

Sampling for Tasmanian devil Facial Tumour Disease

Tasmanian devil Facial Tumour (DFT) is a fatal transmissible cancer affecting the Tasmanian devil (*Sarcophilus harrisii*). DFT1 was first observed in 1996 in the north east of Tasmania, more recently a second transmissible cancer, DFT2, has been discovered in Southern Tasmania. Microsatellite analysis indicates that these diseases arose from two distinct transmissible cancer lineages (Pye *et al.* 2016).

PURPOSE

Where a visible lesion is present, diagnosis for these two tumours can be done using;

- a) histological examination
- b) cytogenetics and
- c) direct PCR

TEST TURNAROUND TIMES (MINIMUM)

Histopathology - 5 days;

Cytogenetics - 5 weeks;

PCR – 1 to 2 months (samples are batched).

If the samples are **urgent** please contact the laboratory.

MATERIALS REQUIRED

- Transport medium (supplied by the Animal Health Laboratory must be kept frozen and defrosted before use)
- RNA later in cryovials (1mL)
- 10% Neutral Buffered Formalin (NBF)
- Needles (21G) and Syringes (5mL)
- 4mm biopsy punches
- Sterile dissection instruments
- Alcohol wipes
- Marker for labelling

SELECTING ANIMALS FOR TESTING

Animals with visible lesions should be tested.

SAMPLING PROCEDURE

Sampling of a Tasmanian devil must be undertaken by a trained individual.

- Choose an area of the tumour which is firm and un-ulcerated (if possible).
- Wipe with an alcohol wipe to disinfect the surface of the skin.
- New sterilised instruments should be used for each animal.

The following samples should be taken from each devil. Ordered by priority;

1. A 4mm biopsy of tumour in **Neutral Buffered formalin**.
2. A 4mm biopsy of tumour in **RNA later**.
3. A fine needle aspirate (FNA) of tumour in **transport medium**. No more than five FNA samples for cytogenetic analysis should be sent in at one time.
4. A 4mm biopsy of skin from host in RNA later (if possible)

(See over)

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If the tumour is large enough to collect all samples they should be collected in the following order.

Fine Needle Aspirate in transport medium

- Defrost a tube of transport medium.
- Insert the needle (attached to syringe) through the skin into the tumour.
- Draw back the syringe to extract cells.
- Withdraw the needle and transfer cells to the defrosted tube of transport medium carefully washing the cells from the needle by pulling the medium through the syringe.
- Recap the tube quickly to avoid contamination.
- Store between 15 and 30°C.

RNAlater samples

- Using the 4mm biopsy punch remove a firm piece of tumour.
- Remove tissue from punch and place in RNAlater.

Formalin fixed sample

- Using the 4mm biopsy punch take a second firm piece of tumour and place in 10% NBF.
- Note: If the tumour is very small a single 4mm biopsy can be carefully cut in half using a sterile scalpel blade and forceps, place half in each of the 10% NBF and RNAlater.

Ear RNAlater sample

- Using the 4mm biopsy punch take a piece of ear and place in a separate cryovial of RNA later (avoid hair as much as possible).
- Label all tubes with the ID of the animal.

TRANSPORTING SAMPLES

Samples in transport medium must be kept between 15 and 30°C and returned to the laboratory within 48 hours. All other samples should be submitted as soon as possible. Samples must be delivered by courier or transferred by departmental staff to the laboratory. Please **do not post** samples.

SUBMITTING SAMPLES

Submit samples to:

Animal Health Laboratory
NRE Tas
165 Westbury Road
Prospect TAS 7250

P: 03 6777 2111

E: specimenreception@nre.tas.gov.au

REFERENCES

Pye, Ruth J., et al. "A second transmissible cancer in Tasmanian devils." *Proceedings of the National Academy of Sciences* 113.2 (2016): 374-379.



www.nre.tas.gov.au/AHLabs

AHL NRE Tas 165 Westbury Road Prospect TAS 7250

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