VERSION 2

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Protocol status: Working
 We use this protocol and it's working

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Integrated Virus Detection System - sample collection - prep - processing V.2

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ABSTRACT

IVDS is uniquely suited to large volume sampling. Collection from saliva or sputum are straightforward – collection in disposable pipette and transfer to a small vial, dilute as needed, filter, and test, all at under 5 minutes including results. Sample collection, storage and processing are straightforward, not requiring sensitive or unstable solutions.

GUIDELINES

none

MATERIALS

MATERIALS

⊗ Ammonium Acetate Sigma Aldrich Catalog #A1542-500G

⊗ Butanol Fischer Scientific Catalog #71-36-3

SAFETY WARNINGS

! none

Collection

- 1 Sample collection:
 - Collect saliva in a disposable pipet by having the subject placing the squeezed pipet into subject's mouth and releasing pressure on the bulb, with patient directing the pipet to the pooled saliva in the mouth. This will collect 1 mL to

• 2.5 µL of sample.

2 Sample processing:

• 1.5 µL of this sample is moved from the pipet into a 1.5 ml centrifuge tube.

3 • 200 µL of this sample is moved to a clean and empty 1.5ml centrifuge tube and mixed with 800 µL of RO water.

Expected result

greatly reduced viscosity, the amount of sample may be reduced if the saliva is highly viscous.

4 • Sample is then agitated for 00:00:05 to 00:00:15 to mix.

Expected result

even mix,

5 • Sample is then placed into a 3 ml disposable syringe with a 0.45µm MCE filter attached and the sample is then filtered into a new 1.5 centrifuge tube.

Expected result

This removes the possible mouth contaminants that can potentially clog the instrument capillary.

6 • This filtered sample is then placed into the IVDS sample holder.

7 • Instrument saturates for 00:00:30 to 00:01:00 and then initiated for instrument scan (testing). Test is 1 scan of 1.5 minutes and sample is processed at 40nl per minute. Test results are a real time read out from IVDS. Data is stored automatically. Sample is assigned a number for processing, this the sample id which will match source id.

8 If the sample does not have enough ionization salt naturally from the saliva, then adding ammonium acetate (50 µL @ 20 millimolar (mM)) is placed in the sample and vortexed for 00:00:05 to 00:00:15 and rerun. (Determined by the voltage and Taylor cone shape.)

9 Instrument setup:

• Air is set to 4psi

• CO₂ and Air flow are set to 2.5 and 1.5 lpm respectively.

• nA is typically set to -300nA and the kV is typically set to 2.05. These are set to balance the Taylor cone on the ESI.

- Scan is set to 00:01:30 and set for 1 to 2 scans as needed. (View Results)
- Other instrument settings are per standard setting in the operators guide.
- These settings do not change for the days run, regardless of the number of samples run.

10 After each sample processing:

- After a positive test a 1.5 μ L of blank buffer is placed into the instrument tube holder and is run as a blank test with the same settings as a sample. This will flush the instrument and the readout will have a zero reading. If not then repeat.
- A sample that has a negative result, proceed to the next sample, a flush is not necessary.

11 At the end of the day run:

Clean the instrument with Potassium Hydroxide 20 millimolar (mM) solution in a 1.5 ml centrifuge tube flowing into the instrument for 00:02:00 .

Remove clean vial and Reverse air flow for 00:02:00 and then return to normal.

Run a buffer sample for 00:01:00 to verify instrument has no counts or is clean.

Remove buffer and reverse air flow for 00:02:00 , return to normal air flow for 00:10:00 . Shut off instrument, air supply, CO₂, and electrical.

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Equipment

Integrated Virus Detection System

NAME

Non Specific Virus Detector

TYPE

TSI, Inc

BRAND

3081, 3480, 3080, 3772, 3032

SKU

components; 3081 - DMA, 3480 - Electrospray Generator, 3080 - Electrostatic Classifier, 3772 - Condensation Particle Counter, 3032 - vacuum pump.

SPECIFICATIONS

Expected result

a negative result will display as no counts by the cpc for the determined "size" or m/z ratio. A positive result is a cpc count at the determined "size" or m/z ratio. It is unknown to determine if there is an active infection since it is unknown what the minimum number of virions are needed to start an infection, so we simply detect them and count the virus particles and not the copies of genetic material that are a secondary detection if at all.