Viral transport media (VTM) preparation.

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This protocol describes the Viral Transport Media (VTM) used by Chua *et al*, 2002 for the Isolation of Nipah virus from Malaysian Island flying-foxes. This recipe includes 1x Hank's balanced salt solution, 1% bovine serum albumin, pH 7.4, containing amphotericin B (15 µg/ml), penicillin G (100 units/ml) and streptomycin (50 µg/ml). Animal specimens potentially harbouring viral particles are to be stored in this media at 4ºC until permanent freezing at -80 ºC becomes available. The infectivity of viruses decreases over time, the decay rate is a function of temperature, so stability is achieved at lower temperature. In some situations, the viruses can be transported in the specimen itself. Examples of this include urine, cerebral spinal fluid, blood, respiratory tract lavages and stools. Viral transport media are designed to provide an isotonic solution containing protective proteins, antibiotics to control bacterial proliferation and buffers to control pH. Earlier studies have shown that virus recovery was higher for Hank's balanced salt solution for parainfluenza virus, enterovirus, adenovirus, HSV, influenzavirus types A and B, respiratory syncytial virus (RSV), varicella-zostervirus (VZV), and rhinovirus when compared to *Stuart and Leibovitz-Emory Media* (SLEM). In a study reported by Gallo and co-workers RPMI-1640 with 10% FBS and 10% DMSO proved suitable at conserving viable HIV at —196 ºC. Hypertonic sucrose solutions exert a protective effect on labile viruses such as RSV. RSV survival was enhanced when stored at 44.5% sucrose solution and stored at -70 ºC (in excess of 2 years with no significant loss of infectivity). For RSV, and perhaps other labile viruses, sucrose provides better stabilization than serum or other protein solutions. At a concentration of 44.5% sucrose is toxic to cell cultures, a 1:10 dilution reduces this toxicity.

Procedure

1. Prepare Hanks balanced salt solution according to the following table

Reagent		1 litre	500 mL	250 mL	50 mL
Hank's balanced salt solution	Calcium Chloride ($CaCl_2$)	140 mg	70 mg	35 mg	7 mg
	Potassium Chloride (KCl)	400 mg	200 mg	100 mg	20 mg
	Monopotassium phosphate (KH_2PO_4)	60 mg	30 mg	15 mg	3 mg
	Magnesium Chloride Hexahydrate ($MgCl_2 \cdot 6H_2O$)	100 mg	50 mg	25 mg	5 mg
	Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_20$)	100 mg	50 mg	25 mg	5 mg
	Sodium Chloride (NaCl)	8 grs	4 grs	2 grs	0.4 mg
	Sodium Bicarbonate ($NaHCO_3$)	350 mg	175 mg	87.5 mg	17.5 mg
	Sodium phosphate dibasic (Na_2HPO_4)	48 mg	24 mg	12 mg	2.4 mg
1% Bovine Serum Albumin (BSA)		10 mL	5 mL	2.5 mL	0.5 mL
Amphotericin B (15 µg/ml)		15 mg	7.5 mg	3.75 mg	0.75 mg
Penicillin G (100 units/ml)		$1x10^5$ IU	$5x10^4$ IU	$2.5x10^4$ IU	$5x10^3$ IU
Streptomycin (50 µg/ml)		50 mg	25 mg	12.5 mg	2.5 mg

2. Add the corresponding amount of BSA, Amphotericin, Penicilin G and Stremptomycin as shown in the previous table.

Notes

3. Once in the laboratory, each swab in VTM should be lightly vortexed and 200 µl of the medium containing the swab from each pooled urine, oropharygeal or rectal specimen carefully transferred into a well of a 24-cell tissue culture plate pre-seeded with 1×10^5 Vero cells (ATCC, CCL-81) in 1 ml of Eagle's minimal essential growth medium for Henipahvirus isolation (here given solely for reference).

4. The plates were sealed and incubated at 37 ºC.

5. Specimens were inspected daily for evidence of cytopathic effect (CPE).

6. When CPE was nearly complete, cells were pelleted and total RNA was extracted by using the RNeasy Mini Kit (Qiagen, Germany).

7. All viruses that stained positively with Nipah & Hendra-specific antibodies were successfully isolated from original specimens 2 months after storage at -80 ºC.

Alternative formulations

WHO also recommends the following locally prepared VTM formulations for animal specimens. The World Health Organization (WHO) keeps human specimen viral stocks in commercially available COPAN Universal Transport Media (http://www.copanusa.com/products/collection-transport/utm-viral-transport/). Another commercially available virus transport/storage media is Eagle's Minimum Essential Medium (E-MEM). WHO recommends the following locally prepared viral transport media (VTM) formulations for human specimens:

Veal infusion broth
Add 10g veal infusion broth and 2g bovine albumin fraction V to sterile distilled water and make up to 400 ml. Add 0.8 ml gentamicin sulphate solution (50 mg/ml) and 3.2 ml amphotericin B (250 µg/ml). Sterilize by filtration.

VTM-199
To 1 L of tissue culture medium 199 containing 0.5% bovine serum albumin (BSA) add $2x10^6$ IU benzylpenicillin, 200 mg streptomycin, $2x10^6$ IU polymyxin B, 250 mg gentamicin and $0.5x10^6$ IU nystatin. Sterilize by filtration and distribute in 1.0 – 2.0 ml volumes in screw-capped tubes.

PBS-Glycerol-VTM
Prepare 1 L of phosphate buffered saline (PBS) by adding 8 g NaCl, 0.2 g KCl, 1.44 g Na2HPO4, 0.24 g KH2PO4 and make up to 1 litre with distilled water. Autoclave this PBS and mix 1:1 with sterile glycerol to make two aliquots of 1 litre. To each liter of PBS-Glycerol add: $2x10^6$ IU benzylpenicillin, 200 mg streptomycin, $2x10^6$ IU polymyxin B, 250 mg gentamicin, $0.5x10^6$ IU nystatin , 60 mg ofloxacin hydrochloride and 0.2 g sulfamethoxazole. Dispense 1.0–2.0 ml of transport medium into sterile plastic screw-cap vials (Cryovials). It is best to store these vials at –20 °C until used. However, they can be stored at +4 °C for 48–96 hours (optimally less than 48 hours) or at room temperature for short periods of 1–2 days.

Notes

1. Prepare Hank's balanced salt solution first and correct pH to 7.4 using HCL of NaOH, drop by drop. Add BSA and antibiotics after pH correction.
2. Distribute 1 to 2 mL aliquots into cryovials and store at -20ºC until use.
3. Aliquots can also be kept at 4 ºC for 48–96 hours (optimally less than 48 hours) or at room

temperature for short periods of 1–2 days.

4. Normal saline (NS) solution should not be used as a VTM. Adding BSA and antibiotics to NS changes the pH and this will destroy viruses.

References

1. Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME, Lam SK. Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes Infect. 2002 Feb;4(2):145-51. PubMed PMID: 11880045

2. World Health Organization (WHO) Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection Guide for field operations. Annex 8. Viral transport media (VTM) October 2006.

Revision history

1.0 Original document.

2.0 Optimized protocol.

3.0 Optimized protocol.

4.0 Changes to document format only.