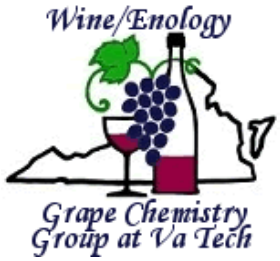


## Enology Notes # 165 June17, 2013

**To: Regional Wine Producers**

**From: Bruce Zoecklein, Head, Professor Emeritus, Wine/Enology-Grape Chemistry Group, Virginia Tech**

**Subjects:**

1. **Future Enology Notes**
2. **Wine Storage and Bottling Quality Control**
3. **2013 Technical Study Tour**
4. **Winery Planning and Design CD 16 available**
5. **Microbial Ecology during Vinification**
6. **In Memory of Dr. Keith Patterson**

### 1. Future Enology Notes

I retired from Virginia Tech in 2010, but agreed to remain part-time for three years while the university attained the resources to fill the two positions created from my own. That is soon to happen. My student Molly Kelly has accepted the Enology Extension position and we are soon to fill the -Enology Research and Teaching faculty slot. Enology Notes will continue. All previous Enology Notes are posted at www.vtwines.info.

### 2. Wine Storage and Bottling Quality Control

The principal quality control difficulties of the wine industry in include the following:

- lack of adequate recordkeeping
- fruit quality
- control of phenol extraction
- oxidative and microbiological degradation

The key to adequate quality control is to monitor how each production activity affects wine palatability and to make adjustments accordingly. Complete and accurate recordkeeping is the cornerstone of a successful quality control program. Only when

proper up-to-date accounts of wine production activities are kept can a full understanding of the parameters affecting wine quality occur.

The development of a HACCP (Hazard Analysis and Critical Control Point) Plan is the key to consistently crafting fine wines. Such a system allows one to use his or her own philosophy to determine which steps in the kaleidoscope of winemaking choices should be monitored.

The following is an outline of some common issues. For the establishment of a detailed quality control or best-practices program, see *Enology Notes* index HACCP at www.vtwines.info.

## Aging and Storage Quality Control

### *Sanitation*

Each winery should have an established sanitation program, and periodically monitor the effectiveness of that program. Such simple procedures as tasting barrel and tank rinse water can be a significant step in ensuring quality. Alcohol is an excellent solvent. Therefore, any off-character in the rinse water may be picked up in the wine.

### *Chemical Analysis*

A procedure should be established for running a specific set of analyses according to a specific timetable. The analysis performed depends somewhat upon the philosophy of the winemaker. Several analyses are essential on newly-fermented wines:

- pH
- free and total sulfur dioxide (by the aeration-oxidation method, see Zoecklein et al. 1999)
- titratable acidity
- reducing sugar
- alcohol
- protein stability (for whites and low-tannin reds)
- potassium bitartrate stability
- MLF status, biological stability
- Proper and controlled sensory analysis

These are the very minimum analyses which the winery should be capable of performing or willing to contract.

It is important that the winemaker know how each processing step affects his product, both chemically and organoleptically. Formal or semi-formal sensory evaluations should be conducted regularly using reference samples. For example, wines should be evaluated for comparison of color and body stripping due to filtration, cold stabilization, etc., both before and after the procedure is conducted.

*Oxygen Pickup*

Most winemakers strive to retain as much of the “grape” as possible in their wines. The loss of aroma components between fermentation and bottle release is a significant problem in this state.

The colder the wine, the greater is the solubility of molecular oxygen in the wine. When the wine is then allowed to warm, oxidation can occur. This is a principal disadvantage of conventional cold stabilization for potassium bitartrate stability. Such procedures often result in prolonged refrigeration of wines, resulting in oxidative degradation and high energy demands.

Each winemaker must know how processing and equipment affect $O_2$ uptake. Free sulfur dioxide analysis is a good indication of $O_2$ uptake, since sulfurous acid is oxidized by the dissolved oxygen in wine. Therefore, a rapid decline in the free $SO_2$ level in a short period of time is indicative of $O_2$ pickup.

Such preventive steps as proper equipment, sulfur dioxide additions *during or just* prior to wine movements, nitrogen blanketing, $CO_2$ sparging, and flushing lines and receiving tanks, all have their place in reducing the likelihood of excessive oxidation. (The use of a “Y'” valve on the suction side of a positive-displacement pump is an easy

way to introduce $SO_2$, gases, fining agents, etc., during racking). For a discussion on the use of displacement gases, see *Enology Notes*.

There is no substitute for storing wines in full containers, with the possible exception of barrels. A partial vacuums is formed in *properly sealed* barrels over time. Many assumed that barrels should be topped regularly to prevent oxidation and biological growth. The frequency of barrel topping should be part of the winery's HACCP program, and determined based on wine chemistry, style (secondary lees volume), and barrel sanitation program.

As stated, wine temperature is important because of its effect on oxygen solubility. Knowing storage temperatures and temperature fluctuations is a key to understanding the aging potential of a wine.

## Pre-Bottling

A checklist should be established to ensure that important factors are not overlooked. These should include the following:

- free sulphur levels ($FSO_2$)
- dissolved carbon dioxide ($DCO_2$)
- dissolved oxygen (DO)
- turbidity
- residue sugar/density
- temperature
- stability: protein, color, bitartrate, and microbiological
- final sensory analysis or review, including screen for volatile sulfur-like off odors.

It should be noted that wines are generally stable with regard to MLF if the malic acid content is less than 30 mg/L. Paper chromatography is only semi-quantitative and will detect malic acid levels in the range of 100 mg/L or more.

Wines to be either bottled unfiltered would without absolute membrane porosity should be tested for viable yeast, acetic acid bacteria and lactic acid bacteria. This includes

*Brettanomyces* spp. Testing procedures include traditional bench top techniques such as plating or molecular methods such as Scorpion ™.

Dissolved oxygen meters must be calibrated regularly can capable of reading to the hundredths. Residue carbon dioxide may increase the risk of foaming, necessitating the use of anti-foam adjuncts.

## *Chemical Analysis*

Has the wine met the proper analytical criteria for bottling? Do you know the accuracy and precision of your analysis?

## *Stability Analysis*

Has the wine met such stability criteria including protein, color, and bitartrate and microbiological stability? Note that any blending, acid or sweetness source addition can change a wine from stable to unstable. If wine was produced from compromised fruit and the calcium level is greater than 40 mg/L there is a possibility of post-bottling calcium tartrate precipitation (See *Enology Notes* Index).

## *Sensory Analysis*

All bottling lots should be reviewed by a panel, not solely by the winemaker. It is easy for those in the commercial wine industry to overestimate their own sensory abilities. The fact that winemakers can distinguish between ethyl acetate and ethyl mercaptans does not necessarily mean they are the best judge of what the buying public desires. It is recommended that the winemaker take a sample of the wine home and have it with several meals in a comfortable, relaxed atmosphere. Many contrast their wine with at least one other.

## *Materials and Quality Control Analysis*

Are all materials needed for bottling present and in the proper condition? Clearly distinguish between critical (functional) and minor (“cosmetic”) flaws.

- Acceptable Quality Levels (AQL): This is a satatistical measures of consistency or quality common to sampling programs. AQL refers to the maximum number of defects that are considered acceptable during inspection of a randomly selected sample.
- AQL sampling fixes the probability of lot acceptance at 95%. For example, establishing a AQL of 1.5% for cork equates to ≤15 defects per 1,000 cylinders. A restrictive AQL such as 0.010 (1/10,000) will require a much larger sample size than a higher AQL such as 1.0 (100/10,000).
- Selection of AQLs depend upon the nature of defect(s) to be detected:
    - Major defect (Critical) : 1%
    - Minor (Cosmetic) defect 2.5 - 4%
    - The set Acceptable Quality Levels (AQL) should be equal to (but not more restrictive) than your processing capabilities.

## Bottling Quality Control

### *Sanitation Program*

The winery should have a set bottling sanitation program and know its effectiveness. Sanitation in the absence of monitoring is faith-based.

### *Biological and Oxidative Quality Control*

Based upon microbiology, is there a need to sterile package? Options include the following:

- sterile bottling (0.45 µm filtration)
- 0.80 µm filtration
- “sterile” pads
- chemicals (preservatives or sterilant)

Aside from packaging, the two most important considerations during bottling are biological and oxidative. Spoilage organisms which are present in the winery can easily find an adequate growth medium in spilled wine, particularly if the wine is not cleaned up properly. The major sources of contamination during bottling include the following:

- Filter pad drip trays. This is of increased importance due to the use of cellulosic pads, which drip heavily. Trays must be drained often during bottling runs if wine is being filtered during bottling.
- Fill bowls. Leaky spouts, wine blown from snifter valves, wine residue on bell rubbers, etc., can harbor wine contaminants. It may be desirable, particularly during long runs, to occasionally mist bell rubbers and filler stems with a 60-70% ethanol solution to inhibit microbial growth.
- Corking machines. Corkers are a significant source of potential sanitation difficulties, due to the likelihood of wine spillage, and are easily contaminated. These units should be completely dismantled and cleaned before and after each bottling. Ethanol misting of the corker jaws during bottling can be a significant asset in minimizing biological problems.
- Work activity. Increased worker activity in the bottling area increases the spread of airborne wine microbes. It is desirable to limit the number of employees around the filling and corking area to as few as possible.

Bottling line sanitation monitoring is essential for minimizing potential problems. Optimally, sampling should occur at 1 hr intervals during operational run. Bottling line samples should be held 2-3 days before biological plating (if that is to occur) to allow sulfur dioxide and other preservatives to impact microbes.

If biological plating is to occur the universal question is how much wine needs to be plated to detect problems?

- Hypothetical Scenario: Assume that >10 cells/L constitutes microbiological instability.
- 100 mL and 750 mL membrane-filtered samples:

$$\text{Case 1: } \frac{20 \text{ cells}}{1000 \text{ mL}} \times 100 \text{ mL} = 2.0 \text{ cells}$$

$$\text{Case 2: } \frac{20 \text{ cells}}{1000 \text{ mL}} \times 750 \text{ mL} = 15 \text{ cells}$$

## *Wine Oxidation*

Another potential problem during bottling is wine oxidation. It is not unusual for bottling to impart from 0.5 to greater than 2 mg of $O_2$/L into the wine. Such addition can have a profound effect on wine quality and shelf life. It is therefore essential to know your bottling equipment and how it affects wine oxidation. Such production practices as sulfur dioxide additions just prior to bottling, ascorbic acid additions, nitrogen sparging, carbon dioxide or nitrogen flushing of bottles prior to filling, vacuum corkers and fillers, etc., can be useful in limiting $O_2$ problems.

The loss of free sulfur dioxide in wine is proportional to the dissolved oxygen content. Producers not using vacuum fillers and corkers, or flushing bottles with gas, can have up to 5 mL of air in the headspace of their bottled wine (750 mL bottles). This amounts to approximately 1 mL (1.4 mg) of oxygen. Four mg of sulfur dioxide are needed to neutralize the effects of 1 mg of oxygen.

Using this relationship, an additional 5-6 mg of free sulfur dioxide is needed to reduce molecular oxygen in the headspace. This represents a rather significant loss of free sulfur dioxide which could otherwise be available as an antimicrobial agent. It should be noted that the reaction of sulfur dioxide with oxygen is not instantaneous. As such, oxidation of desirable aroma and flavor components can occur. An advantage of the use of ascorbic acid is that it reacts very rapidly. For a discussion on the advantages and disadvantages of ascorbic acid see *Enology Notes* # 133 and 144 at www.vtwines.info.

If the extent of potential oxidation is high, wines should not be bottled cold, due to the increased solubility of molecular oxygen. High levels of oxygen are particularly detrimental to wines which contain sorbic acid (potassium sorbate), due to the development of oxidative products which impart an unpleasant character to the wines.

There is a risk in the cellar operation of picking up oxygen during the mixing of wine. The risk of picking up dissolved oxygen can be reduced by ensuring that there is good cover of $CO_2$ gas on the top of the tank during the mixing process, and that all hose and fitting connections on the pump and tank, particularly on the suction side, are air tight.

There is a much greater risk in picking up oxygen in this cellar operation if wine is being recirculated and chilled through a heat exchanger. A good $CO_2$ gas cover on the top of the wine maybe helpful. The risk of picking up dissolved oxygen when using mixing tanks can be reduced by ensuring that there is a good cover of $CO_2$ gas on the top of the tank during the mixing process, and that all hose and fitting connections on the pump and tank are air tight.

In wines with dissolved carbon dioxide, the $CO_2$ level should be monitored prior to bottling to assure that proper concentration has been attained and that there are not foaming issues.

## *Wine Temperature*

The TTB requires proprietors to test representative wines at intervals during the wine bottling operation for correct fill height. Fill height is highly dependent on wine temperature. Ideally, wine temperature should be between 60-70°F at bottling. Thermal expansion of wine between 20°C (68°F) and 40°C (104°F) is 0.08%. As a general rule, wine volume will increase 0.166 mL/1°F in the neck of most 750-mL bottles.

Thus, if a winery bottles at 58°F with 4.5 mL of headspace, that ullage will be reduced to under 3 ml at 68°F, and internal bottle pressure will have risen significantly. This generally is alcohol dependent. The higher the alcohol, the greater is the volume increase, resulting in decreased headspace and corresponding increases in pressure. If lower temperatures are used, the fill points should be adjusted down to compensate for expansion in the bottle when room temperature is reached. General tolerances for 750 mL bottles is 2.0 percent, for 350 mL bottles, 3.0 percent at 20° C/68° F.

### *Label Coding*

Label coding is a means by which the winemaker can extend his quality control into the marketplace. By placing very small notches, one each for day, month, and year on the label, winery personnel can determine the bottling date and, from there, the complete history of the wine.

Label coding can be done by simply placing a stack of labels in a vise and using a saw to cut a small notch on each axis. Using a standard – usually a piece of plastic – the vintner can identify the bottling date. This can be highly important if the winery is forced to have several to many bottling runs of a particular wine lot.

We have had several cases where sheer biological or physical instability occurred with only one bottling date, of a wine with several bottlings. Had these wineries coded their bottles, they could have gone into the marketplace and simply removed only that particular bottling date affected. Instead, they were forced to recall all bottling dates of that particular wine, resulting in a major credibility problem – to say nothing of the direct economic loss.

## Warehousing and Bottle Release

Wines bottled in synthetic closures should be stored upright. Cork-finished wines can be stored on their closures some time after bottling.

Bottle release dates are usually determined based on marketing decisions. However, bottled wines should be periodically tasted by a panel against reference samples (held at < 40°F) to determine how the wine is developing. Too early a release date results in a bottle bouquet that is less than fully developed, too late may mean a large segment of the consumers could receive the wine after its quality begins to diminish.

### *Bottle shock*

Bottle shock is a rather strange phenomenon where a wines aromatic intensity is reduced after bottling only to return sometime later. The cause(s) of bottle shock are

unknown, but widely debated and are likely the result oxidative effects. Generally, if wines are tasted immediately during or shortly after bottling, there is no noticeable effect. However, within the course of a few hours or days the oxygen present can react with wine components causing a reduction in the aromatic intensity. Traditionally this loss has been attributed to the production of aldehydes, a product of ethanol oxidation. Others suggest that bottle shock is the result of another oxidation product, hydrogen peroxide.

Bottle aging is dependent upon the wine chemistry and the warehousing conditions. It is essential that the winemaker understand how each processing step affects wine chemistry and, therefore, wine shelf life. Bottle storage temperature naturally impact wine longevity. For example, it is estimated that a white wine stored at 55 degrees F and expected to continue to improve for 5 years may have that time reduced by one half if stored at 70 degrees F.

Premium wine quality is the result of quality fruit and many processing steps. These steps, viewed individually, may be insignificant. However, collectively they make the difference between standard and outstanding wines. It is the responsibility of the winemaker to understand how production parameters affect wine quality, and to make adjustments accordingly.

For a more detailed discussion on quality parameters, see *Enology Notes* HACCP (Hazard Analysis and Critical Control Points) at www.vtwines.info.

### 3. The 2013 Technical Study Tour.

We are planning another wine industry technical study tour. This tour will go to Burgundy, Alsace and Champagne between December 4 and December 14. This is the 8th technical study tour we have conducted.

These tours have involved winemakers and growers from California, the mid-west, New York and Virginia. The maximum number allowed is 18 participants. Technical study

tours are restricted to commercial winemakers and grape growers. A deposit of $200 is required to secure a slot. More details to follow.

Several write-ups about recent trips are posted at www.vtwines.info under *Enology Notes.*

- AOC's of Provence, *Enology Notes* # 138
- Languedoc, the Rhone, Bandol and Casses, *Enology Notes* # 152
- Spain and Bordeaux, *Enology Notes* # 164

## 4. Winery Planning and Design CD

This publication is in CD format. Below is a listing of the subject index. This CD is the result of a number of workshops, and short courses I have organized on various aspects of winery planning in various regions of the country. The information provided is from a number of authoritative sources and is not linked to specific geographical regions.

*Winery Planning and Design*, Edition 16 is available through the industry trade journal *Practical Winery and Vineyard* (at http://www.practicalwinery.com/bookshelf.htm, phone 415-453-9700 ext 102, email: office@practicalwinery.com ).

## 5. Microbial Ecology during Vinification

Molly Kelly, Extension Enologist, Enology Grape Chemistry Group, Virginia Tech

### Wine Spoilage Yeasts

*Kloeckera apiculata* is the most common occurring yeast species on mature grapes. Other yeasts that may be isolated from grapes and must include species of *Brettanomyces*, *Dekkera* (the sporulating counterpart of *Brettanomyces*), *Candida*, *Pichia, Hansenula* and *Torulopsis* (Lafon Lafourcade, 1983). Diseased and damaged grapes have significantly higher populations of spoilage yeasts that can affect the outcome of the fermentation. These yeasts can metabolize the sugar in the grapes

(which contain 160-240 g/L) mainly in the form of glucose and fructose. These yeasts can result in strong off-flavors and may be tolerant to sulfur dioxide.

### *Kloeckera*

*Kloeckera apiculata* has been reported to grow in the presence of up to 150 mg/L of $SO_2$. It is also cold tolerant (10-15$^{o}$ C) and is able to survive until the end of fermentation. This can result in thick scum formation, ethyl acetate and amyl acetate (banana skin smell) formation. Its presence depletes nutrients needed by *Saccharomyces cerevisiae* for a successful fermentation. *Kloeckera apiculata* and its counterpart *Hanseniaspora* are fermentative yeasts and can grow in abundance early in native fermentations. They can represent the dominant species in unsulfited juice and must.

Using microscopy, if more than 5-10 non-*Saccharomyces* yeast are observed in a field under 40x magnification prefermentation, there will most likely be problems with the fermentation. *Kloeckera* has a distinct morphology when viewed using a microscope. It is described as lemon-shaped due to repeated budding at both poles. It will grow in 1-2 days in culture while *Brettanomyces* requires 3-7 days to grow.

### *Brettanomyces*

The most common form of yeast spoilage is due to *Brettanomyces bruxellensis*. This yeast produces volatile phenols and acetic acid. Examples of flaws include aromas described as “medicinal” in white wines and “leather” or “horse sweat” in red wines. Other aromas descriptors include barnyard, wet dog, tar, tobacco, creosote, plastic and band aid.

*Brettanomyces* can infect red wine 6-10 months after barreling and can spoil bottled wines as well. It can also be transmitted by fruit flies. It also grows on the disaccharide cellobiose, a by-product of toasting in barrel production. Control of this yeast is difficult due to its tolerance to sulfur dioxide.

Microscopically, they resemble *S. cerevisiae* but are usually smaller. The cells are described as ogival in shape. This shape results from repeated polar budding and looks like gothic arches. In older cultures, cells appear elongated and chains may also occur.

Prevention strategies for *Brettanomyces* include:

Grapes: Minimize damage to skins, pick when cool, sorting, add sulfur dioxide to picking bins, minimize transport distance and use adequate hygiene.

Winery: Winery equipment should be cleaned using regular cellar hygiene. Wines should contain adequate $SO_2$ and air/oxygen exposure minimized. Filtration at the 0.6 micron level will eliminate *Brettanomyces*. For additional information see *Brettanomyces* - Practical Monitoring and Management of *Brettanomyces.* Under on-line publications and Enology Notes at www.vtwines.info.

## Wine Spoilage Bacteria

The two major groups of wine spoilage bacteria can be placed in either the acetic acid bacteria (AAB) or the lactic acid bacteria (LAB). The AAB include the genera *Acetobacter* and *Gluconobacter*. Both have aerobic metabolisms and thus their growth generally occurs on wine surfaces as a translucent film that tends to separate into a patchy appearance. In contrast, the LAB are microaerophilic to facultatively anaerobic, requiring low oxygen conditions for growth. The LAB include the genera *Lactobacillus, Pediococcus* and *Oenococcus.*

During fermentation the presence of microbes may be indicated by ethyl acetate, a spontaneous or sluggish fermentation, spontaneous malolactic fermentation (MLF), volatile acidity (VA) or other off-odors.

## Acetic Acid Bacteria (AAB)

Bacteria in this group, *Acetobacter* and *Gluconobacter*, use ethanol (and glucose) aerobically to form acetic acid. Of the two, *Acetobacter* is the most commonly encountered. *Acetobacter* can grow in barreled or bottled wines. It has the ability to grow using small amounts of oxygen absorbed during clarification and maturation. This

organism is strictly aerobic (requires oxygen to grow) and appears as small rods or cocci (round) under magnification typically 0.6-0.9 microns by 1-3 microns in size. It also frequently occurs in pairs and chains.

Moldy grapes have a high population of AAB and this can lead to spoilage after crush. The most serious consequence of spoilage by AAB is the production of high levels of acetic acid (volatile acidity).

In order to control AAB, pHs should be low. Other control methods include minimizing oxygen incorporation, maintaining cool temperatures (< $50^{o}$ F) and maintaining correct free $SO_2$ levels. High VA wines (legal limit reds: 1.40 g/L, legal limit whites 1.20 g/L) can be blended with unaffected wine or treated with reverse osmosis.

## Volatile Acidity (VA)

Acetic acid is formed by *S. cerevisiae* at low levels during alcoholic fermentation. It is also produced by *Oenococcus* during malolactic fermentation (MLF) by the metabolism of citrate by *Oenococcus*. Commercial ML strains produce low levels of acetic acid but spoilage lactic acid bacteria produce more. VA can also be produced by *Brettanomyces* and LAB during primary fermentation.

There are two components of VA: acetic acid (smells like vinegar) and ethyl acetate (nail polish remover). Although we include ethyl acetate, this compound is an ester, not a volatile acid. Ethyl acetate is therefore not measured when using the Cash still. This only measures the level of acetic acid. Ethyl alcohol and acetic acid react to yield ethyl acetate and water. The main source for acetic acid alone is LAB. The main sources for acetic acid and ethyl acetate are *Acetobacter* and wild yeasts. The sensory threshold for these two compounds together is much lower than that of acetic acid alone. Post-fermentation sources of VA include headspace in barrels and oxidation of wine.

## Lactic Acid Bacteria (LAB)

The typical spoilage times for LAB include during stuck fermentations and in finished wines with low $SO_2$, and residual malic or sugar. These organisms appear rod-like using

microscopy. The use of lysozyme will bring about destabilization of the bacterial cell wall peptidoglycan and an therefore be used to control LAB in wine.

In addition to the production of acetic acid through the metabolism of citric acid as well as glucose, LAB can result in a number of other faults. These include mousiness, geranium taint and ropiness. Mousey taint is an aftertaste. It is not volatile at wine pH but when mixed with the neutral pH of saliva, it becomes apparent. The taste is described as mouse urine and rancid nuts. This taint is usually due to LAB but can also be caused by *Brettanomyces.* Geranium taint is caused by the metabolism of sorbic acid by LAB. Sorbic acid is a yeast inhibitor added to prevent refermentation in the bottle. Although generally effective as a fermentative yeast inhibitor, sorbic acid shows little inhibition of LAB, AAB or film yeasts. Another fault caused by LAB, ropiness, is the result of the production of extracellular polysaccharides by LAB.

Some strains of LAB are beneficial such as *Oenococcus oeni*. This bacteria is involved in the decarboxylation of malic acid to lactic acid during malolactic fermentation (MLF). This reaction increases pH resulting in a “softer” mouthfeel. Diacetyl is also produced resulting in a “buttery” character. 1-4 mg/L of diacetyl is considered desirable depending on wine style, while high concentrations (>5-7 mg/L) is considered a spoilage characteristic.

In addition to the sensory implications, acetic acid and products of LAB metabolism act as inhibitors to *Saccharomyces*. This causes a delay in onset of fermentation or may cause a fermentation to stick. A sluggish fermentation should never be inoculated with malolactic bacteria. The bacteria can metabolize glucose and fructose to acetic acid, increasing VA by 1 g/L or more.

Other spoilage organisms that can be present during MLF include *Acetobacter*, *Pediococcus*, *Brettanomyces* and film yeasts. Wines should be monitored for VA increases (>0.15 g/L), surface films and off-odors/flavors.

The methods used to control/prevent LAB and AAB on grapes are the same as those for the spoilage yeasts discussed earlier. Regular cellar hygiene should be used to clean equipment. Grapes suspected to be infected should have short to no skin contact.

Exclusion of air, filtration (0.45 micron) and acid additions can be used to control/prevent spoilage bacterial growth.

## Winery Microbiology Laboratory

Some considerations when planning a winery microbiology laboratory are: space considerations, availability of trained staff to perform testing, willingness to maintain adequate record-keeping, equipment costs as well as the cost of consumables.

## Equipment/Microbiological Methods

A microscope capable of 1000x magnification is needed to view bacteria and yeast. These can cost anywhere from $1000-$3000 but bargains can be found on used microscopes. A phase-contrast microscope requires no staining of slides. This feature also allows for rapid detection and response. The staff in the microbiology lab should have training in the proper use of a microscope as well as identification of microorganisms.

In addition to identifying spoilage organisms, a microscope can be used to monitor yeast populations. By using a simple methylene blue stain, yeast viability can be determined. Bacterial culture media is available for the growth of spoilage organisms for identification. This requires additional equipment including an incubator. This also requires further training in sterile technique and organism identification techniques. Several types of culture media exist for the detection of the organism of interest. For example, media used to plate for *Brettanomyces* exists that contains chloramphenicol (200 mg/L) to prevent bacterial growth while others may contain cyclohexamide to prevent *Saccharomyces* growth.

The membrane filter method can be used to isolate small numbers of microbes from a liquid sample. A sterile cellulose nitrate membrane (0.45 microns for bacteria, 0.65-8 microns for yeasts) is placed on a vacuum flask and filtered. Using sterile technique, the membrane is placed on the culture plate and monitored for growth. This method could be used to check bottle sterility.

### Environmental Monitoring

The cellulose membrane can also be used to perform environmental monitoring on smooth surfaces. The sterile membrane is placed on the surface to be tested and then placed on an agar plate using sterile technique. The plate is then monitored for growth.

The swab test method is used for semi-quantitative analysis. Moist sterile cotton swabs are used to monitor dry areas (moistened with sterile saline or water). Dry swabs can be used to test moist areas. The swabs can then be used to inoculate the proper agar medium, depending on the organism of interest. Agar plates can also be used to detect airborne organisms at critical winery locations. Plates are left open for 30 minutes to 2 hours and then incubated. Airborne organisms that settle on the plate will grow and can be further identified.

### Cellar Hygiene

It should be stressed that cellar hygiene is critical in maintaining wine integrity and quality. Poor wine quality is usually due to poor sanitation practices. Areas of spoilage organism build-up include: the vineyard, second-hand barrels, imported bulk wine and areas of the winery that are difficult to reach.

### Outsourcing

There are commercial enology laboratories that provide all of the microbiological services discussed here. The Enology Services Laboratory at Virginia Tech offers microbiological testing. For further information contact Ann Sandbrook or Ken Hurley at Enology.Services@vt.edu.

## 6. In Memory of Dr. Keith Patterson

Several months ago the wine industry recently lost a true impact player, Dr.Keith Patterson. Keith was the viticulturists for the CalPoly Wine and Viticulture program for 16 years.

Many spend a career looking for a career. Not Keith, who found his passion in teaching,

to say he was a unique academician is a vast understatement. His mantra was - there is so much to learn and so little worth knowing. He taught students practical skills so that they departed college actually knowing how to do something-there is a concept!

Keith knew well that a good like does not mean learning how to avoid the storms, it means learning how to dance in the rain. He had a rare combination of intellect and personality that made him  a very popular and an effective teacher and leader. His students are in positions of prominence in wine regions around the world!

Fortunately for all that knew Keith Patterson, someone who is not completely forgotten never completely dies. His legacy will live on within the countless lives he has touched.

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