SAMPLE PREPARATION FOR ORBIVIRUS ISOLATION		
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1. SCOPE

To describe the processing of clinical samples from animals to get the isolation of Orbivirus (African horse sickness virus, Bluetongue virus and Epizootic haemorrhagic disease virus) in an "in vitro" system, such as cell culture or embryonated chicken eggs

This procedure is applicable to:

-Whole blood collected on anticoagulant (EDTA)

-Tissue samples, specially spleen, lung and lymph nodes

from animals having a disease caused by a virus of the Orbivirus genus, previously confirmed as positive by serogroup specific RT-PCR, more specifically:

-Equines for AHSV isolation

-Sheep, bovine and other wild ruminants for BTV isolation

-Deer and cattle for the isolation of EHDV

It is also applicable to tissue samples from embryonated chicken eggs inoculated with the samples described above.

2. MATERIALS AND EQUIPMENT

Material and reagents:

Disposable pipette sterile tips with filter (100, 200 and 1000 µl range)

Sterile disposable plastic syringes (1, 2 and 10 ml)

Racks to hold tubes

Disposable sterile pipettes with filter (1, 5, and 10 ml range)

Containers for dispensing reagents

Container biosanitary waste

Container stinging waste

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Plasticware (sterile): 1,5 ml tubes with screwcap containing glass beads for automatic homogenization

Plasticware (sterile): tubes of 10 ml

Sterile forceps and scissors

Scalpel blade

Sterile Petri dish

Disposable low protein affinity membrane mini-filters of 0.45 µm

Antibiotic-antimycotic

Phosphate buffer saline (PBS1X)

Virucidal disinfectant (*Virocid 1:50*)

Sterile distilled water

Equipment:

Laminar Flow Cabin Type II

Vortex (stirrer)

Bench centrifuge capable of centrifugation at approximately 3000rpm (2400g). Refrigerated if possible

Automatic Homogenizer (i.e. Magna Lyser)

Single-channel micropipettes in ranges 2 – 20, 100 - 1000µl

Deep freezer (<-60ºC)

Refrigerator (2-8º C)

Chronometer

3. PROCEDURE

Whole blood collected in anticoagulant (i.e. EDTA)

This kind of sample could be inoculated without treating, just diluting 1/100 in PBS1X after a freezing and thawing process. However, to avoid neutralizing of virus with antibodies present in the plasma, it is highly recommended to remove the plasma and promote erythrocyte lysis and the collection of their membranes.

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Process of erythrocyte lysis is carried out by osmotic pressure difference, such as is described below:

1. Centrifuge 1 ml of whole blood at approx. 1000g (~2500 rpm) for 10 min at 4°C if possible.
2. Remove the supernatant fluids and <u>wash the red cells three times</u>:
3. Add 1 ml of PBS1X and vortex
4. Centrifuge at approx. 1000g (~2500 rpm) for 10 min at 4°C if possible
5. Remove the supernatant fluids
6. <u>Disrupt the red cells by osmosis</u>:

   Add 1 ml of sterile distilled water and vortex

   Keep on ice for 10 min.
7. Centrifuge at approx. 12000g (~2500 rpm) for 5 min at 4°C if possible
8. Remove the supernatant fluids
9. Add 500 µl de PBS1X containing 1% of antibiotic-antimicotic. Let stand for 20 minutes
10. Store at +4°C (or -70ºC for a longer time) until required

<u>Preparation of tissue samples</u>

1. Using sterile scissors, remove excess fat, connective tissue and muscle
2. Cut the tissue to about the size of a lentil into a 1,5 ml tube with screwcap containing glass beads and 1ml of PBS1X
3. Grind the tissue in automatic homogenizer (i.e. Magna Lyser: 2 cycles of 20 seconds at 6000 rpm)
4. Centrifuge at approx. 1000g (~2500 rpm) for 15 min at 4°C if possible
5. Filter the supernatant (using a needle, syringe and 0.45 µm pore filter) into a new sterile, labelled tube and add 1% of antibiotic-antimicotic. Let stand for 20 minutes
6. Store at +4°C or -70°C until required

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4. REFERENCES

CLAVIJO A., HECKERT R.A., DULAC G.C. & AFSHAR A. (2000). Isolation and identification of bluetongue virus. J. Virol. Methods, 87, 13–23.

GARD G.P., WEIR R.P. & WALSH, S.J. (1988). Arboviruses recovered from sentinel cattle using several isolation methods. Vet Microbiol, 18, 119–125.

Goldsmit, L. & Barzilai, E. (1968). An improved method for the isolation and identification of bluetongue virus by intravenous inoculation of embryonating chicken eggs. Journal of Comparative Pathology, 78(4), 477–487.

OIE. Manual for Diagnostic Tests and Vaccines for Terrestrial Animals. English version in force at date

Chapter African horse sickness (Infection with African horse sickness virus)

Chapter: Bluetongue (Infection with Bluetongue virus)

Chapter: Epizootic haemorrhagic disease (Infection with Epizootic haemorrhagic disease virus)

SEKAR. P., et al. Optimization and characterization of bluetongue virus in embryonated chicken egg. Advanced Biotech. (May 2008)

THOMAS F.C. (1984). Comparison of some storage and isolation methods to recover bluetongue virus from bovine blood. Can. J. Comp. Med., 48, 108–110.

Verwoerd D.W., Huismans H., Erasmus B.J. (1979). Orbiviruses. In: Comprehensive Virology, Fraenkel-Conrat H., Wagner R.R., eds. Plenum Press, London, UK, Vol. 14, 285–345.

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