STANDARD OPERATING PROCEDURE

Title: Swine Pox Virus- Detection of Swine Pox virus DNA by Polymerase chain reaction

Reference: OIE *Terresterial Manual*, (2008)

1.0 Introduction

1.1 Purpose/Scope Of this Sop

This SOP outlines the method for the detection of Swine Pox Virus DNA from tissue samples. DNA is isolated from samples using the QIAGEN QIAamp® Viral DNA mini-kit or the Nucleo-Puro DNA Sure Tissue Mini Kit (Company name), following which Swine Pox Virus specific DNA sequences are detected using a commercial gel based PCR method.

2.0 MATERIALS:

3.1.1	Consumables	Supplier
	0.2ml PCR tubes	GENAXY
	0.2ml Thin Wall Clear PCR 12-Strip Tubes and Flat Strip Caps	GENAXY
	Filter Tips	GENAXY
3.1.2	**Equipment**	**Supplier, Model**
	Class 2 safety microbiological safety cabinet	LABCHEM & LABORTENIK INSTRUMENTS
	Micro-centrifuge	TARSONS
	VERITI 96-well Thermalcycler	APPLIED BIOSYSTEMS
	Micropipette (0.5-10 µl, 10-100 µl, 20-200 µl, 100-1000 µl)	GENAXY (Nichipette)
	7500 Real Time PCR System	Applied Biosystems

3.0 PROCEDURES:

3.1 Collection Collection Of Fowl Pox Suspected Samples

Clinical as well as post mortem samples were collected from field outbreaks of Swine

Pox for detection of the Swine Pox virus.

3.1.1 Clinical samples:

Field tissue samples (liver, spleen, kidney and esophagus)

3.2 Detection Of Swine Pox Viral Nucleic Acid:

Clinical as well as post mortem samples collected from affected pigs were processed for detection of viral nucleic acid by polymerase chain reaction. Standardization of Polymerase chain reaction was done following the method described by OIE *Terresterial Manual*, (2008). In Swine Pox Virus genome, DNA directed DNA polymerase gene was targeted and anticipated product size was 527bp.

3.3 Extraction of viral DNA:

For extraction of Swine Pox viral DNA various samples were prepared following standard methods:

- Freeze dried Swine Pox virus vaccine was reconstituted in sterile distilled water.
- Field tissue samples (liver, spleen, kidney, oesophagus) were homogenized and 20 percent suspension was prepared by suspending in appropriate volume of PBS (pH7.4) A standard protocol enclosed in the kit was followed:
- Briefly, a volume of 70µl of tissue homogenate, 180µl buffer ATL and 20µl proteinase-K was taken in 1.5ml micro centrifuge tube.
- Mixture was vortexed for 2 minutes and incubation was done at 56^0C for 10 minutes.
- Repetition of the vortex and incubation was done again.
- Vortex of the tube was done and added buffer ATL 200µl.
- Again vortex was done and ethanol 200µl (96 to 100 percent) to the micro centrifuge tube and vortexed again.
- Transferring of the material was done from micro centrifuge tube to spin column and then spinned was done at 8000 rpm for 1 minute.
- Collection tube was discarded and transferred the spin column to another collection tube and addition of the buffer the AW_1 500µl was done and again spinned at 8000 rpm for 1 minute.
- The collection tube was discarded and spin column was transferred into another collection tube and addition of the buffer AW_2 500µl was done.

- The collection tube containing the reagents was transferred and spinning was done at 1000 rpm for 5 minutes and the collection tube was discarded.
- The spin column was placed in 1.5 ml micro centrifuge tube and 200µl buffer AE was put into the membrane of spin column. Incubation was done at room temperature for 1 minute.
- Finally, centrifugation of the micro centrifuge was done at 8000 rpm for 1 minute and then sediment was collected.
- Extracted DNA samples were properly labeled and stored at -20^{0}C until use.

3.4 Polymerase Chain Reaction (PCR):

Amplification of the targeted gene was done using the following reaction condition and primer sets (Table1).

TABLE 1: DESCRIPTION OF THE PRIMERS USED IN THE PRESENT STUDYTO AMPLIFY DNA-DIRECTED DNA POLYMERASE GENE.

Primer	Sequence (5' – 3')	Reference
Primer-1 Sequences (Forward)	**5'-TAGTTTCAGAACAAGGATATG-3'**	OIE *Terresterial Manual*, (2008)
Primer-2 Sequences (Reverse)	**5'TTCCCATATTAATTGATTACT3'**	

3.5 Reaction master mixture:

The targeted gene of the Swine Pox virus was amplified using following reagents (Table:2)

TABLE: 2 DETAILS OF THE PCR REACTION MIXTURE

Total reaction volume : 50µl		
Reagents/Ingredients	**Volume**	**Supplier**
Nuclease free water	6.5µl	Sigma
PCR Master mix (Fermentus)	12.5µl	Fermentus
Forward primer (20pmol)	2µl	XXIDT
Reverse primer (20pmol)	2µl	XXIDT
DNA template	2µl	

The contents were mixed by brief spinning and placed all the tubes in a thermal cycler (Applied Biosystems,USA) using following thermal cycling conditions:

TABLE 3: DETAILS OF THE THERMAL CYCLER CONDITION

Number of cycles	Temperature	Time
1 cycle	94^0C	4 minutes
25 cycles	94^0C	30 seconds
	49^0C	1 minute
	72^0C	1 minutes
1 cycle	72^0C	5 minutes
	4^0C	Stored until use

3.6 Confirmation of PCR amplicons:

The amplified PCR products was confirmed by agarose gel electrophoresis using 1.7 percent agarose (Amresco) containing ethidium bromide in 0.5X tris borate EDTA(TBE) buffer.

- Briefly, gel casting tray was set up by placing the comb in the slot containing 8 wells.
- A volume of 40ml agarose gel was prepared by adding 0.680g of agarose to 40ml of 0.5X TBE.
- The agarose was melted in microwave oven (Haier, India) for 1 minute.
- Thereafter, the melted agarose was allowed to come down to 56^0C and 1 µl of ethidium bromide (10mg/ml) was added.
- After proper mixing it was poured on the gel casting tray and allowed to solidify without disturbing the tray.
- Then 0.5X TBE was poured on the gel casting tray till the gel was submerged and the comb was removed carefully.
- Then 10 µl of each PCR amplified products was mixed with 2 µl of 6X loading dye (Fermentas) and loaded into the wells.
- Along the PCR amplicon a 100bp DNA ladder (Fermentas) was loaded and run parallel to the PCR amplicons.
- Electrophoresis was carried out at 78V for 1hour and 30 minutes. The gel was then visualized on a UV transilluminaor (Kodak, Biostep, Germany).
- **The PCR amplicons shows a product size of 527 bp.**