Direct extraction of viral RNA from clinical swab samples using Nanotrap® Magnetic Virus Particles

Summary: This document describes a protocol using Nanotrap® Magnetic Virus Particle to capture and concentration whole viruses from clinical swab samples followed by a direct RNA extraction. This method is compatible with clinical swab samples stored in viral transport medium (VTM), universal transport media (UTM), or saline, as long as the medium used to collect the swabs and the storage conditions do not lyse viruses. This method has been confirmed with samples that contain infectious virus and with samples that contain virus that has been heat-inactivated at 65°C for 30 minutes.

Materials and equipment

1. Viral samples in VTM, UTM, or saline containing intact virus (i.e. not lysed)
2. Nanotrap® Magnetic Virus Particles, Ceres Nanosciences SKU# 44202
3. 1.5 mL microcentrifuge tubes
4. Deionized water
5. Magnetic separator (For example, DynaMag-2 mL Magnetic Rack, ThermoFisher Catalog # 12321D)
6. Extraction buffer – 0.5% Triton X-100 in PCR grade water
7. Wash buffer – 0.05% Tween-20 in 1X PBS

Procedure

A. Processing clinical swab samples with Nanotrap® Magnetic Virus Particles

1. Take 1 mL of the sample and transfer it into a microcentrifuge tube. *Note: 500 µL of the sample can be used if 1 mL is not available, but it is strongly recommended to use 1 mL sample when possible.*
2. Add 100 µL of Nanotrap® Magnetic Virus Particles to the sample.
3. Mix by pipetting up and down five times.
4. Incubate samples with Nanotrap® Magnetic Virus Particles at room temperature for 10 minutes.
5. Use a magnetic separator to separate the Nanotrap® Magnetic Virus Particles from the sample.
6. Discard the supernatant carefully without disturbing the pellet. *Note: For optimal RT-PCR results, consider using a smaller pipette to remove the residual VTM/UTM/saline supernatant from the tube. Residual VTM/UTM/saline may interfere with the RT-PCR assay.*
7. Add 500 µL of wash buffer (0.05% Tween-20 in 1X PBS) to the Nanotrap® Magnetic Virus Particles pellet.
8. Mix by pipetting up and down five times.
9. Use a magnetic separator to separate the Nanotrap® Magnetic Virus Particles from the sample.
10. Discard the supernatant carefully without disturbing the pellet. *Note: For optimal RT-PCR results, consider using a smaller pipette to remove any residual PBS.*

Note: Washing described in steps 7, 8, 9 and 10 can be omitted if extra care is taken to remove all of the UTM/VTM/saline supernatant in step 6.

B. RNA extraction

11. Resuspend particle pellet in 50 µL of extraction buffer (0.5% Triton X-100 in PCR grade water) by pipetting up and down five times.
12. Heat samples at 95°C for 5 minutes. This step can be performed on a heat block or thermocycler.
13. Use a magnetic separator to separate the Nanotrap® Magnetic Virus Particles from the sample.
14. Collect the supernatant; the sample is ready for analysis.

C. RT-PCR Reaction

15. Follow RT-PCR kit instructions to analyze the supernatent.

Additional Nanotrap® Magnetic Virus Particle Product Information available at www.ceresnano.com/viruscapture

WWW.CERESNANO.COM +1.800.615.0418 (U.S. tel) SUPPORT@CERESNANO.COM