GENETIC TESTING FOR PLN-ASSOCIATED VARIANT GENES

STEP BY STEP GUIDE TO USING AND SUBMITTING CHEEK SWABS

UPDATED – October 2015
Introduction

- After years of research supported by hundreds of Wheatens, their owners and breeders, Drs. Meryl Littman and Paula Henthorn at Penn Vet have identified mutations associated with PLN in two genes.

- As a result, there is now a test using a non-invasive cheek swab (also referred to as a “cheek brush” or a “cytology brush”), which an owner can use and submit to the University of Pennsylvania School of Veterinary Medicine for interpretation – no blood test, no complicated shipping, no trip to the vet!

- All swabs submitted will support ongoing research into PLN as well as PLE. This guide will walk you through using the swab and submitting it to Penn.

- In October 2015, this guide was updated with instructions for using the PennGen website for submission.

For instructions on litters or frozen semen testing, visit the SCWTCA website at http://www.scwtca.org/health/dnatest.htm
First, obtain your swabs

Most individuals will submit cheek swabs (aka cytology brushes). You can get these in a couple of ways:

- Ask your own vet for two (2) “sterile cytology swab packs”. Each should come in a sealed sleeve.
- You can obtain them from http://www.ezswabs.com/ at a cost of $7.79 per dog (eg, 2 swabs)
- You can obtain kits from Penn:
  - For each dog, send a stamped, self-addressed business envelope for each dog to:
    
    Michael Raducha/ PLN Test  
    Ryan Veterinary Hospital – Room 4022  
    University of Pennsylvania School of Veterinary Medicine  
    3900 Delancey St  
    Philadelphia PA 19104-6010  
  - If you are testing multiple dogs, send a stamped, self-addressed LARGE envelope to Penn and tell them how many dogs you will be testing – number of dogs, not number of swabs.
    
    - As a guideline, a large manila envelope that can hold enough for 10 dogs weighs about 6 ounces. You can calculate postage using the USPS Postage Price Calculator.
  - Owners living outside the US only
    
    - Request your kit(s) from Penn as described above. Envelopes need to be self-addressed but you can include a check (US funds only) for the amount of the postage. You can calculate postage using the USPS Postage Price Calculator.
Using the PennGen site

- “Paperwork” is done through the “PennGen” website at http://research.vet.upenn.edu/penngen
- Here’s the process
  - First, create an account for yourself. Once you’ve done this, you can go to your account in the future and add dogs.
  - Then, add information on the dog(s) you are going to submit.
  - Print out the submission form and send it with the swabs to Penn.
  - When the results are ready, you’ll get an email with a link to the site.
  - Log in, pay for the test and then your results will be immediately available.

For detailed instructions on using the PennGen site, see “Using the PennGen Site” at http://www.scwtca.org/health/dnatest.htm
OK, now you can swab...but make sure you have what you need

2 swabs

2 mugs or glasses to hold the swabs while they dry

Ziploc™ type bag

Pen to label the swab sleeves...or 2 pre-printed labels.

Submission form... printed from PennGen

Tape to reseal the swab sleeves

IF YOU HAVE THEM: copies of your dog’s registration form and a 4-generation pedigree.
Wait! Before you start...

- Make sure you’ll be able to mail the swab the next morning. Don’t let it sit around.
- Has your Wheaten eaten in the last 3 hours...including treats? If so, wait.
- Does he have the unfortunate habit of eating poop? Make sure it’s been 3 hours since he’s had access to it.
- If you have multiple dogs
  - Swab your dog 1st thing in the morning before he comes into contact with his housemates. That way, they won’t share a water bowl or toy or lick each other.
  - When testing, separate them so they don’t disturb you or possibly contaminate the swab.

Wash your hands. If you are submitting multiple dogs, do them separately and wash your hands between dogs.

Have someone hold your dog or, if you have a table and grooming arm, put your dog on the table.

OK...now you’re ready...!
Swab the first cheek

**IMPORTANT CAUTION:**

*Never touch the swab end or allow it to touch anything other than the inside of your dog’s cheek. Always hold it by the handle.*

1. At the handle end, peel open the sleeve the swab is in...you only need to open enough to get it out. Save the sleeve

2. Run the swab over the inside of one cheek for about 15-20 seconds.

3. Carefully, put the swab in the mug/glass to dry...swab end up! Remember, don’t let the swab touch the cup.

If you’d like to see a YouTube video of a dog being “swabbed”, click here: [http://tinyurl.com/7gkhmbp](http://tinyurl.com/7gkhmbp)
Swab the other cheek

1. Use the second swab to swab the other cheek.
2. Put this swab in the other mug/glass...don’t let the two touch each other
3. And NOW you can give your good dog a treat!

You must submit two swabs. This serves as a control...the DNA on both cheeks is identical so the results should match. If they do not, there is the possibility one was contaminated and the test will be invalid.
While you’re waiting...

While the swabs are drying, you can do a few things...

1. Label each sleeve with your dog’s name and your first and last names.

2. Gather your documents.
   • Make sure you’ve printed out the submission form.
   • If you have them, enclose copies of your dog’s registration certificate and 4-generation pedigree.

3. Address an envelope to PennGen at the address on the submission form. You may find that a larger envelope (6” x 9” or larger) is easier to handle.

DON’T FORGET:
Never touch the swab end or allow it to touch anything other than the inside of your dog’s cheek. Always hold it by the handle.
When the swabs are dry

So...how long does it take to dry? We recommend waiting a minimum of 30 minutes. **DO NOT BLOW** on the swab...you’ll add YOUR DNA to it.

1. Return each swab to its individual sleeve.
2. Tape each sleeve closed.
3. Put both sleeves in a Ziploc™ bag, marked with your and your dog’s name.

**DON’T FORGET:**

*Never touch the swab end or allow it to touch anything other than the inside of your dog’s cheek. Always hold it by the handle.*
Gather up everything to mail

If you have them, your dog’s registration form and 4-generation pedigree.

Address your envelope to PennGen. You may find that a larger envelope (6” x 9” or larger) is easier to handle.

Completed submission form...do not forget to sign it..

2 swabs, in labeled sleeves with the opened ends taped, and enclosed in a Ziploc™ bag.
Getting Results

- You can log into the PennGen website to confirm Penn received the swabs.
  - If you see “Received”, expect 1-4 weeks for results.
  - If you see “Waiting to be Received”, you can follow up with the delivery service to find out what happened.
- When the test is complete, you’ll receive an email with a link.
- Log in and provide your credit card information.
- Once the card is processed, your results will be available to view.
1. Do I need to send a blood sample or cheek swab (also referred to as cheek brushes or cytology brushes)?

We can use blood or cheek swabs. Semen can also be used. (See question 10, below.)

- Swabs can be sent by regular mail without ice or any need to call first.
- Blood samples should be sent in purple/lavender top tubes (EDTA) with an ice pack, by overnight mail or 2nd day delivery, with a call-in to arrange a submission date.
- Frozen tissue may be sent but requires ice packs, a Styrofoam box, overnight mail, and a call-in to arrange a specific submission date so that the sample will not thaw. We prefer swabs, blood, or semen rather than tissues.
3. Who takes the sample...me or my vet?

   Blood would be drawn by your vet; swabs can be done by anybody.

4. Who runs the test ...my vet’s office, Penn, a commercial lab?

   The test will be run at Penn. (*NOTE from SCWTCA:* The submission form provides the address to send the swabs.)

5. At what age can the test be done?

   Any age, but if a puppy has not been weaned, it needs to be separated from the dam overnight so that there is no chance of contaminating the brush with the dam’s milk. The dam’s milk contains her cells and her DNA. For now we are not accepting puppy tails/dewclaws.

   (*NOTE from SCWTCA:* See our “Directions for Testing Litters” guide at  
   http://www.scwtca.org/health/dnatest.htm for more info on puppies.)
FAQs – continued

6. What is the cost?
   - It is $125 per dog.

7. Is there a “litter discount”?
   Yes, $100 per dog if more than 3 pups from a litter are tested, with a maximum of $500 per litter, regardless of the number of pups. *(NOTE from SCWTCA: See our “Directions for Testing Litters” guide at [http://www.scwtca.org/health/dnatest.htm](http://www.scwtca.org/health/dnatest.htm) for more info on puppies..)*

8. How long does it take to receive results?
   The results will be reported in 2-4 weeks, possibly longer if we are inundated with samples. This may occur when the test is first available.

9. Can owners outside the US test their dogs?
   Yes. It’s probably easiest to send cheek brushes from other countries. SCWT clubs in other countries may be organizing genetic test kit distribution.

10. I have frozen semen on my deceased dog. Can it be tested?
    Yes, but it may take longer to get the results. *(NOTE from SCWTCA: Visit the SCWTCA Website for a **Guide to Testing Semen** [http://www.scwtca.org/health/dnatest.htm#request](http://www.scwtca.org/health/dnatest.htm#request) Please contact Dr. Henthorn for additional information.)*
FAQs – continued

1. I provided samples as part of other research efforts, such as the Geriatric Program or CPP at Missouri. Can I have those samples tested?
   If the sample is sent to us and is in good condition, we can test it.

12. How is the test done?
   We are using standard methods for genotyping as described in the paper we are submitting.

13. Will the genetic test results specify that the dog has one or both mutated genes?
   Yes. (We think it is more accurate to refer to them as predisposing alleles....)

14. Will permission to publicly disclose outcomes be required to test my dog? If not, will de-identified results be aggregated to further research?
   There is no requirement to disclose an individual dog’s result. We plan to aggregate non-identified information and disseminate our results concerning prevalence and distribution of carrier status in the Wheaten community in order to help with breeding decisions.
For more information

Check the SCWTCA website for:

- Audio of Dr. Meryl Littman’s presentation on May 5, 2012
- “How To” Guides
- FAQs for owners and breeders
- Research abstract presented at Tufts in 2011
- Contact information for more assistance
- ...and watch for more resources

http://www.scwtca.org/health/dnatest.htm