

# HOW DO YOU KNOW YOUR TEST RESULTS ARE ANY GOOD?

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## ABSTRACT

Users of forage testing laboratories assume that the test results are of high quality. Unfortunately, high quality cannot be assumed, as these laboratories are not regulated, and may not be certified. Methods vary between laboratories, resulting in a large variation in results between different laboratories. The evaluation of variation between testing laboratories is complicated by the lack of homogeneity of forage samples. Basic quality concepts for forage testing are introduced, and methods for evaluation of laboratory performance are presented.

**Key Words:** laboratory, quality, forage testing, accuracy, precision

## INTRODUCTION

Testing of forage provides the basis for evaluating quality and thus price. Many users of testing laboratories are unaware of the quality of the tests performed, assuming that all laboratories have a high standard of performance. Unfortunately, this is not the case. Agricultural laboratories are generally not regulated. Certification programs exist, but are not typically required. Laboratory users should have a basic understanding of laboratory quality control and quality assurance, and how these concepts are applied to the testing of forage samples. This information can be utilized by the users to verify that laboratories are performing adequately

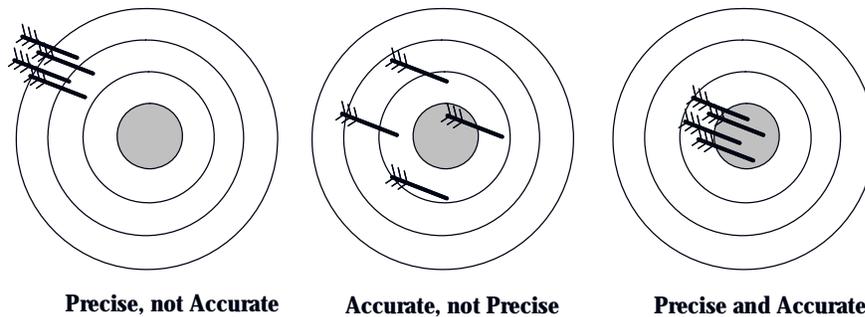
## EVALUATING THE QUALITY OF TESTING

*The need for good test results.* Laboratory testing is necessary due to the unreliability of visual evaluation of forage. Testing provides an unbiased measure for the determination of price between buyer and seller. Quality factors such as protein and fiber are only well evaluated through testing (Putnam, 2004). While visual evaluation is necessary to evaluate the presence of noxious weeds, texture and odor, testing is far superior in evaluating stage of maturity, leafiness, fiber and protein content. USDA hay quality guidelines require laboratory analyses to discriminate between one or two percent fiber or protein, yet laboratories cannot always attain this level of accuracy. The difference between laboratories testing for fiber can be far greater than one to two percent.

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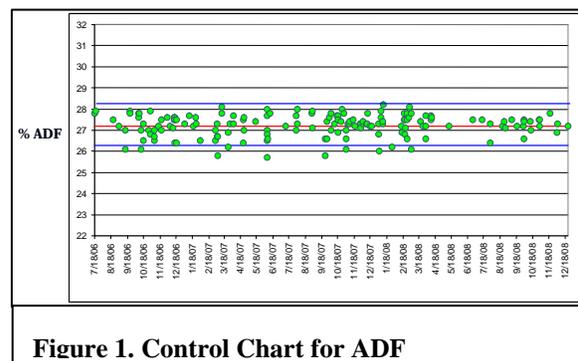
**Definition of a “Good” Test Result.** Precision is the agreement between replicate measurements (2 or more) that are made by the same procedure. Accuracy is the closeness of the measured value to the “true value.” A good test result should be precise and accurate. Tests should be reproducible, in such a way that a re-test of the sample gives the same or similar result. The test should also give the “correct” result, plus or minus expected variation.. Bias is a consistent trend towards a particular result. Testing should not be biased towards a particular high or low result.



All tests have an inherent accuracy and precision, which should be measured or evaluated by the laboratory at the time of testing as part of the quality control process. Laboratories must follow a quality assurance plan to control accuracy and precision. For example, standard methods must be used. Laboratories must verify that the methods are performed correctly. Results must be comparable to those from other laboratories.

**Laboratory testing.** Agricultural laboratories are not regulated in the same way as environmental or medical laboratories and certification or accreditation is often not available or required. Procedures are not always consistent between laboratories. The choice of method can vary greatly from laboratory to laboratory, yet these results are often reported identically, making it difficult to determine exactly how the tests were conducted. For example, fiber and protein can be tested by wet chemistry methods (which themselves can differ significantly) or by Near Infrared Reflectance Spectrometry (NIRS), potentially giving dissimilar results.

Proper use of testing methods requires technique and experience. Testing methods are complicated, with many opportunities for error. For example, the following is a brief description of the wet chemistry acid detergent fiber (ADF) method: Dry and grind the sample. Weigh 1.000 gram onto glass filter/crucible. Boil and wash sample with hot sulfuric acid containing detergent (60 minutes) followed by hot water and acetone washes. Dry 3 hours in an oven. Weigh sample. Potential errors include: Weighing errors, errors in preparing reagents, error in the time of boiling or washing, grinding the sample too finely or too coarsely, and operator error such as sample switches and recording of wrong numbers. Likewise, the NIRS ADF method,



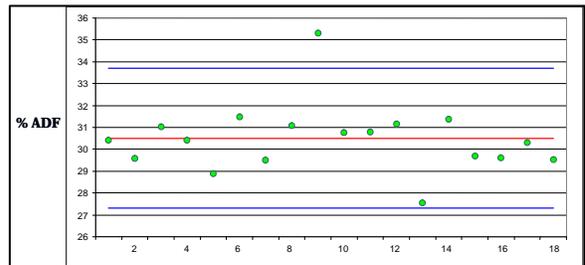
**Figure 1. Control Chart for ADF**

though simpler, also has many opportunities for error. The method appears quite simple: Dry and grind the sample to fine powder. Place the sample in a disk. Insert the disk into the NIRS instrument. Compare the NIRS spectrum to a library to obtain the results. However, many potential errors are possible, such as the sample not being sufficiently dry, the sample not well ground, the disk loaded incorrectly, the instrument not warmed up for 1 hour, the instrument not at 72-82 degrees (F) with controlled humidity, the sample type not matching the library, and operator errors. While NIRS is faster and cheaper to run, the technique depends on the sample matching a library built from wet chemistry testing of sample of similar type. NIRS performs best on pure samples. Labs using NIRS must check their results periodically using wet chemistry, as wet chemistry methods are the reference methods for NIRS.

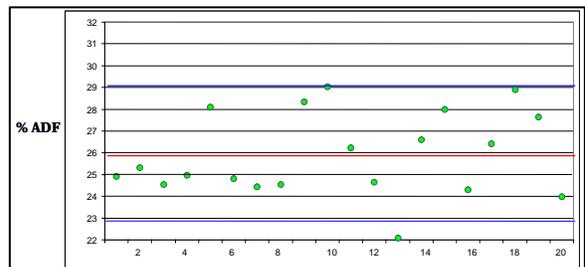
Due to the inconsistent use of methods between laboratories, testing results can differ significantly between laboratories. Figure 1 shows a control chart of one alfalfa sample tested repeatedly over an 18 month period by one laboratory.

Most results were within  $\pm 1\%$  of the mean. However, when 18 certified laboratories tested one uniform alfalfa sample, ADF results varied  $\pm 4\%$  (Figure 2). These large discrepancies can be problematic when proper evaluation of forage quality requires results within one to two percent (Putnam, 2004)

A large component of the variability in the result comes from the sampling. Figure 3 shows the variability in testing 20 separate cores from a hay stack. Results varied by more than  $\pm 3\%$ , emphasizing the need for proper sampling. (Putnam, 2004). Guidance for sampling can be found at the National Forage Testing Association (NFTA) website: [www.foragetesting.org](http://www.foragetesting.org).



**Figure 2. Variation in ADF results between 18 certified laboratories**



**Figure 2. Variation in ADF results between 20 samples from one stack (20 individual bales)**

**Quality Assurance / Quality Control (QA/QC).** All laboratories make mistakes – procedures and practices must be in place to catch errors. Quality control (QC) is the daily measurement of the quality of the testing process. QC includes the use of reference materials (samples with a known value), testing samples in duplicate, running blanks sample and instrument calibrations. Quality assurance (QA) is the system that manages the quality of all aspects of the testing process. QA includes a description of the QC measures used, the use of written procedures, a documented training program, an equipment maintenance program, the use of proficiency programs, charting of QC results and a thorough set of procedures to describe the response when quality control results fail. Forage testing laboratories can be certified by the National Forage Testing Association (<http://www.foragetesting.org/>). NFTA sends the same sample to each participating laboratory, and grades each result. NIRS and wet chemistry are graded and certified

separately. Passing laboratories are given a certificate. Other proficiency programs exist such as the American Association of Feed Control Officials (AAFCO).

***Information to obtain from the testing laboratory.*** Find out if the laboratory is certified by NFTA, and whether the laboratory will share their “grades” with you. It is highly recommend that a certified laboratory be used. Identify which other proficiency programs the laboratory participates and ask to see their performance data in those programs. Laboratories with a strong quality assurance program will have a quality manual, which you can ask to see. Ascertain if the laboratory can or will report quality control results, or if those can be viewed on request. It is important to confirm that the methods used are official methods and that they are well documented. Laboratories should be able to provide a copy of their methods manual for viewing, or as a minimum the reference for the methods used.

***Methods for verifying the quality of testing.*** It is highly recommended to use a NFTA certified laboratory with good certification results. Compare results from different laboratories by sending the same samples to several laboratories. Sample variability can be large, so it is preferable to submit a ground sample if possible, which can often be obtained from a laboratory by requesting the unused sample be returned. To check the precision of one laboratory, split one sample and submit it as two samples. Check the accuracy of the laboratory by submitting a sample with known values, which can be obtained from NAFTA or AAFCO. One means of measuring that the laboratory provides reproducible results is to submit the same sample with each sample submission. Verify that sample results are consistent with your expectations and are reasonable.

## **CONCLUSION**

Agricultural laboratories are not regulated, so the quality of testing results cannot be assumed to be high. Use of a NFTA certified laboratory helps assure higher quality testing results. An informed choice of laboratory should be based on certification and proficiency data, quality assurance program and methods used. Adequate laboratory performance should be continuously verified by submission of samples to check the accuracy and precision of results.

## **LITERATURE CITED**

Putnam, D. Forage Quality Testing And Markets; Where Are We Going?, in National Alfalfa Symposium Proceedings, 13-15 December, 2004. San Diego, CA.