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, homozygous affected animals are entirely blind.

• It is present in Texel or Texel composite sheep only.

• Testing of suspect animals identifies carrier animals, allowing informed breeding decisions to reduce the frequency of the recessive allele over time.

• i-SCAN® is a genetic test that can effectively identify carriers of Microphthalmia, allowing 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.

Recessive disorders like this are hard to remove from a ewe flock without a DNA test because the damaged gene is hidden in carriers. Typically, a small population of unobservable carrier ewes will remain in a flock, even when parents of Microphthalmia-affected lambs are identified and removed.
The impact of Microphthalmia

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.

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 that carry a copy of the damaged gene.

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

The i-SCAN test

i-SCAN was originally developed in a collaborative effort between the New Zealand Texel Society, AgResearch science groups and the University of Utrecht. Over time, there have been a number of iterations of the I-SCAN test. Recently, Microphthalmia test technology has moved from an association (haplotype) test to a more definitive SNP test. The current SNP test is for the presence (reported as Carrier) or absence (reported as i-SCANCLR) of a mutation described by Becker et al. (2010; PLoS One, Jan 13;5(1):e8689) that is associated with Ovine Microphthalmia in Texel Sheep. This mutation is believed to be the causal mutation. While the results from the test are highly predictive, we cannot exclude the possibility there are other mutations or factors that are responsible for this disease that this test will not resolve.

The i-SCAN test examines the DNA profile of each animal at a specific region in the Microphthalmia gene. It looks at a specific change (SNP mutation) in DNA at that region to determine whether an animal has:

- No copies of the damaged gene – i-SCAN clear (i-SCANCLR)
- One copy of the damaged gene – 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.

i-SCANCLR 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.
Interpreting i-SCAN results

The i-SCAN DNA results simply report whether an animal is a carrier of the damaged Microphthalmia gene or whether it is free of any damaged copies.

Table 1 – Explanation of Results

<table>
<thead>
<tr>
<th>Result</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>i-SCANCLR</td>
<td>The animal tested has a clear result and is free from any copies of the damaged gene.</td>
</tr>
<tr>
<td>Carrier</td>
<td>The animal tested has one copy of the damaged gene.</td>
</tr>
</tbody>
</table>

What do i-SCAN results mean for breeding strategy?

The i-SCAN test can help breeders verify which animals are i-SCANCLR and which are carriers. Table 2 summarises the inheritance of Microphthalmia and the consequences of mating animals with different i-SCAN results.

Breeding only from i-SCANCLR 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.

Simulation work has shown that in the absence of testing for carrier individuals, the reduction in gene frequency is minimal over a 10-year period. In contrast, full testing options resulted in a much more rapid decline, with the recessive generally being eliminated within 4 years of full testing.

Table 2 – Microphthalmia Inheritance and Breeding Implications.

<table>
<thead>
<tr>
<th>Sire</th>
<th>Dam</th>
<th>Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>i-SCANCLR</td>
<td>i-SCANCLR</td>
<td>Physical appearance: Normal • All will be i-SCANCLR</td>
</tr>
<tr>
<td>i-SCANCLR</td>
<td>Microphthalmia Carrier</td>
<td>Physical appearance: Normal • 50% will be i-SCANCLR • 50% will be Microphthalmia Carriers</td>
</tr>
<tr>
<td>Microphthalmia Carrier</td>
<td>i-SCANCLR</td>
<td>Physical appearance: Normal • 50% will be i-SCANCLR • 50% will be Microphthalmia Carriers</td>
</tr>
<tr>
<td>Microphthalmia Carrier</td>
<td>Microphthalmia Carrier</td>
<td>75% of animals with normal appearance • 25% i-SCANCLR • 50% will be Microphthalmia Carriers • 25% blind lambs displaying Microphthalmia</td>
</tr>
</tbody>
</table>
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 testing all animals in the flock 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. (Figure 2)

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
- The financial situation of the farm

![Figure 2 – Carrier Lambs](image-url)

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