NCL Method STE-3

Detection of Mycoplasma

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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.

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1. Introduction

Mycoplasma is a form of bacteria that lacks a nucleus and a cell wall, and are thus 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 within the Frederick National Laboratory for Cancer Research, the Protein Expression Laboratory (PEL), within the Cancer Research Technology Program.

Briefly, the NCL will culture 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 PEL for mycoplasma testing via the VenorGem Mycoplasma PCR-based Detection Kit from Minerva Biolab (also available through Sigma, 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.

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.

3. Reagents and Equipment

Note: The NCL does not endorse any of the suppliers listed below; their inclusion is for informational purposes only. Equivalent supplies from alternate vendors can be substituted.

3.1 Reagents

1. Phosphate buffered saline (PBS) (GE Life Sciences, SH30256.01)
2. Fetal bovine serum (FBS) (GE Life Sciences, Hyclone , SH30070.03)
3. Pen/Strep solution (Invitrogen, 15140-148)
4. Trypan Blue solution (Invitrogen, 15250-061)

5. RPMI-1640 (Invitrogen, 11835-055)

3.2 Cell Lines

1. NCI H460 or equivalent

3.3 Materials

1. Pipettes, 0.05 to 10 mL
2. Polypropylene tubes, 50 and 15 mL
3. T25 culture flasks

3.4 Equipment

1. Centrifuge
2. Refrigerator, 2-8ºC
3. Freezer, -20ºC
4. Cell culture incubator with 5% CO_2 and 95% humidity.
5. Biohazard safety cabinet approved for level II handling of biological material
6. Inverted microscope
7. Vortex
8. Hemocytometer

4. **Preparation of Study Samples**

The assay requires 2.2 mL of the test nanomaterial. The concentration of nanoparticles is case-specific. Most samples are tested directly from stock. 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 a solution at 1 mg/mL. The weight information can refer to active pharmaceutical ingredient, total construct, total metal content, or other units. Such information is specific to each nanoparticle and should be recorded to aid result interpretation. When nanoparticles carry cytotoxic drugs or are otherwise toxic to cells, the highest non-toxic concentration should be selected.

Test nanoparticles should be reconstituted in sterile PBS, water or other appropriate vehicle. If vehicle is a buffer or media other than water or PBS, the vehicle control should also be included in the test. 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.

5. **Preparation of Cells for Mycoplasma Testing**
    1. Identify a quickly proliferating cell line (e.g. NCI H460) and grow in the complete culture media appropriate for this cell line.
    2. Grow the cells in a T25 cell culture flask until they are approximately 80% confluent.
    3. Add 1 mL of test nanoparticle formulation and negative control to cell culture media. Use 10 mL of culture media per flask. Test each sample in duplicate.
    4. Incubate cells for 24 hours, then replace growth medium with 5 mL of fresh complete medium appropriate for the cell line.
    5. Split cells as needed and passage 25 times. Use 5 mL of culture media for all passages after initial treatment.
    6. After last passage, collect supernatants for mycoplasma detection by appropriate method (PCR or ELISA).