

# Testing of Oryctes Virus (OrV) in Rhinoceros Beetle Guts

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## Introduction

The rhinoceros beetle (*Oryctes rhinoceros* L (Coleoptera; Scarabaeidae) is a serious pest of coconuts where it has found its way in the Pacific Island Countries and Territories (PICTs). OrV is used as a biological control agent to keep the beetle under control in all of the infested PICTs. Recent releases of OrV, especially in Samoa, have had no effect on the beetle population reduction. This instigated a study into the possible problem of OrV use. SPC in collaboration with AgResearch of New Zealand has developed a method of testing OrV. The procedures for collection and preparation for PCR methods is outlined in this poster.

## Procedures

### Collection of Rhinoceros beetle adults and larvae

The adults and larvae can be found in breeding places. Easiest way is to capture adults in pheromone traps.



The pheromone *ethyl-4-methyl-octanoate* sachet is placed in trap and hanged from tree branch. Adults are separated into collecting jars placed in ice pack containers and taken to laboratory for dissection.



### Dissection – Extraction of Midgut

Live adults brought to laboratory are killed by squirting a very small dose of 95% alcohol with a syringe in the head of the beetle. This should kill the beetle immediately. Remove forewing (elytra) by twisting them with hand. Cut off the hind wing with heavy scissors. Cut off the pronotum. Cut open the abdomen ventral cover and pull it out using heavy forceps. Expose the midgut by cleaning off the fatty tissues. Pull out the gut and cut pieces of 2-3mm.



Remove the elytra (front wings) or by twisting and cut off the hind wings. With fine scissors cut open the ventral part of the abdomen exposing the midgut. Clean off the fatty tissues attached to the midgut. Cut out the midgut into 2-3 mm pieces and place them in fixatives, alcohol, FAA or distilled water as necessary.



Healthy gut, dark and thin



OrV infected gut – milky and swollen

### Storage of the Gut for Diagnostic

The cut pieces of the gut is placed in vials containing; 5 mls of 70-95% alcohol for DNA and PCR studies; in 5 mls of FAA (5mls formalin, 2.5 mls glacial acetic acid, 45 mls of 95% alcohol) for histological studies; or in distilled water for fresh specimen studies.

### Shipment of Gut Samples

Where necessary gut samples need to be packed in hard carton (usually available from local post offices) with Styrofoam lined inner sides. Vials are placed in the carton and cushioned with packaging materials. The parcel must be labeled as "**preserved rhinoceros beetle guts for scientific studies**" and addressed to: **Entomologist, SPC, Suva, Fiji**. The guts must be shipped as soon as possible by courier or any other quickest means.



Special LabPak-1 approved by IATA for transporting of harmful liquids if available, should be used

### Recording of Characteristics of the Guts and other Data

Record the characteristics of the gut (dark or milky, thin or swollen, empty or full of organic matter, etc.) and health of the beetle. Provide full data on the collection site, trap number, date of collection, gender of the beetle, etc.

Beetle no.	Locality	Gender	Insect and gut condition	PCR (ethanol)	Histology (FAA)	Remarks
Example 1 EJ-01-09	Fiji	M	Milky & swollen			
Example 2 SM-10-09	Samoa	F	Thin & dark			

### Performing DNA Extraction for PCR Studies at SPC Laboratory

The preserved insect guts in 70-99% alcohol are used for PCR examination. The DNA is extracted as in detail in this book '*Biological control of rhinoceros beetle in the Pacific Operational Protocols*' by Jackson et al (2009). The DNA obtained is stored at -20°C or kept at 4°C for the PCR set up.

### Documentation of Results

The gel is placed on the surface of the trans illuminator and the UV light is turned on. The bands will be shown in the results. This is then processed and reported.

### Conclusion

The PCR and Molecular laboratory at SPC aims to reduce crop damage to coconut through improved biological control of rhinoceros beetle and to provide environmentally sustainable solutions for pest control. The introduction of appropriate bio-technology techniques will help increase competence amongst the Plant Protection Officers in the Pacific. PCR equipment and laboratory has been set up at SPC with assistance from AgResearch of New Zealand based in Lincoln and funded by New Zealand Overseas Development Assistance (NZ - ODA).

