Pfizer Animal Health
Animal Genetics

TECHNICAL SUMMARY

i-SCAN Technical Information
Testing for Microphthalmia in Your Flock

Key Points

- Microphthalmia is a genetic defect caused by a recessive gene that causes a developmental malformation in the eyes of affected lambs. Typically, affected animals are entirely blind.
- Testing of suspect animals is essential to identify carriers, make informed breeding decisions and reduce the frequency of the recessive allele over time.
- i-SCAN® is a genetic test that can effectively identify carriers of microphthalmia, which allows breeders to progressively remove the recessive gene from the flock.

Background to Microphthalmia

Microphthalmia is a genetic defect caused by a recessive gene that causes a developmental malformation in the eyes of affected lambs. Typically, such animals are entirely blind, although some retain the ability to detect movement.

Microphthalmia occurs when a lamb inherits damaged copies of a gene needed for eye development from both parents. An animal which inherits a damaged gene from only one parent is termed a carrier. Carrier animals have normal, functional eyes because they also carry a copy of the normal, functional gene. If an animal inherits two copies of the damaged gene, it will be born with the eye disorder. When blind progeny are observed, there are likely to be multiple carriers in the flock and DNA testing may be required to remove the condition.

The eye of a microphthalmia-affected sheep.

Recessive disorders like this are hard to remove from a ewe flock without a DNA test. Typically, a small population of unobservable carrier ewes will remain in a flock, even when parents of microphthalmia-affected lambs are identified and removed.

Currently in New Zealand, an estimated 5 to 10 percent of Texel or Texel-cross rams are microphthalmia carriers. The origin of microphthalmia in New Zealand is almost certainly due to the existence of a few carrier animals in the Texels imported to New Zealand during the 1970s. Microphthalmia was first recognised in European Texels in the 1950s and remains an issue with this breed. It has been reported in flocks throughout the world including Australia, New Zealand and South America.

i-SCAN® was developed in a collaborative effort between the New Zealand Texel Society, AgResearch science groups, Dr John McEwan and the University of Utrecht.

What is i-SCAN?

i-SCAN is a predictive DNA test that identifies carriers of the damaged gene that causes microphthalmia. i-SCAN allows sheep breeders to screen their flocks to identify animals with a high probability of carrying the damaged gene.

With i-SCAN results, a breeder can plan matings to avoid breeding blind lambs and to progressively remove microphthalmia carriers from a flock.

i-SCAN clear (i-SCAN ^{CLR}) animals have tested free of microphthalmia. The result assures breeders and ram buyers they will not introduce microphthalmia into their flock by using these rams.

The i-SCAN test examines the DNA profile of each animal in the region of the microphthalmia gene and compares it to information in a large database of profiles from blind lambs and known carriers. The data is analysed to determine the probability of an animal having:

- No copies of the damaged gene – i-SCAN clear (i-SCAN ^{CLR})
- One or more copies of the damaged gene – microphthalmia carrier

i-SCAN can predict the status of an animal without reference to pedigree information, however, inclusion of pedigree, and pedigree links to known blind lambs in particular, can assist in interpretation of the results.

Interpreting i-SCAN results

The i-SCAN DNA results report the probability that an animal is a carrier of the microphthalmia gene. The probability estimates are given in a percent range from zero to one hundred. Zero represents a nil probability and one hundred a near certainty that the animal is a carrier. Based on the probability, results and interpretation are provided as described in Table 1 below.

Table 1 – Explanation of Results.

Probability	Result	Explanation
0%	i-SCAN ^{CLR}	The animal tested is clear of any DNA profiles associated with microphthalmia
1% – 95%	At Risk	There is a chance (indicated by the probability) that this animal carries the microphthalmia gene disorder
>95%	Microphthalmia Carrier	There is a high likelihood this animal carries the microphthalmia gene disorder

Technical Information

i-SCAN® analyses blood blot samples collected using DNA kits. In special circumstances the test may be conducted from other material containing DNA, such as semen or body tissue.

The test uses information from several DNA microsatellite markers close to the damaged gene to calculate the probability that a specific animal is a microphthalmia carrier.

A DNA marker is any identifiable length of DNA sequence that shows variation between animals. Markers can be used to trace the inheritance of certain traits or, more importantly for this test, to identify the presence of a particular gene.

When DNA markers are tested in the laboratory, a wide variety of different DNA types can be found in a population of sheep. Combining several markers provides a distinct profile which individually identifies each animal.

By testing markers close to the microphthalmia gene we have been able to identify particular profiles characteristic of carrier animals. The specific profiles identified are known as haplotypes.

Table 2 below gives the frequency of the haplotype for microphthalmia-affected lambs, parents of microphthalmia lambs and other sheep. It demonstrates the strong association of the microphthalmia haplotype with the condition.

The probability is calculated by comparing the test animal's marker profile to the microphthalmia haplotype in known carrier animals. If the probability that a lamb is carrying the gene is zero, the animal's profile includes none of the sequences associated with the damaged gene. If the probability is high, all the marker sequences associated with the damaged gene are present. An intermediate probability occurs when an animal contains only some of the associated DNA characteristics.

Test Specifications

- 2 to 5 markers analysed
- The markers span a 2.4 centi-Morgan region which includes the microphthalmia gene.
- Out of the thousands of possible marker profiles, only one is associated with the damaged microphthalmia gene

Table 2 – Haplotype Frequency.

Microphthalmia haplotype occurrence	
In affected lambs	100%
In parents of affected lambs	100%
In the overall Texel population	2 – 10%
In non-Texel breeds	0%

What do i-SCAN results mean for breeding strategy?

The i-SCAN® test can help breeders verify which animals are i-SCAN CLR and identify which animals are carriers. Table 3 summarises the inheritance of microphthalmia and the consequences of mating animals with different i-SCAN results.

Breeding only from i-SCAN CLR animals will rapidly reduce the prevalence of the gene carriers in the flock. The most direct breeding strategy is simply to test all animals, identify the carriers and avoid using these in breeding.

As with any gene test, a degree of balance is required; culling simply on the i-SCAN test is not recommended. Some compromise should be made if carrier animals are identified which are of high genetic merit: these are critical to genetic progress within the flock. In this case, the high genetic merit carrier can be mated to i-SCAN CLR stock and the offspring tested.

This type of strategy avoids the loss of genetic progress from culling high-value animals solely on the basis of their i-SCAN status.

While this strategy is direct, the upfront cost of testing may be prohibitive for some breeders. The alternative is testing potential breeding sires only and then mating just the i-SCAN CLR rams. No blind lambs will be born with this strategy, but carrier ewes will persist and pass on the gene within the flock for many generations. Tests would have to be maintained for a long period of time (5 – 10 years) to have any chance of eliminating microphthalmia carriers from the flock.

Possibly the optimum strategy is to focus testing on potential sires and strategically test dam lines where carrier families are detected.

Ultimately, the choice of which strategy to undertake will vary depending on the goals of the breeder, how prevalent the microphthalmia gene is within the flock, genetic merit of microphthalmia carrier animals and the financial situation of the farm.

Table 3 – Microphthalmia Inheritance and Breeding Implications.

Sire	Dam	Progeny
i-SCAN CLR	Microphthalmia Carrier	Physical appearance: Normal • 50% will be i-SCAN CLR • 50% will be Microphthalmia Carriers
Microphthalmia Carrier	i-SCAN CLR	Physical appearance: Normal • 50% will be i-SCAN CLR • 50% will be Microphthalmia Carriers
Microphthalmia Carrier	Microphthalmia Carrier	75% of animals with normal appearance • 25% i-SCAN CLR • 50% will be Microphthalmia Carriers 25% blind lambs displaying microphthalmia