The University of Georgia

College of Veterinary Medicine
Department of Infectious Diseases

DrenchRite® Larval Development Assay:
for the laboratory detection of anthelmintic (dewormer) resistance

DESCRIPTION:

The DrenchRite® Larval Development Assay (LDA) is an *in vitro* test for the detection of anthelmintic resistance in the major gastrointestinal nematode parasites infecting small ruminants (sheep, goats, llama, alpaca, etc). The test evaluates the resistance to benzimidazole (e.g. Valbazen, Panacur, Safeguard), levamisole (e.g. Totalon, Levasol, Prohibit), and avermectin/milbemycin (Ivomec, Cydectin). Nematode resistance to all drug classes listed above are tested for in this assay from a single pooled fecal sample. Nematode eggs are isolated from the submitted fecal sample and placed into the wells of a microtiter plate containing growth media and anthelmintic. The concentration of anthelmintic required to block development of nematode larvae is related to the effectiveness of the drug in the animal.

The DrenchRite® LDA offers a diagnostic alternative to the laborious task of performing fecal egg count reduction tests (FECRT) in order to determine the effectiveness of dewormers. All that is needed to perform the DrenchRite is a pooled fecal sample from 10 or more animals containing adequate numbers of nematode eggs. The test can be done with fewer than 10 animals, but it is recommended to include at least 6. The mean fecal egg count (FEC) in the sample should be > 350 eggs per gram (EPG), but samples with mean FEC >500 are preferred. In general, the higher the mean FEC, the better the assay works. The higher egg counts allow for a much cleaner extraction of the eggs from the feces. If *Haemonchus contortus* (barber pole worm) is your primary concern (this is the most common), and you are using the FAMACHA® system, only select feces from animals scored as 3, 4, or 5. Animals scored as 1 or 2 usually will have low egg counts.

DIRECTIONS FOR SAMPLE SUBMISSION:

Collect a pooled sample made up of feces from 10-20 animals (minimum 6). It is preferable to not use animals that have been dewormed in the last 4 - 6 weeks because worms that survived treatment will bias the result. However, the test can performed at anytime after treatment as long as there are enough parasite eggs being passed in the feces. It is best to collect samples directly from the rectum, however, feces can be collected off the ground if the animals are first put into a shed with a clean floor (free of bedding, grass and dirt). Feces are easily collected from the rectum of mature animals using a latex glove with a little OB lubricant or KY jelly. The size of the sample that is needed to perform the test depends on the number of eggs in the feces (EPG). If FECs are high (>1000 EPG), only 50-60 grams of feces (lemon-sized clump) are needed. If FEC are unknown or < 500 EPG, about 120 grams of feces (orange-sized clump) are needed. We can always dispose of extra feces – better to include too much than too little.

On the day of collection, it is critical that feces be kept cool to prevent hatching of eggs, but care must be taken not to get the samples too cold because this will inhibit hatching. At the time of collection, feces should be placed in a cooler with ice packs to keep the sample cool and can be placed in the refrigerator overnight. However, **feces should not be kept refrigerated more than 48 hr. – *prolonged chilling will inhibit hatching of eggs making it impossible to perform the assay*.** We have also found that samples in direct contact with ice packs for 24 hr often do not hatch well. Therefore, if kept cool with ice packs, keep the samples from direct contact with the ice packs. **Because of this problem with cold-inhibition, fecal collections should be timed so that they can be hand delivered or shipped the same or the next day. If feces are to be shipped to the lab it is important that air be excluded from the feces to prevent the development of the nematode eggs prior to their isolation and testing (see below).**

SAMPLE PREPARATION (for shipping):

We currently recommend two different methods for packaging the sample for Drenchrite® LDA submission:

1. Utilize the "Reynolds Handi-Vac" system which utilizes a small handheld vacuum pump and special zip lock type bags for vacuum sealing. The Reynolds Handi-Vac kit is available at most grocery stores and at Walmart for around $10.00. The sample is placed in the Reynolds Handi-Vac bag and sealed. The Handi-Vac pump is used to evacuate all of the air out of the bag, providing an anaerobic environment that will delay the hatching of the nematode eggs until they arrive at our lab. Samples packaged this way can be sent by priority mail, so long as they arrive in our lab within 3 days of collection. We recommend that you place a piece of tape over the vacuum port of the bag once you have vacuumed all of the air out of the bag.

2. *Wrap the sample tightly in plastic wrap in order to exclude all air*. A small amount of water should be added to moisten the feces making it easier to work with (add small amount of water to a cup containing the feces and let stand 15 minutes). Feces should be moist but not wet so better to add to little than too much water at first. Take a tongue depressor or other instrument and gently break up the feces and mix. Dump the clump of feces onto a sheet of plastic wrap, fold the plastic wrap over the feces and kneed it like dough. You should wind up with a cigar-shaped sample in which individual fecal pellets cannot be readily seen. Wrap tightly in the plastic wrap, then wrap the "cigar" tightly in aluminum foil and place the wrapped feces into a ziploc bag, again excluding all air from the bag. Label the bag with the species (sheep, goat, llama, etc), farm name, and date of collection. ***Ship by overnight express*.*** Samples should not be exposed to extremes of temperatures (i.e. do not freeze or leave in the sun). Refrigeration is not needed and is not desirable after samples are processed to exclude air. Samples need to be processed into "cigars" within 48 hr. of collection and samples should reach the laboratory within 72 hr of collection. If the samples will be hand-delivered to the lab within 48 hr., then they can be kept cool and do not need "air-exclusion processing".

Please understand that, regardless of packaging and shipping method, the DrenchRite® test requires a good deal of time to set up, and samples cannot sit around very long -- so please contact the laboratory prior to sending in a sample to be sure we will be able to process it upon arrival (706-542-0742). *If samples are sent to us without prior communication, it may not possible for us to perform the testing.*

Results with interpretation will be available approximately 14 - 21 days after the sample is received.

* If using the US Postal Service for the overnight delivery, be sure to check ahead of time to make sure they deliver to Athens, GA. With FedEx or UPS there should not be any problems.

INFORMATION TO BE INCLUDED WITH SAMPLE: (Submission form attached).

1. Owner name and contact information (including email and fax if available)
2. Name and contact information of veterinarian
3. Species and breed of animals
4. Number of animals feces were collected from, and manner of collection (from rectum or ground)
5. Date of last deworming and drug used

COST:
The current cost (Dec. 2010) is $450 for clinical assays. Either Sue Howell or Bob Storey can help with questions regarding the assay.

A check must be submitted with the sample. Samples received without payment may be discarded unless prior arrangements have been made. (This policy was required because we have had instances where payment was never received for the services provided despite repeated attempts to collect).

If the sample is not adequate to perform the assay (too few eggs), there will be no charge for the assay, but a $50 handling charge will be charged for processing and conducting preliminary analysis. If eggs do not hatch properly, thereby preventing the collection of good quality test data (rare if samples are handled correctly prior to submission), we will give a $75 credit toward a resubmission. We cannot, however, give a refund because of the time and expense we have already invested in setting up the assay.

FOR MORE INFORMATION CONTACT:

Ms. Sue Howell OR Mr. Bob Storey (in lab of Ray M. Kaplan, DVM, PhD)
Department of Infectious Diseases
College of Veterinary Medicine
University of Georgia
501 D.W. Brooks Drive
Athens, GA 30602
voice: (706)-542-0742
e-mail: jscb@uga.edu OR
bstorey@uga.edu

Please include either: Sue Howell OR Bob Storey's name on the address when shipping the sample.

DrenchRite Submission Form

Client Name : _____
Farm Name (if applicable) : _____
Client Address : _____
City, State, Zip : _____

Home Phone Number : _____
Cell/ Other Number : _____
Fax Number (if applicable) : _____
E-mail Address : _____

Name of Veterinarian / Clinic : _____
Address : _____
City, State, Zip : _____
Phone Number : _____
Cell/ Other Number : _____
Fax Number (if applicable) : _____
E-mail Address : _____

Animal Species/ Breed Submitted : _____
Number of Animals Collected: _____
Last Deworming Date and Dewormer Used: _____
Manner Samples were Collected (from Ground or Rectum) : _____

DATE of COLLECTION : _____

PLEASE READ THE COLLECTION/ SUBMISSION PROTOCOL BEFORE COLLECTION TO ENSURE PROPER SAMPLE SUBMISSION.

PLEASE CONTACT THE LABORATORY PRIOR TO COLLECTION TO ENSURE YOUR ASSAY CAN BE RUN! (706-542-0742)

SEND TO:

Sue Howell or Bob Storey
Dept. of Infectious Disease
College of Veterinary Medicine
501 D.W. Brooks Dr.
University of Georgia
Athens, GA 30602