Cell culture incubator, 5% CO_2 and 95% humidity.

4.2.8 Biohazard safety cabinet approved for level II handling of biological material

4.2.10 Inverted microscope

4.2.10 Vortex

4.2.11 Hemocytometer

5. Preparation of cells for mycoplasma testing

5.1 Identify a quickly proliferating cell line (e.g. NCI H460) and grow it in the complete culture media appropriate for this cell line.

5.2 Add 1mL of test nanoparticle formulation and negative control to the cell culture media. Use 10 mL of culture media per flask. Test each sample in duplicate.

5.3 Incubate cells for 24 hours, then replace growth medium with 5 mL of fresh complete medium appropriate for your cell line.

5.4 Split cells as needed and passage 25 times. Use 5 mL of culture media for all passages after initial treatment. Some detection methods may require the use of antibiotic free media. Mycoplasma is typically resistant to common cell culture antibiotics; however, the presence of antibiotics can produce erroneous results in certain tests. Consult your testing method for guidance.

5.5 After last passage, collect supernatants for mycoplasma detection by appropriate method (PCR or ELISA).

Note: This method is not intended to certify the nanoformulation as mycoplasma free. It is used to ensure that no mycoplasma is introduced into cell culture and transmitted to *in vitro* or *in vivo* (in case of xenograft studies) assays.