

# OPP TESTING 101

Since the OPP Concerned Sheep Breeders Society is often asked where samples should be sent for testing — and which test should be used — we have compiled the following general information to assist you and your veterinarian in deciding what's best for your flock. It is important to remember that no diagnostic test is 100% accurate in all cases. Over time, however, you should see consistency in test reports. If you experience wide, unexplainable swings in results from test to test, you may want to consider another lab.

## **AGID (agar gel immunodiffusion):**

The AGID, which detects viral infection by identifying antibody response, has proven effective in eradicating OPP for more than 20 years and is the prescribed test for international trade. It is simple, reliable, inexpensive, and “standard” in that most labs use a USDA-licensed test kit — including OPP antigen — from the same manufacturer.

Since it can take 12 weeks or longer for detectable antibodies to develop following infection with the OPP virus, two or three subsequent negative tests done at 6-12 month intervals will increase confidence in the negative status of a given animal. The AGID does not give false positives. And seroconversion is permanent, meaning that an AGID test-positive animal will remain positive on future tests.<sup>1,2</sup>

For the highest degree of accuracy, the AGID must be done by a properly trained, experienced technician with good eyesight. We recommend that samples be sent only to a lab that is accredited by the American Association of Veterinary Laboratory Diagnosticians ([www.AAVLD.org](http://www.AAVLD.org)). Our members report excellent results from the University of Minnesota, Cornell University in New York, Colorado State University-Fort Collins, and Washington State University. The cost for AGID testing typically runs from \$4.75/sample and up, depending on the lab. Submit 1 ml of serum for the AGID.

## **ELISA (enzyme-linked immunosorbent assay):**

Like the AGID, the ELISA detects viral infection by identifying antibody response. The primary advantage of this test is that it is read by a machine rather than by a human, thus eliminating the subjectivity factor. One drawback is that ELISAs can be overly sensitive, sometimes giving results that are falsely positive.

The first ELISA for OPP, using antigen produced by tissue culture, was developed at Cornell University in the mid-1980s but it was discontinued after the initial study due to its high cost. Subsequently, various more sophisticated ELISAs have been used in research facilities. A few have become commercially available, the most recent being a “competitive” ELISA (cELISA) which was developed for CAE/goats but has also been validated for use on sheep<sup>3</sup> and is currently undergoing further validation studies. Again, we recommend samples be sent only to an AAVLD laboratory.

For accurate ELISAs, antigen purity and adequate lab controls are extremely important. Sample quality may also be a factor. When collecting blood for ELISA tests, every effort should be made to avoid hemolysis (handle tubes with care, never freeze whole blood, and remove clot prior to shipment of serum to the lab). Submit 1 ml of serum for this test.

## **PCR (polymerase chain reaction):**

The PCR has long been used for OPP testing in research facilities and first became available to producers in 1999. While no silver bullet — like other tests, the PCR is not infallible — this test detects the actual presence of the virus rather than relying on antibody response to infection. Due to its relatively high cost, the PCR is usually reserved for especially valuable animals, or those for whom other tests have been inconclusive.

While no formal validation studies have been done on the PCR for OPP testing, Colorado State University has published recommendations for its use as well as results of a test run comparing AGID and PCR.<sup>4,5</sup> They suggest using the AGID for initial whole-flock screening, then verifying with PCR those animals already tested negative by AGID.

To our knowledge, the only lab offering this test for OPP is Colorado State University-Fort Collins, at a charge of \$25.00/sample. Draw 5-10 ml of blood into lavender top EDTA tubes. Immediately after collecting each sample, mix thoroughly by gently rocking the tube back and forth several times. Ship whole blood with ice pack, but do not freeze.

## References

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3. Herrmann LM, Cheevers WP, Marshall KL, McGuire TC, Hutton MM, Lewis GS, Knowles DP: Detection of serum antibodies to ovine progressive pneumonia virus in sheep by using a caprine arthritis-encephalitis virus competitive-inhibition enzyme-linked immunosorbent assay. *Clin Diag Lab Immunol*, Sept. 2003; 862-865.
4. Collins J: Eradication of OPP or CAE: Testing with AGID vs PCR. In *Lab Lines, Newsletter of the Colorado State University Veterinary Diagnostic Laboratories, Volume 4, Number 1, Fall 1999*.
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## OPP Concerned Sheep Breeders Society

### VOLUNTARY GUIDELINES FOR OPP TESTING

Note that adoption of these guidelines is not meant to imply certification of OPP-free status. The OPP Society cannot guarantee any flock to be free of the virus, nor can OPPCSBS confirm the validity of laboratory submissions or the accuracy of test results. Recommended testing guidelines are provided solely for the assistance of those who wish to control the spread of OPP. In addition to requesting test reports, buyers should also inquire about flock management practices.

- **Beginning at the age of 6 months, all animals believed to be OPP-negative are blood-tested at regular intervals of no more than 12 months (6 month intervals preferred) — and breeder is willing to provide copies of original laboratory reports, showing complete flock test results, for prospective buyers.**
- **Or — if a flock is believed to have achieved OPP-free status and is no longer being tested regularly — breeder is willing to provide lab reports to verify (a minimum of 2, and preferably 3, consecutive annual tests during which all animals in the flock were determined to be negative). It is further recommended that breeders continue to monitor OPP status by testing at least 10-20% of the flock each year (preferably ewes 2 years of age or older that have been in the flock for at least 12 months).**
- **In addition to following one of the above testing plans, breeders who advertise their flocks as “OPP Tested According to OPPCSBS Guidelines” agree to allow prospective buyers to have bled, at the buyer’s expense, any animal being offered for sale as well as the dam and/or sire of that animal (assuming that the dam and/or sire are still in seller’s possession).**

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### RECOMMENDATIONS FOR PARTIAL FLOCK TESTING

While whole-flock tests are the norm for small numbers of animals and for seedstock producers wishing to eradicate, partial screening can be used to estimate level of infection in larger flocks. The following, from the Scottish Agricultural College and used in the U.K. Maedi Visna Accreditation Scheme, is based on a 95% confidence of detecting a seroprevalence of 2%.

# of Animals	Sample Size	# of Animals	Sample Size	# of Animals	Sample Size
1-50	All	120	85	350	120
60	55	140	90	400	125
65	60	160	95	500	130
70	65	180	100	700	135
80	70	200	105	900	140
90	75	250	110	1000	145
100	80	300	115	1000+	150

### COLLECTING BLOOD & SUBMITTING SAMPLES FOR OPP TESTING

Regardless of which lab you and your veterinarian select, high quality samples and carefully completed paperwork can help to ensure fast turnaround.

#### TUBES FOR AGID OR ELISA:

Preferred are glass tubes with red rubber stoppers/caps, 16 x 100 mm size (10 ml, finger diameter, 4” long). For the serum, drawn off after blood has been allowed to clot in the red-top tubes, plastic tubes with snap caps are O.K., but push-in caps are difficult to remove. Avoid plastic tubes with push-in caps, as well as very slim (pencil diameter) or short (5 ml “stubby”) glass tubes.

#### BLOOD DRAW:

A 3 ml blood draw is adequate for AGID or ELISA. Use a new, sterile needle/syringe for each animal. If collecting into syringes, blood must then be transferred into red-top glass tubes to clot. While transferring, be sure to direct blood gently down the inside of the tube to avoid “splashing” (splashing may make your serum pink instead of straw colored — O.K., but clear serum makes for easier reading of the test). Submit 1 ml of serum to the lab.

#### ‘SERUM ONLY’ FOR AGID OR ELISA:

Send only serum drawn off of blood clots for AGID or ELISA. Your vet can spin these down, or blood can be allowed to clot at room temperature in the red-top tubes and then serum transferred to a fresh tube. Serum may be refrigerated or frozen, but *NEVER* freeze whole blood. *If serum samples have been frozen, be sure to make note of this when submitting to the lab.*

#### ‘WHOLE BLOOD’ FOR PCR:

If you will be shipping samples for PCR testing, you must use glass “EDTA” tubes with lavender rubber stoppers/caps. The lab will need 5-10 ml of whole blood (not serum). Immediately after drawing each sample, gently rock the tube back and forth several times to thoroughly mix the blood. Ship with frozen gel pack.

#### LABELING:

Number tubes 1 through XXX. Number should be dark, easy to read, and placed at the very top of the label (nearest the rubber stopper/cap) while the tube is held in an upright position. Then, turning the tube on its side (horizontal), write the date drawn, the individual animal ID, and your last name on the label.

#### PAPERWORK:

Use a submission form (available on the Internet) from the lab to which you will ship samples. All information on the form must match the labels on the tubes. Submit under your veterinarian’s name, noting whether the blood was drawn by you or by the vet.

#### PACKING/SHIPPING:

Pack carefully in a strong carton to avoid breakage, enclosing absorbent material as well as a leak proof inner wrap. Tubes may be shipped in a horizontal position. For highest quality samples, ship with a frozen gel pack and mail early in the week to avoid weekend layover. U.S. Priority Mail is fast and economical.