STANDARD OPERATING PROCEDURE			
Title:	**DETECTION OF PORCINE CIRCO VIRUS (PCV) BY PCR**		
Reference:	**G Saikumar, IVRI**	*Author:*	**Dr N N Barman, Dr(Ms) Arpita Bharali**

1. INTRODUCTION

Purpose/Scope of this SOP

1.1. This SOP outlines the method for the detection of PCV DNA from tissue samples. DNA is isolated from samples using the DNASure Tissue Mini Kit.

All PCR reagents are stored at -20ºC. Primers purified either by desalting or HPLC purification are acceptable for this protocol.

2. MATERIALS

2.1

Consumables	Supplier
0.2ml PCR tubes	GENAXY

2.2

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)
Filter Tips	GENAXY

2.3 TABLE 1: Primer sets used:

Primer designation	Primer sequence
PCV2F	CGGATATTGTAGTCCTGGTCG
PCV2R	ACTGTCAAGGCTACCACAGTCA

F= Forward Primer; **R**= Reverse Primer

3. PROCEDURE/METHOD

3.1. Isolation of DNA from samples using kit

3.1.1. Isolate DNA from samples using DNASure Tissue Mini Kit.

3.1.2. Once the DNA has been eluted, store at -18°C or below or keep on ice and proceed to detection of PCV by PCR.

4. Preparation of PCR master mix

4.1 Preparation of PCR master mix must be carried out in the PCR clean room.

4.2. Prepare a master mix containing the following reagents and preferably add the reagents in the order given.

Reagent	Volume per reaction
PCR MasterMix (Fermentas)	12.5 µl
Forward primer (10uM)	2 µl
Reverse primer (10uM)	2 µl
Nuclease free water	6.5 µl

4.3. Thoroughly mix the master mix and aliquot 22µl into the appropriate number of Optical Flat Cap 8/Strip or plates.

4.4. Addition of templates

4.4.1. Add 2µl of the DNA samples to the 22µl master mix.

4.4.2 Once DNA is added, fit caps to all wells. It is important that the caps are fitted firmly and correctly onto the wells before being used on the PCR machine.

5. PCR THERMAL CYCLING CONDITION

5.5.1. Place the reaction tubes in PCR system (Applied Biosystems).

5.5.2. Incubate the reactions with the following thermo cycling profile:

PCR step:

1. 94^0C for 5 min
2. 40 cycles

   94^0C for 30 sec
   56^0C for 45 sec
   72^0C for 30 sec

3. 72^0C for 5 min and hold at 4^0C

6. RESULTS

6.1. Confirmation of PCR amplicons : Gel Electrophoresis

The confirmation of PCR amplicons was carried out by their sizes in agarose gel. The PCR products were electrophorosed in 1.7% agarose gel containing ethidium bromide in 0.5X tris borate EDTA and visualized on a UV transilluminator as per standard procedures. For size comparision, a 100bp DNA ladder marker was run parallel to the PCR amplicons.

The PCR amplicon PCV shows a product size of 481 bp.

Interpretation: The amplified product visualized as a single compact fluorescent band of 481bp under UV light and documented by gel documentation system is considered positive for BVDV.