First WHO International Reference Panel for adventitious virus detection in biological products using high-throughput sequencing technologies

DRAFT INSTRUCTIONS FOR USE

Custodian

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1. DIRECTIONS FOR OPENING THE SCREW CAP VIALS

The material is stored in polypropylene for stability at low temperatures (-70 °C or colder). The vials have a screw cap with a rubber gasket. Vials should be opened in an appropriate biosafety cabinet. The cap should be removed by turning anti-clockwise. Care should be taken on removal of the cap to prevent the contents spilling (such as placement in an appropriate holder).

2. DIRECTIONS FOR STORAGE AND HANDLING

The material is shipped in dry ice and should be stored immediately on arrival under controlled temperature monitoring at -70 °C or colder and avoiding any changes in temperature during transfer to vials.

When handling the virus, use safety procedures according to your laboratory guideline: for example, work in a suitable biosafety cabinet, wear lab coat, gloves, inactivate all surfaces exposed to virus using appropriate virocides before autoclaving or incinerating disposal items. Used vials and all materials exposed to the virus during the handling or in performing the studies should disposed according to laboratory's guidelines for infectious agents.

3. PREPARATION OF VIRUS STOCK ALIQUOTS

- Remove virus from storage and transfer to lab in dry or wet ice
- Thaw virus by gently swirling in 37 °C water bath until only a tiny piece of ice remains (should be less than 1 min; each vial contains ≥ 0.5 mL). Transfer to wet ice to continue complete thawing.
- In biosafety cabinet work with one virus at a time, clean surfaces and allow 45 mins in between different viruses with biosafety blower on, to avoid cross-contamination of the stock viruses. Make appropriate single-use aliquots of each virus based on future work. Use sterile screw capped cryogenic vials with a rubber gasket in the cap (RNase and DNase free) and store at -70 °C or colder. Aliquots should not be less than 10 uL for long-term storage. It is best to store undiluted virus in serum-free medium for long-term stability.
- This initial freeze-thaw step will ensure that the virus used in all of the studies had undergone 1 thaw and freeze after being received in the laboratory and no change in the potency of virus due to difference in sample handling.

4. A SCHEMA FOR PREPARATION OF VIRUS DILUTION FOR A SPIKING STUDY

- All reagents and materials used must be sterile, RNAse and DNAse free.
- To make a dilution, remove volume from one tube, deliver to a new tube, change to a new pipette tip or pipette and mix up and down 3 times (avoid rigorousness and bubbles), then use a new tip or pipette to remove volume and continue till last dilution where final volume is discarded.
- Store each dilution in aliquots to be used for nucleic acid extraction in screw cap tubes at -70 °C or colder (volumes in table are rounded to the nearest whole number). Avoid further freeze-thaw.

Virus Stock	GC/mL in virus stock	Dil. A: $1x10^9$/mL		Dil. B: $1x10^8$/mL		Dil.C: $1x10^7$/mL		Dil. D: $1x10^6$/mL etc…..	
		Virus Stock (uL)	*Diluent: No-serum Medium (uL)*	*Virus Dil A (uL)*	*Diluent: No-serum Medium (uL)*	*Virus Dil B (uL)*	*Diluent: No-serum Medium (uL)*	*Virus Dil C (uL)*	*Diluent: No-serum Medium (uL)*
REO	1.50×10^{10}	67	933	100	900	100	900	100	900
RSV	5.53×10^{10}	18	982	100	900	100	900	100	900
FELV	4.01×10^{10}	25	975	100	900	100	900	100	900
PCV	8.07×10^{09}	124	876	100	900	100	900	100	900

EBV	2.80×10^{07}	-	-	-	-	357	643	100	900
OC43	2.64×10^{10}	38	962	100	900	100	900	100	900
MVM	1.18×10^{10}	85	915	100	900	100	900	100	900

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