

# CARLA® SALIVA TEST

## Measuring parasite immunity in sheep

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### Questions and Answers

#### **What is CARLA®?**

CARLA® is a molecule found on the surface of all internal parasite larvae (L3s) infecting livestock. It is very tough and able to withstand passage through the rumen. CARLA® is only present for a few days after worms are ingested. Later stages of the worm life-cycle (L4 and adult) do not have the molecule.

#### **What are CARLA® antibodies?**

CARLA® antibodies are produced by the sheep's immune system in response to larval challenge. In immune sheep, high levels of CARLA® antibodies are present in saliva and gut mucus; these antibodies bind to CARLA® on the surface of ingested L3 and prevent establishment.

#### **What is the CARLA® Saliva Test?**

Some sheep produce more CARLA® antibodies than others. The saliva test is a simple method to test for the presence and level of these protective CARLA® antibodies in saliva. Testing involves taking a swab of saliva and sending it to AgResearch for analysis. The test result gives the level of CARLA® antibody present at the time of sampling.

#### **What is a “good” result for the CARLA® Saliva Test?**

The test results (example below) provide a numerical read out of the concentration of CARLA® antibodies in saliva and an interpretation of what this means in terms of protection from incoming parasite larvae.

Tag	CarLA IgA		Larval Protection
4	0.00		None detected
5	4.05		Medium
18	0.37		Trace
43	0.00		None detected
61	1.71		Medium
63	0.00		None detected
72	0.53		Low
78	5.14		High
85	0.00		None detected

When larval protection level is medium or high (above 2.0 antibody units), animals have good protective immunity to incoming parasite larvae. However animals will only maintain such high larval protection levels when there is plenty of larval challenge from pasture (for example in autumn). Where pasture parasite levels are lower (e.g. in spring and early summer) a result above 0.5 antibody units (low larval protection) is a good result.

**How do sheep differ in their level of CARLA® antibodies?**

There is large variation in the time at which spring-born lambs develop a protective CARLA® response:

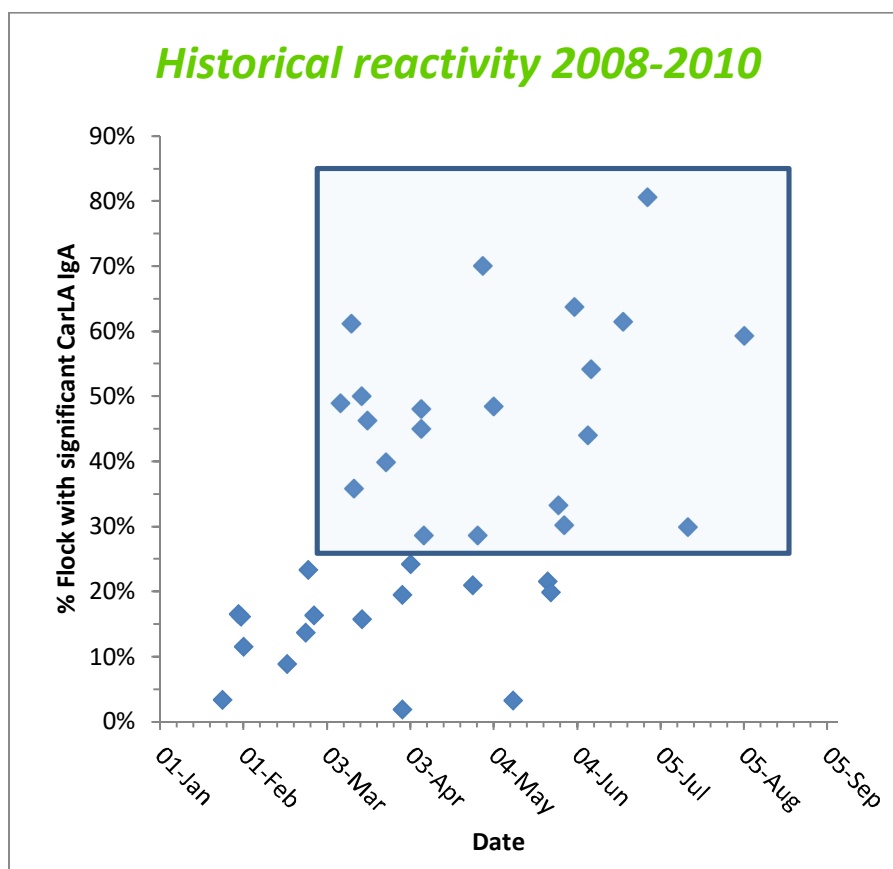
- About 10% of animals produce CARLA® antibodies by February.
- In April, 30-40% of lambs will have high levels of antibodies.
- In June, 50-60% of lamb will have high levels of antibodies.

Figure 1 graphs the proportion of animals with good larval protection in flocks sampled from January to August.

Eventually most hoggets develop some sort of protective immunity, although, even in flocks with high challenge, there are typically still 10-20% of animals where CARLA® levels remain low.

The timing and strength of an individual antibody response is strongly influenced by an animal’s genetics. The heritability of the CARLA® antibody response is high (about 0.3 or 30%).

**Figure 1:** Historical flock CARLA® reactivity for January - August for 2008-2010 based on percentage of animals >2.0 units of CARLA® antibody. The blue box represents flocks with greater than 25% of the animals testing above this level.



### ***How does CARLA® antibody level relate to parasite challenge on pasture?***

The overall size and timing of a CARLA® response in a flock is related to pasture larval challenge; in flocks with low larval challenge (*e.g.* merinos on dry hill county) lambs develop the immune response much more slowly. Even in this case, however, some individual animals will develop the response sooner than others.

Once CARLA® immunity is established, the level of CARLA® antibodies in saliva goes up and down according to the level of larval challenge. If challenge is removed (*e.g.* putting sheep onto crop), the level of CARLA® antibody in saliva drops away. If these sheep are then shifted to pasture with high larval challenge, saliva antibody levels will rapidly rise to high levels within a few days.

### ***How does CARLA® antibody level relate to faecal egg count?***

Lambs with CARLA® antibodies in saliva tend to have a lower faecal egg count. The antibodies neutralize incoming larvae so fewer establish, resulting in less adults and less eggs produced. Animals with a “good” CARLA® antibody response by March are typically shedding 30% less parasite eggs than their non-responsive flock mates. The genetic correlation between CARLA® antibody level and faecal egg count is about -0.5 (this means that it is a useful genetic predictor of faecal egg count).

### ***How does CARLA® antibody level relate to growth rate?***

Overall there is a positive relationship between CARLA® antibody level and production under challenge. Animals with a good CARLA® response are expected to be more resilient to larval challenge because they neutralize larvae on entry and therefore reduce the negative down-stream effects of established worms.

In practice, the impact on production traits is dependent on drenching and how quickly the CARLA® response develops. Once CARLA® antibodies develop in summer and autumn, the CARLA® response limits the establishment of worms between drenches. The impact on growth will be highest where there is high larval challenge and a long interval between drenches.

### ***Does drenching affect the CARLA® antibody level?***

No. The CARLA® antibody response relates only to the number of larvae being ingested by the sheep. Lambs with a good protective antibody response can still be identified in a flock drenched every 28 days. Drenching will affect the CARLA® level only in as much as it reduces pasture contamination and therefore the number of larvae seen by the sheep.

### ***Does CARLA® work on all worm species?***

Yes. The CARLA® antibodies detected by the test bind to CARLA® on all the common infectious internal parasites of lambs (e.g. Haemonchus, Trichostrongylus, Teladorsagia, Nematodirus). There is a question as to whether all worms are equally good at priming the CARLA® immune response in lambs.

### ***Are CARLA® antibodies detected in all sheep breeds?***

Yes. Animals with elevated CARLA® antibody levels have been detected in all breeds tested to date (incl. Romney, Coopworth, Perendale, Texel, Wiltshire, Composite, Merino).

### ***Can I use CARLA® to breed sheep which perform better in the presence of larval challenge?***

Yes. Selection of breeding stock with higher levels of CARLA® antibodies will result in progeny with a better response to larval challenge. These progeny will tend to have lower faecal egg counts and similar or better performance under high larval challenge than the progeny of unselected animals. Because of the good heritability (30%), substantial year-on-year gain is expected. CARLA® results can be used to identify individual rams and ewes with a better immune response to parasites. However, the best way to apply CARLA® is within a planned programme that calculates CARLA® breeding values (through Sheep Improvement Limited (SIL)) and which tests a cross-section of the breeding flock (e.g. at least 20 animals per sire used).

### ***What are CARLA® breeding values?***

Sheep Improvement Limited (SIL) can generate a CARLA® BV using the raw CARLA® results and pedigree information from stud animals. This CARLA® BV is the best estimate of an animal's genetic potential to generate CARLA® antibodies in response to a larval challenge (given the data available). The breeding values can be read directly as a percentage so, for example an animal with a breeding value of "80", will tend to produce 20% more CARLA® antibodies than an animal with a breeding value of "60" (*i.e.* 80%-60%=20%). Breeding values are most accurate where a large number (*i.e.* hundreds) of lambs are measured each year in the stud.

### ***How do can I compare CARLA® tested animals to those on the standard dual purpose index on SIL?***

There are no parasite traits in the standard dual purpose index. CARLA® is a separate development trait which is not included in any of the SIL indexes. The best solution at the moment is to look at CARLA® alongside the dual purpose index. Our results show that progeny from rams with a high CARLA® Larval Protection Breeding Value will tend to maintain their performance better during a parasite challenge and have lower FEC. So for rams with a similar dual purpose index it is better to pick the ram with higher CARLA® BV.

### ***Wouldn't it be better not to have any immune response and put all the effort into production?***

In practice, all sheep are exposed to worms and develop some sort of immune response to worms. The question is whether this immune response is efficient and protective or not. The worst situation is when sheep put a lot of energy into immune responses (such as persistent scouring) that may not protect them, and which leads to reduced production. We are targeting the CARLA® immune response because it is at the other end of the spectrum; it is protective and, because it acts on larvae, it cuts down the workload for downstream immune responses against established worms.

### ***When is the best time of year to test?***

Useful results for the CARLA® saliva test can be obtained from lambs between February and June (*i.e.* 5-9 months of age). The best results are obtained at the peak of larval challenge (typically in late May or June). This is the best time to sample if breeding values are not being used. Timing is less critical if CARLA® breeding values are being calculated because breeding values from February - June samplings are all highly correlated with each other.

The recommended sampling strategy is to check the larval challenge level by sampling 20-30 animals from the flock, approximately a week before the intended flock sampling date. These samples are sent to AgResearch to confirm there has been sufficient larval challenge to deliver good results. Following confirmation, a sample is taken from each individual in the target flock and the tag number of the animal recorded on the tube.

Breeders using CARLA® are typically using one of two strategies:

- Strategy 1: Test recorded lambs in late February to get breeding values for CARLA® before mating decisions are made, or
- Strategy 2: Sample in May at the peak of challenge to select two-tooth sires and calculate breeding values for use in the next years mating decisions.

Both strategies will deliver good genetic gain and the decision largely depends on the age structure of sires to be mated and the workflow in the stud operation prior to mating.

### ***How long will sampling take?***

Saliva sampling itself takes about 30 seconds per animal. A dental swab is rubbed in the cheek pouch for approximately 7 seconds and then placed in a labelled vial. It combines well with other tasks where the tag is recorded (e.g. muscle scanning and weighing). An efficient sampling team involves three people; one person to handle tubes and record tag numbers and two people sampling. Once the workflow is set up, it is reasonable to sample over 120 animals per hour. With EID and barcode systems, this rate is increased.

### ***Do animals need special management before sampling?***

No. The only requirements are that animals are on parasite-infected pasture (not crop) and have had a significant larval challenge before sampling. For example, grazing animals to be sampled on shorter pasture that has been previously grazed by lambs will usually give a good larval challenge. Animals to be sampled need to be grazed in one mob or, if there are several mobs, the mob of each animal recorded. Animals can be drenched at any time, using any short acting drench.

### ***How does CARLA® breeding selection relate to “Resistance” or Faecal Egg Count (FEC) breeding selection?***

CARLA® larval protection level has a high genetic correlation with FEC (-0.5), so selection using CARLA® will reduce the average faecal egg count in a flock. Modelling suggests CARLA® selection will reduce FEC at over half the rate of a well implemented FEC programme. The key advantage of CARLA® is that it typically has better (more favourable) correlations with production traits than FEC.

Overall, these results mean there is potential to achieve faster gains in growth and other production traits in a CARLA®-based programme than in a FEC based programme.

### ***How does CARLA® breeding selection relate to “Resilience” (days to drench) breeding selection?***

In large flocks under high larval challenge, significant positive associations have been observed between CARLA® antibody and weight gain in ewes. Further work is underway to quantify these effects in terms of the “days to drench” used by resilience breeders. These results, as well as the way CARLA® antibody works, suggest the response is one mechanism by which animals remain healthy and productive though periods of high larval challenge.

## ***Can CARLA® be combined with FEC or Resilience selection programmes?***

Yes. CARLA® is effective on its own, but a combined approach is even better.

For a WormFEC® breeder, CARLA® will add to FEC data, with little overlap or redundancy. This is because CARLA® and FEC have a high genetic, but relatively low phenotypic, correlation. In general, addition of CARLA® to a FEC based programme will add accuracy and improve the rate of progress for reduction of FEC. This statement is particularly true for breeders using FEC1a and FEC1b (rather than the full FEC1 and FEC2) as these traits have a lower heritability ( $\approx 0.18$ ) than FEC2 or CARLA®. The options for a combined programme are to sample CARLA® in late February at the same time as FEC1b or to measure CARLA® in retained animals after mating in May.

For FEC breeders who have managed to substantially reduce pasture larval challenge in their own flocks (and so have little data on “resilience” or growth under high larval challenge), CARLA® data may also give insight into which low FEC animals would handle a higher larval challenge with lower production losses (*i.e.* be more resilient).

For a resilience breeder, CARLA® can also add accuracy and give insight into animals with high resilience and reduced FEC. Work is currently underway to better define the genetic relationship between the resilience measure, days to drench and CARLA®.

## ***Can I sample replacement ewe lambs in an unrecorded flock?***

The CARLA® Saliva Test can be used to identify replacement ewe lambs in unrecorded flocks. The best time to select ewes is in late autumn which is typically when larval pasture challenge is highest. In our trials, which compared ewes with high and low CARLA® larval protection in summer-autumn, the high CARLA® ewes had lower FEC, and their lambs also had lower FEC in the following season. Currently several organic and conventional farms are evaluating large scale application of CARLA® ewe selection using a MAF Sustainable Farming Grant. If you want to be kept up to date with developments please email us at [carlasalivatest@agresearch.co.nz](mailto:carlasalivatest@agresearch.co.nz).

## ***Can I sample my adult sires?***

The best option is to purchase rams from a breeder providing CARLA® larval protection level breeding values and regularly CARLA® testing lambs as a part of their breeding programme.

Determining CARLA® levels in adult animals gives no indication of when these animals developed the ability to handle larval challenge as measured by the CARLA® Saliva Test. If an animal develops the response relatively late, say at 12 months of age, it could potentially produce off-spring that also has a delayed protective CARLA® antibody response. We are currently evaluating the relationship between larval protection levels in lambs and older animals which may validate the application of CARLA® to test in older ewes and rams.

## ***Does the test work in other livestock species?***

We can identify cattle and deer under larval challenge with good CARLA® antibody levels. We are undertaking research to validate whether the test can be used to identify animals in other livestock species with enhanced protective immunity to internal parasite infection.

## ***For more information on the CARLA® SALIVA TEST***

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**Web:** <https://www.agresearch.co.nz/doing-business/products-and-services/carla-saliva-test/>

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## *Grazing options to maximise CARLA® responses in sheep*

### **Background**

The CARLA® response (IgA antibody in sheep saliva) is developed in sheep following exposure to parasite larvae (L3s) ingested with pasture during feeding. Development of this protective response takes time and is likely to be related to the amount of L3 ingested. The size of the L3 population on pasture generally increases over summer and autumn, reaching maximum levels in March to May under NZ conditions. Once the CARLA® response is developed in an animal, its level of antibody will then go up and down depending on the number of L3 ingested. Development of L3 from eggs excreted with sheep faeces, and then their survival on pasture, is strongly influenced by climatic conditions. The greatest period of larval development is in summer under New Zealand conditions (*e.g.* development to L3 over summer can occur in 7-10 days). However excessive periods of hot dry weather are generally detrimental to L3 development or survival.

The use of crops, new pastures, hard feed, quick rotations and short periods between drenches (3-4 weeks) can all lower livestock exposure to parasite larvae. While these management practices may maximise production in young animals, they are likely to slow development of reasonable CARLA® antibody responses due to lowered L3 challenge.

**So how can a farmer maximise his CARLA® response in young animals without greatly affecting animal production?**

### ***Grazing plan to maximise CARLA responses in young animals***

Generally, farmers graze lambs post-weaning by rotation around a number of paddocks. Paddocks that are likely to contain elevated larval parasite challenges are:

- Those used for lambing (eggs excreted from ewes that have had a relaxation of immunity around the period of lambing).
- Paddocks grazed by undrenched hoggets / two-tooths.
- Paddocks grazed by lambs post-weaning and at least 21 days since last drench.

If you can identify those paddocks where any of these events have occurred, then grazing animals to be CARLA® tested 10-14 days before a monitor saliva sample would be ideal. If there is good pasture cover, try to graze those paddocks more extensively over the 10-14 days before saliva sampling. After carrying out a monitor sample, if possible continue to graze the animals on wormy paddocks. Grazing “clean” paddocks for more than 5-7 days will see a lowering of the CARLA® response due to a reduction in exposure to L3s on pasture.

Even when larval challenge is good, our trials suggest that it is not until late February or March before reasonable numbers of animals are showing a good CARLA® response (>25% with >2 units of CARLA® antibody and assuming reasonable periods of warm moist conditions).

## Saliva Sampling Instructions

### Monitor Sampling

Before saliva sampling all intended animals in a flock, it is important to take monitor samples, to ensure that the flock has developed a reasonable CARLA response after parasite larval challenge. Monitor sampling involves collecting saliva samples, at random, from ~20 animals from the flock. Freeze samples in an individual bag with sampling date and any other details you may like to record. We suggest collecting a monitor sample about a week before the intended sampling date, freeze then send the next day along with any other previous monitor samples taken. Use the pre-paid courier bag included with the kit to send the samples to the CARLA® Saliva Test Unit. If you inform us via e-mail or phone that you have sent a monitor this will assist us in provide a quick response on the monitor sample often within 48 hours.

We will report back to you via e-mail on your flock's CARLA® Saliva Test reactivity profile and recommendations on whether the flock is ready for sampling. Individual animal results will not be provided on monitor samples.

### Flock Sampling

When your flock's reactivity for the CARLA® Saliva Test is adequate, collect saliva samples from all the appropriate animals. For each animal, clearly record the animal tag number on the sample tube. Pack the samples into a plastic bag and freeze the at least overnight before sending to the CARLA® Saliva Test Unit. Please use the cooler bag supplied to send flock samples back to us. Non-urgent samples can be kept frozen until analysis is required.

Record your details, flock and mob information in the CARLA® Saliva Test Sample Submission form. Read the terms and conditions and sign the submission form.

**Important Note:** If the animals are in more than one mob, collect and store samples from each mob separately. Keep them in separate bags, clearly labelled (within the larger cooler bag) so that sampling mob can be recorded when they reach the laboratory.

## Saliva Sampling Technique

Using forceps, clasp a cotton roll approximately 5-10 mm from one end.



Restrain sheep to be sampled, then place the cotton roll between inside of cheek and jaw of the animal. Gently move forceps backwards and forwards for 7-10 seconds to collect saliva



Remove forceps from mouth and place cotton roll into a plastic vial. Replace lid. Clearly label the vial with the animal's tag number.

While you are working your way through a mob, place the vials in the cooler bag with a couple of ice packs to chill them down. After you have finished for the day **freeze** the samples as soon as possible. Once all sampling has been completed courier the frozen samples in the cooler bag, along with a couple of frozen cold packs, to the **CARLA Saliva Test Unit**.

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## *Pricing* (excl. GST)

For client sampling more than 50 animals the cost of sampling is \$10.50 per sample

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