MP Biomedicals

Case study - *Viruses*

Health

Determination of virus titers in lungs of Influenza A Virus infected mice.

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Introduction

Various virology institutes reported recently a new method for the isolation of intact virus particles from infected animal tissues for studies of pathogenic viruses (*ex: avian Influenza A viruses, i.e H5N1*) and development of vaccines. This simple and reproducible method allows to accurately measure the viral load in tissues, follow the spread of the virus in mouse organs and assess the effect of vaccination.

Overview

- **Keywords:** Virus isolation, influenza A virus, infected animal tissues, pathogenic viruses
- **Aim of the study:** Isolation of intact viruses from infected animal tissues
- **Application:** Virus titration
- **Sample type:** Tissue
- **Sample name:** Mouse lung tissue
- **Material:** FastPrep-24™ instrument, 2 ml lysing matrix tubes containing ¼ inch ceramic beads
- **Buffer:** Hank's balanced salt solution containing 0.5% lactalbumin, 10% glycerol, 200 U/ml penicillin, 200 µg/ml streptomycin, 100 U/ml polymyxin B sulfate, 250 µg/ml gentamycin, and 50 U/ml nystatin.

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Protocol and Parameters

1. Snap freeze the weighed lung of a mice (100-150 mg) in a Lysing matrix M tube and store at -70°C.
2. Add 1 ml of ice-cold Buffer to the Lysing Matrix M tube.
3. Homogenize the tissue with the Fastprep-24™ Instrument for 20 seconds at a speed setting of 4.0 m/s.
4. Incubate the tube on ice for 2 minutes.
5. Homogenize the tissue a second time with the Fastprep-24™ Instrument for 20 seconds at 4.0 m/s.
6. Add 0.5 ml of medium to the Lysing Matrix tube and centrifuge 1 minute at 10.000 rpm to pellet the tissue debris.
7. Transfer the supernatant containing the virus particles to a new microcentrifuge tube.
8. Infect MDCK cells with quintuplicated 10-fold serial dilutions of the supernatants as previously described (1).
9. HA activity of the culture supernatants collected 5 days post inoculation are used as indicator of infection. Titers are calculated according to Spearman-Karber's method 3.

Conclusion

- The Fastprep® System together with Lysing Matrix M tubes (2 ml tubes containing one ¼ inch ceramic bead) were successfully used to homogenize infected tissues and release intact viral particles as a first step of this experimental procedure.