The History of Scientific Testing at Alpaca Registry, Inc.

BY DARBY VANNIER
ARI EXECUTIVE DIRECTOR

Throughout its history, Alpaca Registry, Inc. (ARI) has utilized various forms of scientific testing through multiple testing laboratories. This testing has included blood typing, DNA testing, and Bovine Viral Diarrhea Virus (BVDV) testing.

The founders of the industry had tremendous foresight to utilize scientific testing almost from the very beginning. Initially, ARI used blood typing as a primitive means of parentage validation testing. At that time, recognizing that science evolves, no specifics about the type of testing were written into the ARI bylaws; they only refer to "scientific testing." This ensured that ARI would be able to continue to adapt to new technologies and make changes that help ensure the most up-to-date and robust testing possible.

In 1998, the Registry shifted from blood typing to the more advanced DNA testing. This testing was conducted by the University of California, Davis (UC Davis) utilizing a 10-marker DNA panel. At that time UC Davis was responsible for both conducting the testing as well as making the determination of whether parentage of the dam and sire was validated for a particular cria. The UC Davis scientists set the standard for validation, determining whether adequate markers were present to confirm a cria's sire and dam. Although UC Davis tested on a 10-marker DNA panel, there were occasions when parentage validation was completed using fewer than 10 markers.

The Registry has taken scientific testing very seriously, and has consistently tried to plan for the future by periodically increasing the number of markers tested for and utilized to validate parentage. Each time we have done so, we have evaluated how such a change would affect the membership, and the Board has tried to make changes that do not require retesting of all previously tested alpacas. Here is a timeline for changes in validation over the years:

- Until 1998 – Blood Typing
- 1998-2005 – 10-marker panel
- 2005-2010 – 14-marker primary panel, 10-marker additional secondary panel if needed
- 2010-present – 18-marker primary panel, six-marker secondary panel if needed

Throughout this history, each Board has understood that in order to continue to take advantage of new technology and provide the most accurate parentage validation possible, some retesting would be inevitable. The expansion of markers over the years has become important due to the fact that ARI is a closed Registry and many alpacas are closely related. We could have continued to test for only 10 markers, but eventually it would have led to an increase in false qualifications because there would not be enough data to distinguish between closely related alpacas. The challenge has been to time the decision to expand the number of markers used in the panels well before we begin to have any issues. This strategy ensures that more alpacas will already have data from the larger panels prior to those issues arising, thereby reducing the volume of retesting required, lowering the potential for added costs to members.

In 2003, the testing contract with UC Davis was expiring and a committee was set up by the ARI board to research DNA testing vendors for Registry use moving forward. Using the expertise of the committee led by Dr. Shauna Brummet, the decision was made to change vendors, and Geneseek was selected. Geneseek served as ARI's validation company until 2010. As many breeders might remember, in 2004 the ARI Board chose to move the management of ARI to a new office vendor, Association Management Group. This move did not go well and thousands of registrations were delayed. Geneseek worked very closely with the ARI Board when the ARI headquarters

Photo © 2013 Jennifer Clark, background images: stock.xchng.com

opened in Lincoln, Nebraska, and worked through that testing backlog, which was finally completed in 2005. It was a very difficult and trying time for ARI and our members, but while registrations were delayed due to office issues, validation testing continued to follow the strict set of testing rules that had always been in place.

Throughout its contract with ARI, Geneseek worked with ARI to continue to refine the DNA panel, and assisted in the research and testing of new markers, allowing the primary DNA panel to expand from 10 markers to 14 markers. Additionally, a new secondary testing panel of a further 10 markers was added for use in cases where multiple, closely related, sires qualified.

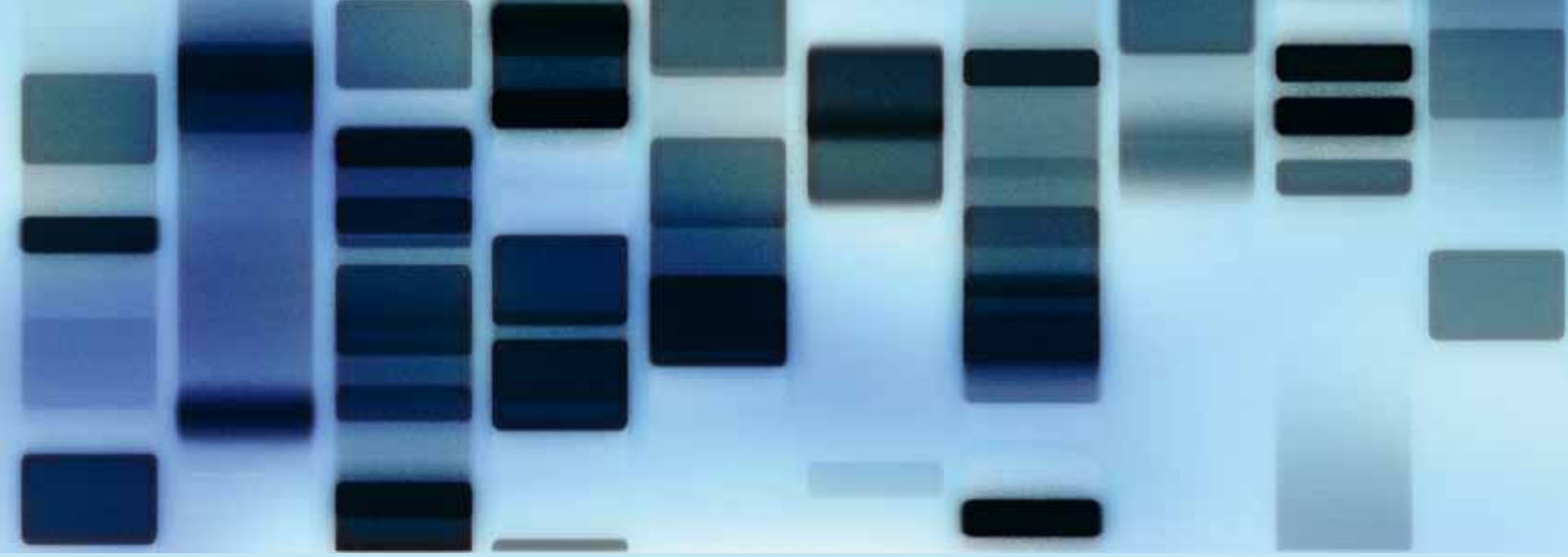
As Bovine Viral Diarrhea Virus (BVDV) became a concern in the alpaca industry, Geneseek also worked with ARI to develop a PCR-based BVDV test which could be completed at the time of registration directly from the same DNA blood card. The ability to have both the DNA test and BVDV test completed utilizing the same DNA blood card has been a huge convenience for members.

In 2010, the second Geneseek contract was expiring and a Request For Proposals was released to other DNA testing firms. The ARI Board ultimately selected DDC Veterinary as their new testing vendor, and DDC remains our current DNA testing vendor, chosen because of their access to a vast array of parentage validation scientific talent, as well as strong testing procedures, and great turnaround time.

When considering the DNA testing itself, it is important to know that since 2003, our DNA testing vendors have conducted blind testing. That is, they have no knowledge of who owns any of the alpacas being tested or validated, and work directly with ARI, not the alpaca owner

While the agreement with UC Davis does not specify the testing requirements of the validations during that time, we do know that many alpacas tested on the original 10 marker panel had fewer than 10 markers reported. The testing requirements with subsequent labs have been:

- **2004-2005**—Ten markers were tested and it was required that a minimum of eight markers were able to be reported or the lab would request a retest.



- **2005-2010**—Fourteen markers tested and it was required that a minimum of 12 markers were able to be reported or the lab would request a retest.
- **2010-present**—Eighteen markers are tested and it is and it was required that a minimum of 17 markers are able to be reported or the lab will request a retest.

It is important to understand that in cases where fewer markers were reported than were tested for (e.g., eight of 10 or 12 of 14), these missing markers are not "lab errors." In any rapid throughput DNA testing method there is the occasion that a marker does not produce enough "signal" to be measured by the instrumentation, and is therefore reported as "no data." This is especially true in animal DNA testing, which doesn't use as many repeats as human testing does. The missing markers simply mean that these don't amplify in a particular test, and therefore are not able to be reported. This absence of markers can also be affected by the sample itself, which, in the early years, was more problematic until members learned how to use the DNA blood cards. These cards are highly effective and have been proven to be just as accurate as whole blood and follicular (root ball) testing. The blood cards utilized for ARI testing provide a larger amount of DNA than follicles, are much more convenient than whole blood, and are far easier to store for archival purposes. In fact, similar cards are utilized by crime labs around the world and by the United State military for human testing.

We currently test for up to a total of 24 DNA markers (primary and secondary) in order to complete parentage validation, and ARI has the largest camelid DNA database in the world by far, with approximately 230,000 animals with DNA profiles. As a result, our DNA testing laboratories have been able to utilize our vast amount of data to make adjustments to improve both the accuracy and the robustness of our panel, which provides a much better and more consistent result for our members. In 2005, based on this analysis, it was determined that one marker (YWLL44) was not a very informative marker. This means that the marker had only a small number of alleles occurring in very high frequency in the population, therefore rendering it of little value to differentiate between animals.

With this information, it was decided to drop that marker and replace it with one that would be more informative. This change was not made casually, for the Board of Directors at that time knew that removing YWLL44 meant a large set of alpacas would now have that marker removed from consideration in parentage validation. However, because that marker was less informative, it was determined that it would be better to switch it out immediately, rather than having the issue compound itself over subsequent years.

While all alpacas at that time were being tested on a 14-marker panel, this change meant that alpacas which had previously only been tested on the original 10-marker DNA panel could have as few as seven markers for comparison in parentage validation. As mentioned before, when the 10-marker panel was utilized, the rule up to that point for parentage validation was that at least eight markers must be held in common (dam, cria, sire) in order to confirm parentage. With eight markers in common, the Registry allowed one marker to mismatch and still validated the parentage.

The lab was consulted, analysis was done, and it was determined that seven markers could be allowed, and still provide an accuracy exceeding 99 percent reliability. However, to guarantee that reliability, no mismatches would be accepted when there were only seven markers in common. Keep in mind that this

only occurs if the dam, cria, and sire only have seven markers in common. In all other cases, the regular rules apply. An alternative approach would have required the retesting of thousands of alpacas at great expense to breeders. While some sires and dams still have to be retested from time to time because they don't have a sufficient number of markers in common with the cria, that number has been greatly reduced by using this approach.

The parentage validation rules are black and white. They are very specific, and have been completed using computer software for more than 10 years. Prior to 2007, the parentage validation process was also completed by the DNA testing vendor. However, in order to streamline the process of completing parentage comparisons after the fact, the validation process was built directly into the ARI software system. As such, the DNA testing vendor currently completes the testing and returns the results, and ARI's software completes the parentage validation process. This ensures reduction in human error, and that the testing process is entirely blind, unbiased, and free of any potential for corruption.

All testing and parentage validation rules that we use are consistently revisited. This particular policy (accepting a seven-marker match) was revisited in 2009. At that time, after having reviewed the number of alpacas that would be affected, the Board determined that since it still provides a sufficient level of accuracy, we would continue the practice.

Lastly, over the years, every ARI Board has to reexamine the parameters surrounding ARI's tradition of rigorous scientific testing. There are three primary considerations that play a role for any livestock registry when looking at testing. They are cost, quality, and turnaround time. Each one affects the other and livestock registries around the world do their best to choose a balanced approach that allows for good quality, good turnaround time, and a reasonable cost. If we try to adjust one of these three, the others are also impacted. For example, increasing accuracy even closer to 100 percent could be achieved, but that would significantly increase the cost of a test, and turnaround time may increase to many weeks, perhaps even more than a month. Neither of those would be acceptable to most ARI members. As a result, we have selected a level of cost, quality, and turnaround time that is appropriate and reasonable to our members.

ARI currently tests for up to a total of 24 DNA markers, and has the largest camelid DNA database in the world.

The ARI Board and staff are committed to operating the organization in the most responsible way possible. It is not always easy making decisions that will affect things for years to come. Every Board member takes their responsibility to the organization and the industry very seriously. They, too, are alpaca breeders and are affected by the decisions they make. Over the years, the Registry has taken advantage of developments in science to ensure accurate validation of parentage. No doubt new technologies will become available for the ARI's consideration to perpetuate the robust pedigree database achieved thus far. There will always be a balancing act to weigh the benefits of those new technologies against the accuracies they provide and the cost to breeders, but the Registry will always be focused on ensuring its process is the most robust in the world.