Vinmetrica Alcohol-by-Volume User Manual

The Vinmetrica Alcohol-by-Volume (ABV) Kit provides a simple, accurate and affordable way to determine alcohol content in any beverage or liquid sample.

Cautions: The ABV kit contains potassium dichromate/sulfuric acid as one of its reagents. This material is corrosive and a strong oxidant. It also contains hexavalent chromium, a potential carcinogen. For your health and safety, please pay close attention to the recommended manner of use and disposal of this reagent as stated in this manual!

Materials provided in the kit:

1. Oxidant (0.062M potassium dichromate/2M sulfuric acid) (PN: SC-60-3)
   Danger: corrosive, oxidizer, carcinogen! See below for safe use and disposal instructions!
2. ABV Titrant (0.2M sodium thiosulfate) (PN: SC-60-4)
3. ABV Developer solution (PN: SC-60-5)
4. Starch Indicator solution (PN: SC-60-6)
5. Reaction bottle with cap assembly (2) (PN: SC-60-8)
6. 5.0 mL volumetric pipette (PN: SC-60-9)
7. Pipetting safety bulb (PN: SC-300-16)
8. Calcium hydroxide ($Ca(OH)_2$, neutralizer and sequestrant) (PN: SC-60-7)
9. Transfer ("Squeeze-bulb") pipettes (2) (PN: SC-60-10)
10. Microliter Pipettor (PN: SC-60-11)
11. 100 µL Pipette tips (25) (PN: SC-60-11-2)

Figure 1. The Vinmetrica ABV Kit

Things you will need:

1. A 10 or 25 mL burette; available from Vinmetrica (PN: SC-300-7-10 or SC-300-7-25)
2. Lab Support Stand; available from Vinmetrica (PN: SC-300-3)
3. Burette Clamp; available from Vinmetrica (PN: SC-300-6)
4. Distilled water (DI water), which can be found at most grocery stores. Use within 2 weeks.
5. (Optional) Rinse bottle; available from Vinmetrica (PN: SC-100-17)
6. (Optional) Additional Reaction bottle and cap assemblies (for running more than two tests at one time. Available in sets of two (SC-60-8) or in sets of five (SC-60-8-5).
7. Baking soda (sodium bicarbonate) and KMBS (Potassium Metabisulfite) to neutralize spills

8. (Optional) Potassium Iodate standard (PN: SC-60-12). For re-standardizing your ABV Titrant. See Appendix C for details
9. (Optional): A heating pad, like the Sunbeam model 756-500 that has no auto shutoff. Or a warm heating area to achieve 35-45ºC (98-113ºF).

How it works:

Ethanol in the sample reacts with the Oxidant to form acetic acid. Each molecule of ethanol consumes 2/3 of a molecule of the dichromate in the Oxidant. The amount of dichromate remaining after the reaction is determined by conversion to iodine and titration with the Titrant (sodium thiosulfate). This gives a simple calculation for the amount of ethanol.

$$2Cr_2O_7^{2-} + 3CH_3CH_2OH + 16H^+ \longrightarrow 4Cr^{3+} + 3CH_3COOH + 11H_2O$$

Oxidation of ethanol to acetic acid by dichromate (Oxidant)

$$Cr_2O_7^{2-} + 14H^+ + 6I^- \longrightarrow 2Cr^{3+} + 3I_2 + 7\,H_2O$$

Conversion of leftover dichromate to iodine by iodide (Developer)

$$2\,S_2O_3^{\,2-} + I_2 \longrightarrow S_4O_6^{2-} + 2I^-$$

Titration of iodine by thiosulfate (ABV Titrant)

Most wine, beer, cider and other samples that we analyze for alcohol content have nonvolatile components (sugars, tannins, etc.) that can also react with the Oxidant. Therefore, unless your sample is a relatively clean distillate that lacks these components, you can't get accurate results if you just introduce the sample directly into the Oxidant. The kit's Reaction bottle assembly (Figure 2) provides a separate chamber for the sample so that only the volatile components of the sample can enter the Oxidant. Since few volatile components other than ethanol react with the Oxidant, you get a very accurate determination.

Figure 2. The ABV Reaction bottle assembly. The sample is suspended above the Oxidant in a small vial (the "bucket"), so only volatile components (ethanol) can react.

Assay Notes:

- If you are analyzing distilled spirits with very low non-volatiles content, you may use the direct method which gives results in little over an hour. See step 9 of the Reaction step under Procedures below (page 4).
- Some volatiles present in your sample may be quantified as ethanol. Although these are rarely produced in significant enough quantities to affect wine or beer results, distilled spirits may have amounts that are significant; this is especially the case for methanol. If you suspect appre-

ciable amounts of methanol, higher alcohols and /or some aldehydes to be present, bear in mind that these may be detected as ethanol, making the assay results higher.

Setup

1. To prepare for this assay you may want a warm location for incubating your assay bottles. An ideal temperature is 40ºC (110ºF). The heating pad mentioned above is a good way to achieve this; use an inverted cardboard box or similar artifice to keep everything warm. Cooler temperatures will require longer incubation times, up to 24 hours at 23 ºC (73ºF).
2. **Cleanliness is important**! Especially note that any residues in the Reaction bottle can react with the Oxidant and produce erroneous results. Rinse Reaction bottles and cap assemblies as in step 2 of the Procedure below. Also, high levels of alcohol vapors may cause erroneous results. Avoid running the assay in close proximity to distillery, winery or brewery operations at times when ambient alcohol levels may be elevated, or in labs at any time when any alcohols (especially methanol, ethanol, or isopropyl alcohol) are being used extensively.

Procedures

The test consists of 4 stages. The *Reaction step* goes from 4 to 24 hours. After the reaction, you perform a *Titration*. From this you can do the *Calculation*. Finally, you'll be *Finishing up* with a safe disposal step. **Note: keep sodium bicarbonate (baking soda) and sodium or potassium metabisulfite handy to neutralize an Oxidant spill. Do not pipette by mouth!**

Reaction step: as per below, place Oxidant in Reaction bottle. Place samples in cap assembly, insert into Reaction bottle and incubate. **Wear gloves and eye protection! If any chemical contacts skin or eyes, flush immediately with plenty of water. Seek medical attention if symptoms develop.**

1. **Accurate pipetting is essential** to success and accuracy. See Appendix A for explanations of pipetting techniques.
2. Remove the cap assembly from each Reaction bottle to be used. Rinse Reaction bottles and cap assemblies thoroughly with freshly-purchased distilled water. Place inverted on a lint-free surface and allow to drain completely. Do not attempt to dry them afterwards. Examine the bottles carefully to be sure they are clean before using them in the assay. They do not have to be perfectly dry.
3. With the 5.0 mL volumetric pipette and pipetting bulb, introduce exactly 5.00 mL of the Oxidant into the bottom of a Reaction bottle (see Appendix A). It is important that this be done in a reproducible manner each time. Prepare as many Reaction bottles as you have samples.
4. Review Appendix A for sampling tips. If your sample is actively fermenting, centrifuge or filter to remove yeast before proceeding.
5. For most wine or beer samples with ABV between about 4 and 16%, use the pipettor set to "100" to deliver exactly 0.100 mL (100 microliters or 100 µL) of each sample into a sample bucket. Immediately lift the whole assembly by the cap carefully to avoid spilling the bucket and put

the cap on the Reaction bottle, allowing the bucket to enter the bottle so it is suspended above the surface of the Oxidant in the bottle. Be sure the contents of the bucket do not spill into the Oxidant. Secure the cap firmly by hand. Label the bottle to indicate the sample.

6. If you are analyzing a sample with low expected levels of alcohol (2% or less), you can use a larger volume of sample. Your pipettor can be set to deliver up to 250 µL precisely; you also can pipette up to 1.00 mL sample into the bucket if you have accurate pipettes for this purpose.
7. If your alcohol level is expected to be above 16%, we recommend diluting the sample quantitatively with pure water to bring the approximate level to between 8 and 16%. Then proceed from step 4. above.
8. We recommend also including a blank, in which the sample is just pure DI water (100 µL). You can use recently determined blank values in lieu of running one each time, but highest accuracy results from a fresh blank.
9. *Direct method* (only recommended for distilled spirits if a quick assay is desired): Dilute the sample to approximately 16% ABV or less with distilled water. Use the pipettor to introduce 100 µL of diluted sample directly into the Oxidant in the Reaction bottle and quickly put on the cap assembly. It is important to close the bottle quickly because acetaldehyde that is initially (and rapidly!) generated is very volatile and can escape, causing erroneously low values. Note the shorter incubation time in step 10.
10. Place all finished assemblies in a warm location for 4 to 18 hours (1-2 hours if doing a *Direct method*) if possible. A temperature of 38-40°C (101-104°F) is ideal; on top of a heating pad with an inverted cardboard box for a cover is one good way to do this. Most beer and wine samples will be complete in 4 hours at this temperature (longer times don't hurt). If you run the test at lower temperature (down to 23°C or 73°F), or if you use a volume greater than 100 µL, let it go 24 hours.
11. Occasionally giving the bottles a gentle swirl will speed up completeness of reaction. Be sure not to let the Oxidant and sample come into contact.
12. Samples with appreciable alcohol content will show darkening of the Oxidant because the orange dichromate is converted to green-blue chromic ion (Figure 3).

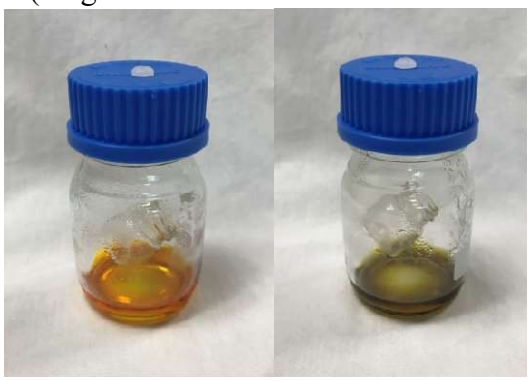

Figure 3. Blank (L) and 13% ABV wine sample (R)

Titration: Remove samples carefully from warm area and allow to cool 5 minutes. As per below, add water and ABV Developer and titrate with the sodium thiosulfate (ABV Titrant) to a starch end-point. **Wear gloves and eye protection!**

1. Fill your burette with ABV Titrant and zero it (or write down the starting volume value). **See Appendix B** for tips on using your burette.

2. For each sample and blank, remove cap assembly carefully to prevent spillage. Examine the cap assembly to be sure only water condensate is present, but no colored Oxidant solution has splashed up onto it. [If this has happened you can try rinsing the Oxidant from the outside of the assembly's bucket back into the Reaction bottle with a stream of a few ccs of DI water from a rinse bottle. However, your results may be compromised.] Set cap assembly aside.

3. Add about 10 mL DI water and 2 mL ABV Developer (for the latter, use the squeeze bulb pipette and note the markings to be sure you deliver at least 2 mL) to the bottle and swirl briefly. A reddish-brown color (due to iodine) will form. (Figure 4, left). If any drops of colored oxidant solution are present on the sides of the bottle, try to swirl the contents to mix them in. A few mL of pure water can also be used to rinse these down.

4. Immediately titrate the bottle until the color changes from reddish brown to a deep olive-green. Don't overrun the endpoint! See Figure 4, right.

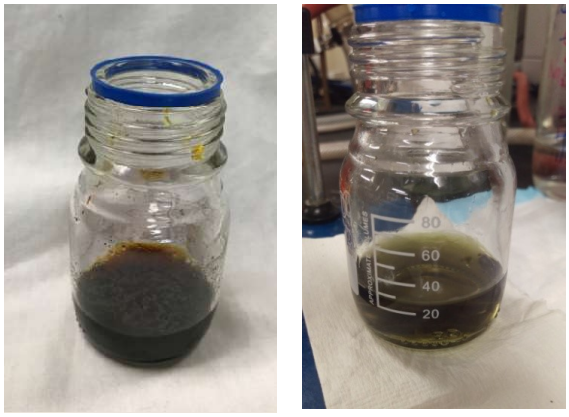

Figure 4. Sample after addition of Developer (L) and after subsequent titration to near the endpoint (R). Note the olive-green color near the endpoint.

5. Add 1 mL of Starch Indicator solution (use a separate squeeze bulb pipette for this) and continue the titration dropwise until the deep blue/black color suddenly clears to a light blue-green. (Figure 5) For best accuracy, try to hit this endpoint with incremental additions of half a drop.

Be sure to set aside this spent reaction for proper disposal (see below).

6. Note the final volume on the burette. Subtract the initial volume to get V, the volume of Titrant used.

7. Table 1 below gives approximate titration volumes you should expect. This is just a guide. Your results may differ slightly.

Figure 5. Titration near endpoint after addition of starch (L), and at endpoint (R).

Table 1. Approximate expected titration volumes (100µL sample)

Sample %ABV:	0 (Blank)	5	10	15
Volume of ABV Titrant (mL):	9.3	7.6	5.8	4.1

Calculation: The calculation is simple for most cases. If you use the standard sample volume of 100 µL, take the strength of the ABV Titrant 'M' as 0.200 (default value), and the density of ethanol 'ρ' as 0.789, then Eq 1. can be used. Call your blank titration volume V_b, your sample titrations V_s; then

$$\%ABV = (V_b - V_s)\ 2.88 \qquad \text{[Eq. 1]}$$

(i.e., take the difference between your blank and sample titration volumes, and multiply by 2.88).
*Example: A blank titration was 9.20 mL. A 100µL wine sample gave a titration of 5.06 mL. So ABV = (9.20 – 5.06) * 2.88 = 11.923% or 11.9% after rounding to the nearest 0.1%.*

The full calculation (Eq. 2) uses V_b, V_s, the density of ethanol **ρ**, the Titrant strength (molarity **M**), and the sample volume **S** (in µL). This may be useful when the assay is adjusted for non-standard conditions.

$$\%ABV = \frac{100\% * (V_b - V_s)\ ml * M\ mmol\ Na_2S_2O_3/mL * 46.05\ mg\ EtOH/mmol\ EtOH}{(\rho\ mg\ EtOH/\mu L\ EtOH * 4\ mmol\ Na_2S_2O_3/mmol\ EtOH * S\ \mu L\ sample)} \qquad \text{[Eq. 2]}$$

If you have diluted your sample prior to the assay, multiply the %ABV by the dilution factor to get the final result. For example, if you diluted a sample with an equal volume of water prior to taking a 100 µL sample, then the dilution factor is 2.

Keep in mind that the ABV Titrant strength M can decrease over time, and ethanol's density ρ changes somewhat with temperature. So,

1. It's a good idea to check the value of M periodically (see Appendix C). Adjust your calculations if M is significantly different from 0.200. For example, if M is actually 0.190, multiply your final result from Eq. 2 by the factor (0.190/0.200).
2. Since the density of ethanol does not change much over the temperature range 15-25 ºC (59 – 77 ºF, the error in assuming it's 0.789 over this temperature range is less than 0.1 % ABV at 16% ABV. If you want to adjust your result for temperature, you can look up standard values of ethanol density online.

Finishing Up:

SAFE DISPOSAL OF SPENT REACTIONS: The spent reaction contains sulfuric acid and chromic ion (Cr^{+3}). Use the calcium hydroxide in the kit to neutralize the acidity. This also sequesters the Cr^{+3} into a solid form that can be disposed of in solid waste.

1. Carry out the following steps in a well-ventilated area. Use gloves and safety glasses.
2. Pour the spent reaction contents of the Reaction bottle into a glass or polyethylene plastic waste bucket; rinse the Reaction bottle with about 20 mL of tap water and add this to the bucket. If you have multiple spent reactions, add them together into the bucket.
3. For every spent reaction, add 5 g (about 2 tsp) of calcium hydroxide. Stir well every so often over the course of 1 to 2 hours until the settled mass contains all the green-blue color of the trivalent chromium (Cr^{+3}), and the supernatant is clear. This waste bucket and contents can be left covered indefinitely. To dispose, follow the next steps.
4. Pour off the supernatant into a separate container. This liquid should be clear and colorless; it contains negligible amounts of chromium. It can be disposed of in most sewer systems with plenty of water flushing. Note that it will be somewhat alkaline, about pH 12. In some locales, you may want to add sodium bicarbonate (about 15 g per 5 g of $Ca(OH)_2$ that was added) to bring the pH to around 9. If in doubt, check local regulations.
5. The sludge that is left is mostly calcium sulfate (gypsum) that contains small amounts of trivalent chromium (Cr^{+3}) in an insoluble form that does not leach into the environment and can usually be put out with solid waste disposal. Again, if in doubt, check local regulations.
6. Rinse all glassware with distilled water and let air dry.

SAFE DISPOSAL OF LEFTOVER OXIDANT: If you have leftover ABV Oxidant to discard, neutralize its acidity and reduce and sequester its dichromate as follows:

1. **Carry out the following steps in a well-ventilated area. Use gloves and safety glasses.**
2. For every 5 mL of Oxidant, place 20 mL of water, 5 g of calcium hydroxide, and about 0.5 g of a sulfite (sodium or potassium metabisulfite is fine) in a waste container of glass or polyethylene composition.
3. Add the Oxidant to be discarded to the container slowly with stirring. Ensure that the orange color changes entirely to blue-green. If there is still some orange (i.e. the color is still showing some olive-green character), add 0.25 g increments of sulfite with stirring until the color becomes blue-green (see above on page 5, figure 5, right side, for approximate color).
4. Stir well every so often over the course of 1 to 2 hours until the settled mass contains all the green-blue color of the trivalent chromium (Cr^{+3}), and the supernatant is clear.
5. Dispose of the container's contents as in step 4 and 5 of the section above.

WARRANTIES AND LIABILITIES

1. The materials provided in the kit, as described on page 1 above, ("Materials") are warranted as follows: All non-reagent components are warranted against defects in workmanship for 1 year from date of purchase. The reagents are warranted to perform as described herein up until any stated expiration date or 6 months after purchase, whichever is later, provided storage recommendations are followed. THE WARRANTIES IN THESE TERMS AND CONDITIONS ARE IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY WARRANTIES OF MERCHANTABILITY, NONINFRINGEMENT, OR FITNESS FOR A PARTICULAR PURPOSE, SAID WARRANTIES BEING EXPRESSLY DISCLAIMED.

2. Buyer agrees that its sole and exclusive remedy against Vinmetrica shall be limited to the repair and replacement of Materials or parts of Materials, provided Vinmetrica is promptly notified in writing, prior to the expiration of the warranty period specified above, of any defect. Vinmetrica's liability for any damages due Buyer shall be limited to the purchase price of the Materials.

3. VINMETRICA'S MAXIMUM LIABILITY FOR ALL DIRECT DAMAGES, INCLUDING WITHOUT LIMITATION CONTRACT DAMAGES AND DAMAGES FOR INJURIES TO PERSONS OR PROPERTY, WHETHER ARISING FROM VINMETRICA'S BREACH OF THESE TERMS AND CONDITIONS, BREACH OF WARRANTY, NEGLIGENCE, STRICT LIABILITY, OR OTHER TORT WITH RESPECT TO THE MATERIALS, OR ANY SERVICES IN CONNECTION WITH THE MATERIALS, IS LIMITED TO AN AMOUNT NOT TO EXCEED THE PRICE OF THE MATERIALS. IN NO EVENT SHALL VINMETRICA BE LIABLE TO BUYER FOR ANY INCIDENTAL, CONSEQUENTIAL OR SPECIAL DAMAGES, INCLUDING WITHOUT LIMITATION LOST REVENUES AND PROFITS.

HAZARDS AND TOXICITY

All Materials offered by Vinmetrica are intended for use by individuals who are familiar with laboratory procedures and their potential hazards. The Materials contain chemicals which may be harmful if misused. Due care should be exercised with all Materials to prevent direct human contact. Glassware can break and chemicals can splash during experiments; ***always use safety glasses***. We strongly recommend using nitrile or latex gloves and wearing long pants, long sleeves and closed-toed shoes. Keep out of reach of children.

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Appendix A: Pipetting accurately

ALWAYS use eye protection and gloves (latex or nitrile) when using glassware and chemical reagents. NEVER pipette by mouth! Use the provided pipetting safety bulb as described below!

It is important to develop a consistent, reproducible procedure for sampling and distributing reagents and samples. There are 2 steps in the ABV assay that require high precision pipetting for accuracy.

1. Pipetting the Oxidant. You will use your 5 mL volumetric pipette to dispense the Oxidant into the Reaction bottles.

a. Be sure the pipette is clean and dry at the beginning of the session.

b. Holding the pipette in one hand, the bulb in the other, immerse the pipette into the bottle of Oxidant so the tip is about 1 inch below the surface.

c. DO NOT Pipette by Mouth! Use your pipetting bulb as shown to suck the orange-colored liquid into the pipette slowly until it rises to above the mark.

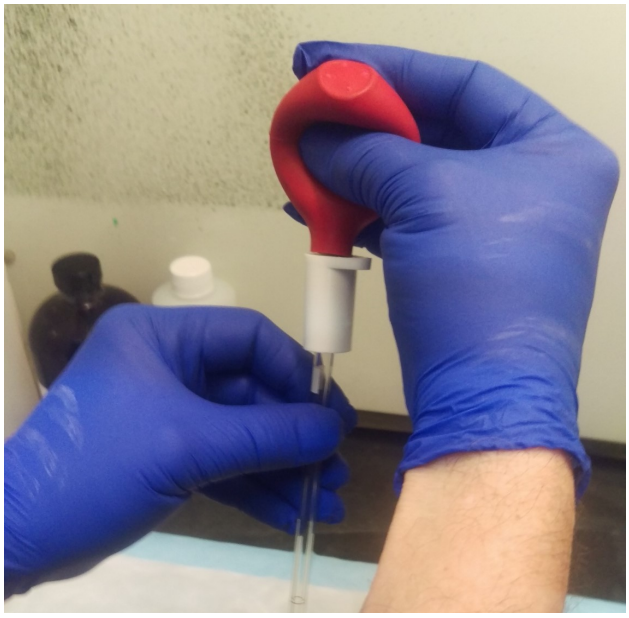

Figure 6. Use the pipetting bulb to safely draw up the ABV Oxidant into the 5.0 mL pipette.

d. In one quick movement, remove the bulb and place your index figure over the end of the pipette to control the release of the liquid.

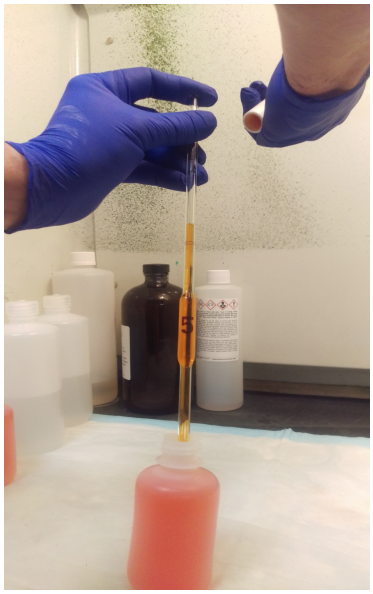

Figure 7. In a quick but smooth motion, take off the pipetting bulb and use your index finger to control the flow.

e. Allow the liquid to flow back into the bottle of Oxidant until the meniscus touches the mark (Fig 8).

Figure 8. Allow the liquid to flow back into the bottle of Oxidant until the meniscus touches the mark (red arrow).

f. Touch both sides of the pipette tip to the inside lip of the Oxidant bottle to drain off any droplet that may be clinging to the outside of the pipette tip.

g. Place the pipette tip against the inside wall of the Reaction bottle you are filling (Figure 9).

h. Allow the liquid to drain completely. DO NOT blow out the liquid!

i. At the end of the draining, keep the tip lightly held against the wall of the bottle and twist/rotate the pipette back and forth five times to complete the delivery.

j. Do not rinse the pipette between successive fillings of Reaction bottles; rather, place the pipette carefully in a clean glass container tip down, or lay it on its side in a manner to prevent the tip from touching anything. When all Reaction bottles have been filled, carefully rinse the pipette into the waste container used for spent reactions (see "Finishing Up" on page 7). Rinse the pipette twice with distilled water and allow to drain.

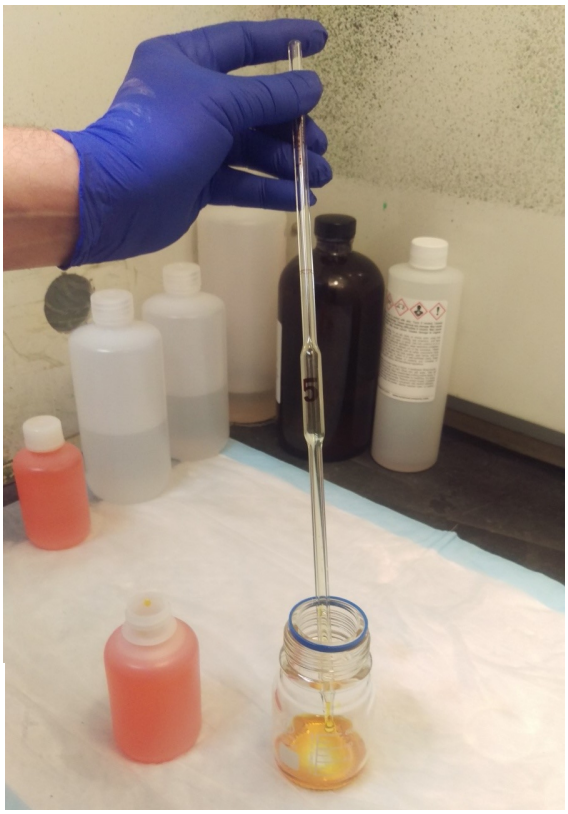

Figure 9. Delivering the Oxidant to the Reaction bottle. Touch the tip of the pipette to the side of the bottle to get complete, reproducible delivery.

2. Pipetting the sample. It is critical that this volume is accurately delivered (100 ± 1 µL). We recommend the micropipettor with disposable tips provided in the kit. Be sure it is set to the 100 µL setting [unless you are taking a larger sample, as in step 6 of the Reaction step on page 4; in that case, be sure to set the pipettor to the desired volume]. Follow manufacturer's directions to deliver the proper volume of sample to a bucket in a cap assembly[1]. For most pipettors, including ours, the following techniques produce the best accuracy:

a. If your sample is carbonated (like beer or sparkling wine), degas it by placing 25 ml or so in a suitable container and shaking or agitating until effervescence ceases. You can also do this by applying a good vacuum for 30 seconds with moderate shaking.
b. If your sample is actively fermenting, centrifuge or filter to remove yeast before proceeding.
c. Use the correct tip for your pipettor. In particular, the diameter of the tip's orifice can affect proper functioning. Use a fresh tip each time.
d. Wet the tip once by drawing up the sample and dispensing it back.
e. Now take the sample by drawing up slowly and pausing for 2 seconds before removing the pipettor from the sample container.
f. If need be, wipe the outside of the tip with a tissue to remove excess droplets, but don't touch the open end of the tip.
g. Dispense the contents of the tip into the bucket of the cap assembly, stopping for 2 seconds at the first stop position of the pipettor. Touch the tip to the side of the bucket, then blow out the tip by going all the way to the second stop. Hold for 2 seconds, then remove the pipettor.
h. As quickly as possible, place the cap assembly over a Reaction bottle and close firmly to avoid loss of ethanol from the sample.

[1]Check pipette calibration once a year if possible. Using a milligram balance, weigh 100 µL of distilled water dispensed by your pipettor. Adjust for density to determine volume dispensed. At 20ºC, 100 µL of water should weigh 99.8 mg (i.e., the density of pure water at 20ºC = 0.9982 g/cc). If the pipettor is within 1.0 µL (i.e., 1 mg) of this value it should be good enough. If it is reproducibly higher or lower (i.e. it is *precise* enough - with coefficient of variation under 1% - but not *accurate* enough), then adjust it if possible, or adjust the calculations in Eq. 2, p 6.

Appendix B: How to use your burette

ALWAYS use eye protection and gloves (latex or nitrile) when using glassware and chemical reagents.

To get the most accurate results when titrating, there are a few things to keep in mind.

1. Filling
    a. Each day of use you should first rinse the burette with a few milliliters of Titrant to remove any excess water or contaminants that may remain from a previous titration. Allow the burette to drain completely. Discard this rinse.
    b. When filling the burette, make sure the Titrant has completely filled the bottom of the burette, including within its tip. Sometimes bubbles can be trapped in the tip of the burette but can usually be dislodged by opening and closing the stopcock while the burette is hovering over a waste container.
    c. Make sure there are not any large bubbles in the burette after filling. If there are, wrap the top of the burette with some plastic wrap (or Parafilm if you have it) and make sure the stopcock is in the closed position. Then take the burette out of its clamp and hold the wrapped end tightly with your thumb. Rotate and invert the burette to allow the bubbles to move out of the column of Titrant.
    d. Once all bubbles have been displaced, replace the burette in its clamp and set the level to 0.0 or slightly below. Make sure that no drop is hanging from the burette's tip, as this will contribute to error. Now you are ready to titrate.

2. Reading:
    a. A simple trick makes accurate reading easy. Draw a 1-inch black band down the center of a thick sheet of white paper, note card, or the back of a business card (Figure 11).
    b. When taking a measurement, hold the paper about an inch behind the burette with the black band about a half an inch below the meniscus (Figure 12). This provides a clear view of the bottom of the meniscus which helps make a precise, consistent measurement.
    c. NOTE: the gradations on your burette may be different than shown in the figures below. These photos are of a 10 mL burette with 0.05 mL gradations; the 25 mL burette will have 0.10 mL gradations.
    d. You should be able to read to a resolution of half a gradation, or 0.025 mL on a 10 mL burette (0.05 mL on a 25 mL burette); with a little practice, you can read to within a fifth of a gradation (0.01 mL on a 10 mL burette, 0.02 mL on a 25 mL burette).

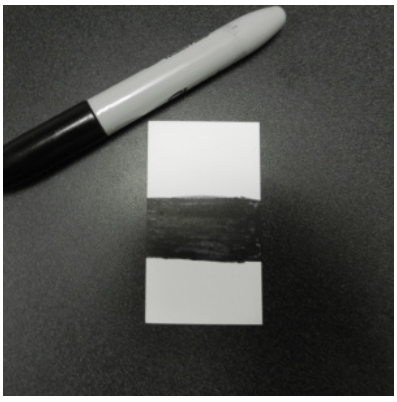

Figure 11. draw a 1-inch black band on card paper. This card will assist you in reading the burette accurately.

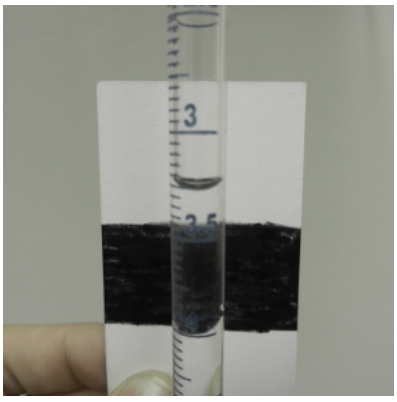

Figure 12. Reading the meniscus of the liquid in the burette. In this picture the value is 3.26 mL.

3. Titrating:
    a. Before beginning the titration, read the starting titration value, using the thick white paper with the black band. Record this value in your notebook.
    b. Add the Developer to your Reaction bottle as instructed. Begin titrating, slowly. As the endpoint nears, the solution's reddish color will give way to an olive-green color. (see Figure 4, page 5)
    c. At this point, add the starch Indicator solution as instructed. Now add the Titrant one drop at a time with swirling of the bottle for a few seconds in between each drop, to give the reaction enough time to respond.
    d. Try to hit the endpoint with half-drop increments for best accuracy. A half drop can be gently shaken off the burette tip, or can be touched off to a point on the inside of the Reaction bottle with swirling to mix. The endpoint is reached when the deep blue-black starch complex clears suddenly to reveal the light blue-green color of the chromic (Cr^{+3}) ion (see Figure 5, page 5).
    e. When you have reached the endpoint, immediately read and record the final titration value. The volume of Titrant used is the difference between the final value and the starting value.
4. Cleaning:
    a. Always rinse your burette out with distilled water at the end of the day. Store the burette inverted and/or covered with a cap to prevent particles and contaminants from entering.
    b. If the burette becomes dirty, you will see that droplets of Titrant remain clinging to the walls of the burette. Since these droplets are supposed to be part of the titration, your results will be inaccurate. In that case it is necessary to clean the burette.
    c. Try filling the burette with Alconox or other glassware cleaning solution (available from Vinmetrica, PN: SC-300-12) and allowing it to soak overnight in the solution. Rinse with plenty of distilled water. Repeat as necessary until water drains smoothly without drops remaining behind.
5. You can also check out these websites for more burette info:
   http://www.titrations.info/pipette-burette or
   http://www.csudh.edu/oliver/demos/buretuse/buretuse.htm

Appendix C. How to re-standardize your ABV Titrant.

ALWAYS use eye protection and gloves (latex or nitrile) when using glassware and chemical reagents.

Sodium thiosulfate, the active chemical in the ABV Titrant, can lose its strength over time, although in our hands we find it stable for at least 2 months at a time. There are two ways to check the strength of the Titrant (M):

1. Blank method. The nice thing about this method is that it's something you usually will do as part of your assay anyway. This method assumes that the Oxidant strength M_o is constant at 0.062 mol/liter. This assumption is largely true for up to 8 months of Oxidant life.
2. Potassium iodate (KIO_3) method. This is the best way to re-standardize sodium thiosulfate. It requires primary standard KIO_3 that has been dried at 105 ºC for 2 hours and stored desiccated. You can obtain pre-weighed samples from Vinmetrica (PN: SC-60-12) that are ready to use.

Blank Method

When you run a blank in the normal assay procedure, use the value V_b (see Reaction step 8 of the Procedure on page 5 and Calculation text at the top of page 6).

This equation applies: $M = 6 * V_o * M_o / V_b$

V_o is the volume of Oxidant (usually 5 mL) and M_o is the concentration of the Oxidant (0.062 mol/liter), so this is simply

$$M = 6 * 5 * 0.062 / V_b = \mathbf{1.86/V_b}$$

KIO_3 method

1. Fill and zero the burette with the ABV Titrant.
2. Weigh accurately (at least to the nearest 1 mg and preferably to the nearest 0.1 mg) about 65 mg of potassium iodate (primary standard KIO_3, AR grade or better) that has been dried at 105 ºC for 2 hours and stored desiccated. Write down the weight in mg, call it 'W'. If you use a Vinmetrica standard (PN: SC-60-12), use the weight on the package as 'W'.
3. Place the KIO_3 quantitatively into a suitable 100 mL titration vessel. Add 40 mL DI water and stir until the potassium iodate is all dissolved. This can take several minutes.
4. Add 1 mL 2M HCl (Vinmetrica Acid solution for SO_2 for example) and 2 mL ABV Developer.
5. Use the ABV Titrant to immediately titrate the iodine formed until the solution changes from red-purple to a pale straw color.
6. Add 1 mL Starch Indicator and complete the titration dropwise until the dark blue color disappears.
7. Determine V, the net volume of ABV Titrant used.
8. Calculation

$$M = W * 6 / (214 * V)$$

Adjustment

If either method determines that M is between 0.197 and 0.203, there is no need to adjust your results. If it is outside this range, you should repeat the procedure once to be sure. If M is still outside the range, then correct your ABV results by multiplying by the factor (M / 0.200).