Do You Really Know the Alcohol Level in Your Wine?

Two laboratory instruments can help

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IN THE PAST DECADE, there have been many innovations in laboratory equipment that offer a variety of ways for small- to medium-sized wineries to modernize a winery lab and increase the value of their analytical data, all while remaining within the winery's budget. But there is one test that all wineries must perform, regardless of lab sophistication, and that is measuring the level of alcohol. While many wineries use the traditional ebulliometer for the alcohol measurement by volume (ABV), there are new methods for wineries to consider for this analysis. Here we will review an update to an enzymatic method of ABV determination by CDR WineLab and compare it to another enzymatic method of analysis from Megazyme's MegaQuant.

The ebulliometer is the standard method used by the Alcohol and Tobacco Tax and Trade Bureau (TTB) for alcohol measurement to determine tax class and the declared alcohol measurement by volume (ABV) on the label. A winery can use any method as long as it can show the ABV is within accuracy limits, i.e., +/- 1.5% for wines under 14% ABV and 1% for wines over 14% but under 24% ABV. The traditional ebulliometer has a coefficient of variation (CV) of 1%, so any analysis for label approval should be evaluated for use based on this standard.

However, there are significant problems with the ebulliometer. First, the analysis can be slow and cumbersome if the alcohol flame version is used, which is the most common method. Electronically heated versions are available but cost more than $2,000. Second, these instruments cannot accurately measure sweet wines without going through a secondary process to prepare the sample by distillation. This step will increase equipment cost and can increase the number and/or severity of errors significantly due to the distillation process and the skill of the technician running the sample.

Several companies have developed laboratory equipment that can perform the necessary alcohol test, as well as many other tests that should be conducted during the winemaking process. While the equipment is more expensive initially, if a winery needs accurate results from a variety of tests for many wines, the speed and accuracy of testing may more than compensate for the cost.

In an article titled "Putting Modern Lab Equipment to the Test," published in the January 2018 issue of *Wines & Vines*, I reviewed several pieces of lab equipment that *W&V* had obtained from some of the major suppliers, including CDR WineLab and Megazyme's MegaQuant instrument, and compared the different tests, including the alcohol analysis, that they could perform and the results the instruments provided.

In tests conducted in 2017 and reviewed in the article, the accuracy of the CDR WineLab analyses were very comparable to other instruments in common usage in the wine industry, such as Megazyme's MegaQuant for enzymatic tests and the Hanna Instrument suite for titratable acidity, sulfur dioxide analysis and pH. However, the CDR WineLab alcohol test gave a wider range of results than should have been expected. At the time, I shared my results with the company's technical staff. They reported that others had a similar comment about the analysis and the company was actively looking at ways to get more consistent results.

Recently, the CDR WineLab released an updated version of its analysis of alcohol in wines and other beverages. The main purpose of this article is to review this improved alcohol test and to look at the similarities and differences between CDR WineLab's instrument and Megazyme's MegaQuant instrument so readers can understand how these two instruments can meet the winery's needs for accurate laboratory equipment.

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CDR WineLab Instrument

CDR WineLab can perform many more tests than the 30 different analyses that are routine in wine laboratories. It is a relatively simple instrument to operate as it was designed on the basic principle that if you can follow the recipe to bake a cake, you can perform any of the analyses the CDR WineLab offers.

The CDR WineLab instrument comes in two sizes, Touch and Junior. The Touch instrument can perform several more tests than the Junior version; the Junior has only four heated blocks to hold tests in the process of analysis while the Touch instrument has 16. For most smaller wineries, the Junior is quite useful and is significantly less costly than the Touch unit. However, if a winery needs to run the additional tests the Touch instrument analyzes, then that instrument should be purchased.

In addition to the tests mentioned above for titratable acidity, sulfur dioxide analysis and pH, CDR WineLab also can perform a copper analysis, which can be useful information.

The new alcohol analysis is a more accurate representation of the true alcohol in the wine. There were several tweaks to the alcohol test protocol, but the main improvement was the long-term stability of the enzyme for the test by changing the storage temperature of the test kit.

This particular test requires that the lab technician strictly follow all of the steps to assure that the analysis returns a good result. I know that when a lab technician becomes comfortable running these lab analyses, that lab person may not always follow the instrument's instructions as closely as he or she should. This may not be crucial for some analyses, and the results may not deviate far from a more carefully run test, but it is particularly important when running the ethanol test to follow the directions. Not doing so may cause more than a minor misstatement of the results.

The physical properties of the ethanol being analyzed are the main reason for the variation in results. Ethanol is volatile. When even a small amount of a solution is exposed to air, the evaporative loss in the small volume induces a significant variable in the analysis. All the necessary steps should be taken to minimize that possibility during the testing process.

I would recommend that if any alcohol test shows that the wine is within 0.2% alcohol by volume for a change in tax class, that analysis should be repeated at a TTB-certified laboratory in order to assure that the winery is covered as much as possible if the TTB takes a bottle to test for compliance requirements.

A Comparison of the CDR WineLab and MegaQuant Operations

The MegaQuant instrument does not do several of the tests that CDR WineLab can analyze, including titratable acidity and pH. Wineries that consider the purchase of either of these instruments usually have another way to conduct these analyses with equal or better results, as well as faster and cheaper on a per analysis basis.

Similar to the CDR WineLab instrument, the MegaQuant has fixed wavelength filters to provide the analytical means to evaluate the tests. For MegaQuant, its standard is a set of six wavelengths, and then there are up to two slots where a custom set of wavelength filters can be ordered, which does increase the price of the instrument. It is important to note that the linear range of the MegaQuant instrument is long, extending the maximum absorbance range to 3 instead of the normal 2.

The heat block in the MegaQuant holds 12 round borosilicate test tube sample slots, which is usually adequate for most situations. The sample chamber is designed to hold both the test tubes and quartz cuvettes. For the tests run today, quartz cuvettes are not now necessary due to the double wavelength filtering of the test. As with the CDR WineLab, the instrument is controlled by a touchscreen where the tests are selected, and parameters are entered for analysis. Included in the total number of tests available with the MegaQuant instrument are 24 wine industry-related tests.

Test procedures are the biggest differences between the CDR WineLab and the MegaQuant instruments. The CDR tests are simpler to perform in that the test materials are pre-mixed to minimize steps the lab technician must undertake. Megazyme enzyme kits have each component placed in separate vials, and the test is performed by adding the proper sequence of components.

These differences mean that the average shelf life of the CDR kits is six to 12 months from the time they are received, and kits are ordered in packets of 10 analyses per test. Megazyme tests have a useful shelf life of two or more years, with either 50 to 100 tests at full volume. It is important to note that most Megazyme tests can be run at half volume although there may be some increase in error range.

Many of the Megazyme test kits require storage of some components at freezer temperatures to get the long-term shelf life. If a winery uses enough tests and long-term storage is not needed, then test kits may be stored at refrigerator temperatures for six to 12 months before changes in the accuracy of the test may occur.

Megazyme Test Protocol

The generalized Megazyme test protocol is composed of three to five enzyme/buffer mixtures. As in the CDR WineLab, microliter pipettes are used to inject samples and reagents. The reagents are taken from the vials and introduced to a sample tube (a borosilicate test tube). Water is frequently added as the mixture solvent. When entering information on the sample, sometimes there needs to be a dilution of the sample to keep the test within linear range. That dilution factor is also entered at the beginning of the process. The procedure usually has two time periods for reaction incubation after which a reading from the instrument can be obtained. After the last reading of a test or group of tests, a final calculation of the analysis is displayed and printed. The final readout from the instrument of the analyte's concentration is stored in the instrument; and if a computer is connected to the instrument, it will be delivered to a folder on the computer.

An important note must be included here. When I began this trial, I ran out of test tubes and re-ordered more from the same supplier. When I received the test tubes, I began my testing procedure as normal, but the results were totally out of spec from test to test. After a significant review of the instrument, procedures and materials, it was determined that the source of the problem was the test tubes. The instrument uses borosilicate glass test tubes, not quartz test tubes. The second batch of the old tubes had a 3X increase in absorbance in just the empty test tubes. That resulted in an incorrect analysis of the results. Bottom line: high-quality borosilicate glass, such as Pyrex, is required.

CDR WineLab Test Protocol

Both MegaQuant and CDR WineLab instruments follow a similar protocol to perform their tests. The following description of technician protocol is technically the number of steps the analysis takes. To minimize redundancy of explanation, all of the precautions mentioned in the following discussion are relevant for both instruments.

The test protocol for the WineLab instrument follows a simpler set of steps than the MegaQuant instrument. All of the analyses come prepackaged as a set of 10 tests. Most tests are stored at conventional refrigerator temperature. However, there are a few, such as the copper test, that can be stored at room temperature. The new alcohol test has one part of the test kit that has to be stored at freezer temperatures while the dilution kit can be stored at room temperature. Relative to other enzymatic analyses, the CDR WineLab has a shorter window of use, in the order of several months, not years. That is probably the reason the tests come packed in groups of 10.

The best procedure to follow is to turn on the instrument about 15 minutes before it will be needed, as it takes several minutes to get all systems warmed up. It also helps to take the test out of storage temperatures in sufficient time for it to warm to lab temperature before proceeding with the test. Warming by holding the sample cuvette in the hand can hasten this process.

Most CDR WineLab tests have one or two testing reagents in addition to the sample. The analyses are run by using micro-pipettes to add a fully prepared sample to the cuvette. If two reagents are needed, then one is added at this time. After a wait for the reaction to complete, the cuvette is entered into one of four measuring chambers, and the sample is read. The last reagent is then added with a micro-pipette. After an additional wait time, the sample is read again. When multiple samples are needed with the same test, the holding slots keep the samples in order so they won't be mixed up. At the conclusion of all additions, a printout shows the test results, which are then stored in the instrument. If a computer is connected, the data can be stored in an Excel spreadsheet.

The first step in the alcohol analysis is to dilute the sample in a dilution vial that comes as part of the test. It is through this step that one analytical test kit can cover dilutions from the legal upper limit of alcohol concentration: 0.5% for dealcoholized products up to 17% alcohol by volume. This is accomplished by adding 50, 100 or 200 µL of wine sample to the sample cuvette.

As mentioned above, this test is highly sensitive to deviations from protocol. In some respects, this part of the test is the most important step and the one that must be done properly. The best practice is to develop a systematic method of process. The lab technician must try to use the same speed and the same series of movements to keep the timing as consistent among tests as possible to perform the test accurately. The important aspect is to minimize the time the undiluted sample is "exposed" to ambient air conditions. If the movements are standardized, then errors will be reduced to a minimum. This makes a real difference when accuracy is important.

In order for these tests to be performed accurately, the lab technician must use micro-pipettes. When taking the sample from the source, the tip should be placed 3 to 6 mm into the wine. Normal micro-pipette procedure should be followed, i.e., take the sample (and when taking a reagent), press to the pipette's first stop and let it draw the sample. The sample is then delivered either to the dilution vial or to the sample test cuvette by pushing to the second stop. Between taking the sample but before introducing it to the dilution vial, quickly and carefully wipe the tip from the large side of the tip to the narrow side with a small wipe. This is to remove any residual sample or reagent from the outside of the tip but be careful not to have the wipe adsorb any wine or reagent from the tip opening.

When introducing the sample to the dilution vial, it is important to be sure the tip is below the dilution vial liquid surface AND to cycle the pipette delivery two or three times. This is not normal pipette technique. These pipette types are made to be TD (to deliver). My tests have shown and verified the claims of the manufacturer that there is some variation in the analysis results if this step is not done properly. Finally, cap and gently shake the dilution vial.

For the other parts of the test when adding reagents, if you are careful to be sure all reagent has been added, then inserting the tip into the test vial is not necessary. My suggestion is to rest the pipette tip on the outside edge of the test cuvette with the tip against the opposite wall and cycle the pipette one or two times. Also settle on either one or two cycles to reduce variability. However, if the reagent tip is placed into the sample, then a new pipette tip must be used after every delivery.

Once the correct analysis has been selected from the instrument touch-screen menu, the test is ready to prompt you through the analysis. Depending on the expected dilution of the alcohol in the sample, the instrument directs you to add the appropriate amount of diluted sample to the test vial that has been properly warmed by the heated temperature block of the instrument. Remember to clean and cycle the pipette in the same manner as the initial dilution.

There are two test reagents that are added in sequence to the test cuvette. The first provides the blank reading and takes only about one minute. The second reagent is then added, which is the alcohol dehydrogenase enzyme. That enzyme reacts quantitatively with the alcohol present, changing the absorbance of light at a specific wavelength. This reaction takes 10 minutes. The bank of 16 heated blocks in the CDR WineLab Touch means that up to 16 tests can be run sequentially. The Junior version has four blocks. A reading is taken between the two reagents and after the last reagent. As soon as all tests have been entered, the instrument prints the results on tape. The instrument can also deliver data to a computer if one is connected.

Alcohol Test Results

It was reassuring to find that the results I obtained in the alcohol tests agreed well with ETS Laboratories' results shown in TABLE 1. In TABLE 2, I selected an additional three samples to test and selected two of them for triplicate analysis, the results of which are shown in TABLE 3. For this type of analysis, a variance of 0.1 to 0.2% is generally a good sign of repeatability. Both MegaQuant and CDR WineLab have a CV of 2%, which translates into a range of 0.29% alcohol by volume at 14.5%. As you can see in TABLE 4, on protocol observance, the effect of not following the steps rigorously does make a difference. The more important the accuracy of the result, the more important it is to run several tests to be sure that a single result is not an outlier.

TABLE 1: Outside Sample Comparison

ETS - Dry Wine	10.0
CDR - Dry Wine	9.9
MegaQuant - Dry Wine	10.1
ETS - Sweet Wine	15.4
CDR - Sweet Wine	15.6
MegaQuant - Sweet Wine	15.2

Two wine samples, one dry and one sweet, were tested by three methods.

TABLE 2: Comparison of Wine Alcohol Levels and Sweetness

Low Alcohol Level	Dry	Sweet
CDR WineLab	10.6	11.8
MegaQuant	10.7	11.6
High Alcohol Level	Dry	Sweet
CDR WineLab	15.2	16.6
MegaQuant	15.2	16.9

Three wines were tested, and these two were selected for triplicate analysis. (TABLE 3)

TABLE 3: Triplicate Analysis

Wine 1	**A**	**B**	**C**
CDR WineLab	15.1	15.2	15.2
MegaQuant	15	15.1	15.1
Wine 2	**A**	**B**	**C**
CDR WineLab	11.8	11.6	11.8
MegaQuant	11.9	11.7	11.7

Results of the triplicate analysis

TABLE 4: CDR WineLab and MegaQuant Protocol Trial

Process Infraction	CDR WineLab	MegaQuant
Initial % Alcohol = 11.8	Alcohol % Volume	
Tip not immersed in sample cuvette	11.5	11.4
Tip not wiped before immersed	11.6	11.7
Tip not cycled three times	11.4	11.6
Cuvette/test tube not mixed	10.8	11

This is not an exhaustive study on test protocol. The results show only that technique is important for the alcohol test.

TABLE 5: CDR WineLab Degradation Test

Days at 75° F	Alcohol Level
Day 1	11.6
Day 2	11.8
Day 3	11.5
Day 4	11.0
Day 5	10.8
3 in storage, 4 out	11.4

A test kit was placed in a 75° F room for the time period, or alternating 3 in, then 4 out of normal storage.

Finally, TABLE 5 shows the results of what happens when a test kit is left out on the counter-top at laboratory temperatures of 68° to 71° F. The degradation of the alcohol dehydrogenase is affected by temperature in a progressive way. It is important to keep the test kits at the correct temperature for storage. If results don't look right, probably the test has not been stored at the correct temperature.

For most wineries, this testing procedure will be good for label approvals, with the exception of when a sample is near the change in tax class. To repeat, the best advice is to get a certified lab result to go with your result when the wine's alcohol level is close to the change in tax class.

From an expense standpoint, it is possible to run about five tests for the same cost as one obtained from a certified lab. The convenience of also being able to run from one to many tests on this analyte, and the many others that either the WineLab or Megazyme supports, makes it worthwhile to have one of these instruments as part of a winery's laboratory.

Summary

The differences between the two instruments are not so much in how they work but more the style in which they work. If the lab technician is a dedicated person who performs the winery's laboratory analyses regularly and where multiple similar analyses are performed as a group on a frequent basis, the Megazyme system may be better suited for that work flow for the following reasons: first, the cost per analysis will, in general, be lower for the kit, especially when the technician is comfortable with using the half-analysis volumes; and second, the kits last for longer periods of time than the CDR WineLab kits.

If the users are not full-time lab technicians and do no more than four or five analyses at a time, the analysis protocols are similar enough between the different materials evaluated that the user may feel more comfortable with the procedural steps of the CDR WineLab than with the Megazyme unit. This simplicity may cost a bit more per analysis, but the comfort level in running the tests will outweigh the cost difference. WBM