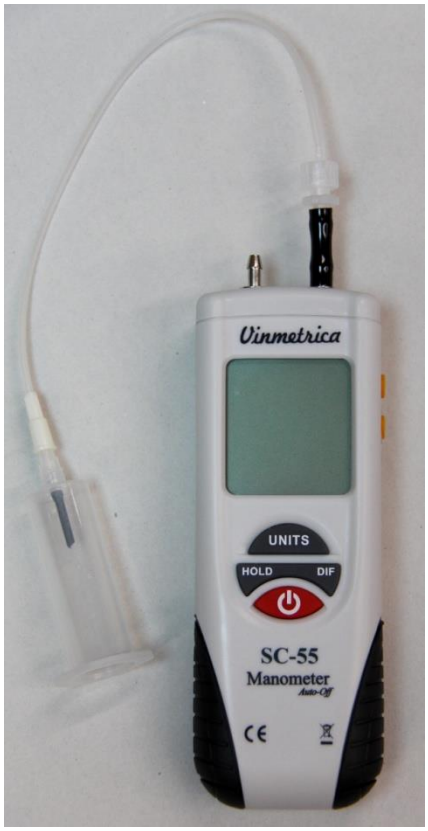
Vinmetrica SC-50 MLF Kit
User Manual

The Vinmetrica SC-50 MLF (malolactic fermentation) Kit provides high accuracy in determination of malic acid concentration levels in wine, an essential parameter to control in the effort to make high quality wines. Note: this version (4.0) contains a small change involving a new reagent. See step 2 and 6 in the Procedure on pages 5 and 6.

Table of Contents:

Materials provided in the kit:



1. Vinmetrica SC-55 Manometer (Part number SC-55)
2. Malic Reagent set ***Keep in Freezer***:

   Malic Acid (1M) concentrate (Part Number: SC-50-5)
   Malic Acid Standard Solution (0.40 g/L) (PN: SC-50-11)
   Malic Acid Standard Solution (0.00 g/L) (PN: SC-50-16)
   Biopressure Agent (PN: SC-50-3-10)
   Assay Diluent (PN: SC-50-3-18)

3. Reaction vials with Septa Caps (6)
4. 10 ml serological pipette
5. 5mL serological pipette
6. 15 ml conical tube (2)
7. Plastic bulb transfer pipette (1)
8. 100 mL polypropylene beaker (2)
9. Plastic Scoop
10. 5 mL Syringe (luer lock type) (for needle clearing)
11. Dual Female Luer Lock Connector (for needle clearing)

Things you will need:

1. Distilled water (DI water), which can be found at most grocery stores
2. Rinse bottle (PN: SC-100-17)
3. Microwave oven, hot plate or stovetop (for boiling wine samples).
4. (Optional) Safety Pipetting Bulb for collecting wine sample. (PN: SC-300-16)
5. (Optional) Additional Reagent Refill Kit for running more than 10 samples, as provided in the kit. Comes in sets of 10 (SC-50-2) or 20 (SC-50-2-1).
6. (Optional) Additional/Replacement reaction parts can be purchased in sets of 10 or 20 for running more than 5 wine samples at one time.

   Septa only (SC-50-10) or Septa caps (SC-50-9) or Reaction vials (SC-50-14-10)
7. (Optional) water bath for temperature control. Set to 25°C if possible.

Why Test for Malic Acid?

In winemaking, malolactic fermentation (MLF) converts malic acid to lactic acid, with CO_2 being the byproduct of the reaction. MLF, which is typically carried out in most red wines and in some white varietals, plays an important role in the finished wine's feel and taste. MLF reduces titratable acidity, increases pH, and produces flavors often characterized as "soft" or "buttery".

Sulfite, as free SO_2, inhibits the bacteria that carry out MLF. Therefore free SO_2 levels must be kept low during MLF, carrying risks that the wine is left unprotected against oxidation and microbial contamination. As soon as MLF is done, then, SO_2 should be raised to appropriate levels for protection of the wine. Thus it is important to know when MLF is done, and the best way to do this is to measure malic acid levels in the wine.

Theory of operation:

The SC-50 MLF Kit relies on the biochemical MLF reaction caused by enzymes found in certain bacteria, including lactobacilli and oenococcus strains, and in the Biopressure agent component of the kit. These bacteria live on a variety of nutrients, but their production of CO_2 results almost entirely from the enzymatic transformation of malic acid to lactic acid:

Malic Acid		Lactic Acid
$HOOC–CH_2–CH(OH)–COOH$	$\rightarrow$	$CH_3–CH(OH)–COOH + CO_2$

The CO_2 creates pressure, which is read by the SC-55 manometer. The CO_2 pressure is directly proportional to the amount of malic acid in the sample. The level of malic acid can be calculated from the pressure values by one or more calibrators of malic acid provided with the kit. Detection limit is below 0.04 g/L. The assay takes 45 minutes, or up to 90 minutes if the Confirmation Procedure is performed (page 8).

Setup

Setting up the SC-55 Manometer for the first time:

1. The SC-55 runs on one 9 volt battery. To insert the battery, open the battery housing by removing its single screw on the back and gently lifting it out. Insert the battery in the correct orientation. Replace the cover and re-tighten the screw.
2. Low Battery/Auto off:
   a) When the battery level is too low, the LCD screen will indicate BAT on the lower left side of the screen. Replace the battery.
   b) After 25 minutes of inactivity, the unit will power off to save battery life. Press Power button to resume. See Appendix B under "Operation", step 8, for override instructions.

Instrument Operation:

1. Press the Power button to turn the device on or off. All the characters on the LCD screen will appear briefly, then the current pressure reading will appear.
2. The UNITS button allows you to select various units for display of your pressure for your data – e.g., bar, psi, in Hg, etc. Observe that by pressing the button repeatedly, the selected unit cycles across the bottom of the LCD screen. We recommend you use kilopascals (kpa), which is located in the bottom left corner of the display.
3. The HOLD button will freeze the display on the current reading. This can be useful when you want to accept the current value and thus want to keep it displayed until you can record it. Pressing the button again will release the hold.
4. The DIF button puts the device in differential mode, subtracting the current reading from all subsequent ones. You can use this to "zero" your instrument if you want.
5. On the side of the instrument are two buttons.
   a) The one with a light bulb icon toggles the backlight.
   b) The one marked REC starts recording mode. You will not normally need to use this mode. You start the mode by pressing this button; press and hold to exit the mode.

Prepare all Reaction Vials:

1. Remove Biopressure Agent from freezer and allow to come to room temperature.
2. Using the small plastic spoon provided, measure out two (2**)** level scoops (approx. 35-40 mg) of Biopressure Agent into each Reaction Vial.
   a) Due to the consistency of the Biopressure Agent, we recommend rinsing and completely drying the plastic spoon after filling approximately 5 vials.
3. Cap all Reaction Vials with provided septa caps.
4. Keep out all Reaction Vials needed for MLF assay.
5. Place all remaining reaction vials in freezer until use.
   a. Thoroughly rinse plastic measuring spoon with DI water.

Procedures

The SC-55 is designed to give a read out of pressure in kilopascals (kpa) created by the reaction in the reaction vials. This information can be used in two modes: 1. In "Quick check" mode you only need to run the wine sample and use the results against a table of values for a quick assessment of MLF status. 2. In "Quantitative" mode you run at least one malic acid standard, such as the 0.4 g/L, a "zero" (0.00 standard, i.e., buffer only), as well as your wine sample(s). These data can be used to calculate how much malic acid is in your wine.

If you believe your wine is at or near completion of MLF, we suggest you run the Quantitative mode using the 0.4 g/L standard. Otherwise, you DO NOT need to run a standard; see the Data Interpretation section below.

Note: the following procedure is designed to be run at room temperature, preferably 78 °F (25 °C). Temperatures below about 73°F are not recommended, as test results may be less sensitive if temperatures are below this range. Make sure all reagents and reaction vials are at <u>room temperature at all times</u> in the procedure! If temperatures fluctuate in your environment, you may want to set up a tray with water at room temperature to serve as a temperature-stabilizing water bath – see step 5 below. See Appendix A for more information on temperature.

1. Take out all Reaction vials needed for assay from freezer. One Reaction vial per sample is necessary. <u>If you wish to quantify the results you will need at least two additional reaction vials for a Malic Acid Standard (e.g. the 0.40 g/L; see step 9 below) and for a 0 level response (0.00 g/L standard)</u>. Allow Reaction vials to come to room temperature before initiating assay. Note that you can run multiple wine samples at one time.

2. Take out all reagents from freezer and allow to come to room temperature: Assay Diluent (NOTE this is new, see step 6 below), Malic Acid Standard (if quantifying results), and 1M Malic acid (if running confirmation procedure, see below). You may put these reagents in a room temperature water bath. Mix well after thawing completely.

3. Using the 10 mL serological pipette, measure a 10 mL wine sample and dispense into the 100 mL plastic beaker or other suitable container. (Serological pipette can be rinsed with DI water for re-use; we recommend that you remove and discard the cotton plug in the end of the pipette)

4. Place wine sample in microwave and boil for about 60 seconds at medium power. Actual time depends on your microwave oven's power; the object is to get the wine sample down to about 7-8 mL due to a gentle boil for about 60 seconds without having the wine splash out of the beaker. If you are unsure about the power of your microwave, we suggest you experiment with a couple of wine samples beforehand to determine what the best settings are for your microwave oven. You may also bring wine to a gentle boil on a stove top for 60 seconds.

5. Let wine sample cool to room temperature, then pour the sample into the 15 mL conical tube. Add DI H_2O to restore the volume to 10.0 mL, then pour the sample back into the 100mL beaker or other clean tube.

6. Now use your 5 mL serological pipette to mix 3.0 mL of boiled, restored wine sample with 3.0 mL of Assay Diluent [NOTE: this is the change from earlier versions that used the 0.0 standard for diluting wine]. Repeat steps 3 through 6 for each wine sample. **Make sure to rinse the pipette between wine samples. Be sure any restored, diluted sample is completely cooled to room temperature before proceeding to step 8!** If you are using a water bath, place the reagents, vials and restored wine samples into the bath and allow their temperature to stabilize for at least 10 minutes.

7. Disconnect the needle assembly from the SC-55 by unscrewing the luer lock connector on the bottom of the instrument. (Figure 2.) The disconnected vial insertion assembly will be used to zero the pressure immediately after capping the sample. Note: The luer lock connector should be reattached tightly to the SC-55 (step 10) before taking a measurement in step 15.

Figure 2. Disconnecting the vial insertion assembly

8. **[Reminder: be sure the wine sample is at room temperature and not warm to the touch!]** Using the 5 mL serological pipette, transfer exactly 3.0 mL of the diluted wine sample from step 6 into the reaction vial with the Biopressure agent. Do not mix yet! Immediately cap the vial tightly, then quickly stand the vial upright and position the disconnected vial insertion assembly such that the needle punctures the septum of the reaction vial (see figures 3 and 4, but note that in this case the SC-55 is NOT connected to the vial insertion assembly). Keep the vial insertion assembly with its needle inserted through the septum for about 8 seconds. Immediately remove the needle and shake the vial vigorously for 5 seconds.

   ☞This step equilibrates the vial's internal pressure to ambient pressure, i.e., it "zeroes" the initial pressure. Then shaking the vial disperses the Biopressure Agent and starts the reaction. NOTE: be sure the needle is NOT plugged. See troubleshooting in Appendix A.

9. If you are performing a Quantitative mode test, follow step 8 above, using the 0.4 g/L **and** 0.0 g/L Malic Acid Standards, in the same manner as a wine sample. If you want to run other standard levels of malic acid, you can prepare these separately. See Appendix B. Do not heat the standards.

10. Repeat step 8 for each sample or Malic Acid Standard to be analyzed. When done, be sure to reconnect the needle assembly to the SC-55 by firmly screwing its luer lock fitting back on to the mating partner.

11. Shake all vials again, gently but thoroughly for approximately 10 seconds.
12. Allow sample to incubate at constant temperature for 45 minutes. While incubation is occurring, gently shake the vials at about the 15 minute and 30 minute mark.

13. Turn on the SC-55. **Press the MODE button, if needed, to select kpa as units to display.**

14. Immediately following the 45 minute incubation period, shake the vial vigorously once more for 5 seconds and then proceed to the next step immediately. It is best to shake the vial by holding the cap, as holding the glass vial transfers heat, resulting in a less accurate pressure reading. DO NOT remove the cap of the vial.

15. This is the CO_2 pressure reading step. [**Note:** Make sure all the parts of the vial insertion assembly are secured tightly before proceeding.] Place reaction vial upright on your work surface. Position the opening of the vial insertion assembly over the vial (Figure 3). Gently push the assembly down onto the vial as far as it will go and hold it in place (i.e. so that the assembly's rubber-sheathed needle is inserted into the vial's septum – see Figure 4.)

Figure 3.

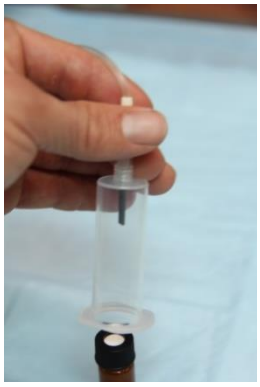


Figure 4.

16. Record the highest value that appears on the meter right after insertion of the needle. This process should take about 5 seconds.

17. Release the vial from the assembly. If you are not performing multiple reactions, continue to the next step. When performing multiple reactions, once the vial is removed, the manometer should stabilize back to the original zero value. If it does not, remove the assembly from the instrument and try to blow it out to clear the needle as in the Troubleshooting section, Appendix A.

18. If you did not test a Malic Acid Standard: if the value is above 2.0 kpa, MLF may not be complete. If below this, MLF is near or at completion. See "Data Interpretation" (page 9).

19. If you did test a malic acid standard alongside your sample(s) you can perform the quantitative mode calculations (see page 8):
    a) For each wine sample, call its value "**a**".
    b) Call the 0.00 g/L Standard response "**b**".
    c) Call the 0.40 g/L Malic Acid Standard responses "**c**".
    d) Follow the Malic Acid Concentration Calculation section below.

Confirmation Procedure

To ensure your Biopressure assay is working correctly, you can confirm the validity of the assay result using the 1M Malic Acid concentrate with a "just-completed" wine sample.

1. Add 1M Malic Acid concentrate: after recording the CO_2 response in steps 15 and 16 above, open up the Reaction vial and, using the plastic bulb pipette, add 1 drop (30 µL) of 1M Malic Acid to the sample. This is equivalent to 1.3 g/L malic acid in the 3mL wine sample.

2. Immediately re-cap the reaction vial and equilibrate it with the disconnected insertion assembly as in step 8 above. Mix the vial thoroughly.

3. Let the sample incubate for 45 minutes, then repeat steps 13-17 from above, performing the CO_2 pressure reading step. Record the data.

4. At the end of the confirmation procedure the reading should increase to over **4 kpa**. This confirms that when malic acid is present, the system responds appropriately.

Malic Acid Concentration Calculation (Quantitative mode)

If you ran a Quantitative mode test, i.e., a malic acid standard as well as a sample, you can calculate concentration.

1. You should now have three values **a**, **b**, and **c,** from steps 15 - 19 of the Procedure, in order to calculate the concentration of malic acid:
   a = Wine sample results
   b = 0.00 g/L Malic Acid Standard result
   c = Standard Vial result for standard concentration **S** (e.g., 0.40 g/L)

2. The malic acid (MA) content, in grams per liter, is given by

   MA, g/L = 2 ∗ S ∗ (a - b)/(c - b) (eq. 1)

   i.e. subtract **b** from **a**, subtract **b** from **c**, divide the first difference by the second, then multiply that result by **S**, the concentration of your standard (0.40 g/L). Multiply this by **2** (to account for the dilution of the wine sample by half).

Example: For a red wine: **a** was 1.05, **b** was 0.71, and **c** was 3.81 for the 0.40 g/L standard, (i.e., S was 0.40). So the malic acid concentration was

2 * 0.40 * (1.05 – 0.71)/(3.81 – 0.71) = 0.088 g/L malic acid

Having determined the concentration in g/L, you should use Table 1 as a rough guideline for status of MLF in your wine:

Table 1. MLF Malic Acid concentration and status

Malic Acid Concentration, g/L	**MLF status**
Above 1	Not started or just started
0.4 – 1.0	Incomplete, probably started
0.1 – 0.4	Progressing well
0.05 – 0.1	Nearly complete, probably OK
Below 0.05	Complete

Note: see Appendix B for more information on quantitative mode, standards, and calculations

Data Interpretation (Quick-check mode)

In Quick-check mode you are just looking at the result to get an idea if your MLF has started, or is nearly finished. The SC-55 kit is designed to give a readout of **2 ± 0.2 kpa** when the level of malic acid in the wine is at 0.2 g/L, where MLF is considered to be almost complete. When malic acid levels are higher than this, you should consider MLF not done. For example if you read a value of 5 kPa, you can tell that this wine sample has a malic acid concentration near 1 g/L and has not completed MLF. If the malic acid level is below 0.1 g/L, a signal around 1 kpa will be seen. At this point we recommend you verify this result by following the confirmation procedure above or repeating the analysis of the sample in Quantitative mode with a standard to confirm low malic acid levels. See Table 2 below for further details.

Table 2. Example data for wine samples with the MLF Kit. Your results may differ.

Malic Acid Concentration in wine (g/L)	**Kilopascal (kpa) Reading**
1.0	7.5 ± 0.4
0.4	3.5 ± 0.3
0.1	1.3 ± 0.3
0	0.8 ± 0.2

Note: The kpa readout values for the various Malic Acid levels are averages and can vary slightly unit to unit. Table 2 is only an example. Your results may be different.

Finishing Up:

1. Turn off the SC-55 manometer.

2. If you have unused reaction vials, place these back in the freezer as well as the 1M Malic Acid and the Malic Acid Standard Solutions.

3. Rinse all plasticware with DI water and store the unit and plastic accessories in a safe place.

4. Thoroughly rinse out all used reaction vials with DI (distilled) water two to three times and air dry with lid removed. Save all reaction vials and caps. Reaction vials and septum caps can be re-used and replaced if/when necessary.

5. NOTE: We recommend replacing the septa in the caps after three uses. For best precision in quantitation, replace the septa after each use.

Representative Data:

Analyses performed at Vinmetrica Labs on an SC-55. This table is for reference purposes only; your data may be different. The Red Wine had completed MLF; the White wine had not.

Table 3. Representative data from concentration calculations

Representative Data			
Sample	Sample response (**a**)	Standard response **(c,** for **S = 0.4**)	Zero Standard response (**b**)
White wine	9.22	---	0.80
Red Wine	1.22	---	0.80
Red Wine Pre- MLF	8.87	---	0.80
0.4 g/L malic acid		3.7	0.80

See Appendix B for more information on quantitation with standards.

Technical assistance: info@vinmetrica.com tel. 760-494-0597 x102

Ordering Refill Kits for your MLF Analyzer:

In an effort to eliminate waste the MLF Kits contain parts that are reusable.

All Reaction Vials are reusable as long as they are not chipped, broken, or contaminated. Reaction vials (SC-50-14-10) are sold separately in packs of 10 or 20.

The septum caps are reusable up to six (6) punctures, following the procedure, you may use a septum cap for up to three (3) different tests (2 punctures per test). Replacement septum caps (SC-50-9) (10 or 20 per pack) and replacement septa (SC-50-10) are also available in packs of 10 or 20.

MLF Refill Kits are sold with all the reagents, the Biopressure Agent and replacement septa. You have the option to buy the refill kits with or without the plastic scoop provided with the SC-50 MLF Kit. Refill Kits will be sold in amounts enough for 10 or 20 tests.

The user must now fill reaction vials before beginning the MLF analysis.

Protocol for preparing reaction vials:

1. Remove Biopressure Agent from freezer and allow to come to room temperature.
2. Using the small plastic spoon (PN: RS-12), measure out two (2**)** level scoops (approx. 35-40 mg) of Biopressure Agent into each reaction vial.
3. Due to the consistency of the Biopressure Agent, we recommend rinsing and completely drying the plastic spoon after filling approximately 5 vials.
4. Cap all reaction vials.
5. Keep out all reaction vials needed for MLF assay.
   a. For Quick Check Mode, you will need one (1) reaction vial per wine sample.
   b. For Quantify Mode you will need at least three (3) reaction vials, one (1) for the Malic Acid Standard, one (1) for the Zero Response and one (1) per wine sample. (See Concentration Calculation, pg 8 of the manual, for further information).
6. Place all remaining reaction vials in freezer until use.
7. Thoroughly rinse plastic measuring spoon with DI water.

NOTE: If you have been testing MLF using the SC-50 and have not kept your reaction vials, we recommend purchasing the MLF Refill Kit (in the increment you need) plus a set of reaction vials.

You will only need to replace the reagents, biopressure agent and the septum caps or septa when necessary.

For more information, please visit our website at www.vinmetrica.com or call our Tech Support, (760) 494-0597.

WARRANTIES AND LIABILITIES

1. The materials provided in the kit, as described on page 2 above, ("Materials") are warranted as follows: The SC-55 Manometer instrument and non-reagent accessories are warranted against defects in workmanship for 24 months 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-toe shoes. Dispose of unwanted material by adding baking soda to wine samples and dumping down the sink with plenty of water. Keep out of reach of children.

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Appendix A – Troubleshooting and FAQs

1) The SC-55 does not power on, or behaves erratically on the display: Check the battery and replace if necessary.
2) The instrument reading does not return to 0.0 after releasing a sample vial; or, little or no response is seen on inserting a vial into the assembly; or a response is low and steadily increasing over the course of a few seconds: Check to be sure the insertion needle is not plugged or partially obstructed.
   a) Disconnect the needle assembly as shown in Figure 2 above.
   b) Draw back the plunger of the 5 mL syringe and connect the needle assembly to the syringe end.
   c) Push the syringe plunger to force obstructing material out of the needle. Check for free flow by suspending the needle in water while pushing the plunger. A steady stream of bubbles should flow easily out the end of the needle. You can order replacement needle/assemblies from Vinmetrica (part number SC-50-15).
3) Values drift up or down when reading
   a) It is normal for values to *decrease* slowly; that is why we recommend taking the initial highest value.
   b) If values keep increasing past about 5 seconds after the insertion event, take a reading at the same time point (15-20 seconds later) for all samples.

FAQs

1. What are possible interferences in the assay?

Very high alcohol levels (above 20% ABV) may change the response of the system somewhat. The boiling step generally reduces levels far enough to limit this problem. For very high alcohol levels, you can further dilute the wine in distilled water to bring the concentration below 10% ABV.

High free SO_2 levels (>30ppm) may impact the Biopressure agent. Again, boiling will help this, but if needed you can dilute the wine sample as above.

2. What if I did not shake the vial during the 45 minute incubation period? Do I need to start over? Is this vial no longer good?

We recommend that you shake your vial at the 15 minute and 30 minute mark during incubation to ensure that the Biopressure agent gets thoroughly mixed and remains so. However, the agent stays pretty well in suspension provided it is shaken well at the beginning as in step 8. And make sure that your sample has had a good thorough shake before performing the CO_2 pressure reading step (step 16).

3. What if I let the incubation period go for longer than 45 minutes? Is my sample no longer good?

Timing in this step is not critical but needs to be as close to the 45 minute mark as possible. Accuracy of the technique may be affected by letting the reaction go for excessive periods of time.

4. What is the effect of temperature on the assay?

Temperature can have a significant effect on the assay. Whatever temperature you are using, it is important to be sure that all components of the assay start and finish at the same temperature. In particular, be very sure that your wine samples have completely cooled to room temperature after restoring their volume to 10.0 mL as directed in step 5 and 6 of the procedure. A water bath with controlled temperature is a useful option – put all your reagents, vials and restored samples in this bath for 5-10 minutes before starting the assay, and leave the reaction vials in the bath until you do the final pressure measurements. Even a simple tray with water at room temperature can help to stabilize the temperature.

The recommended temperature is standard room temperature, or about 78°F (25°C). Temperatures down to 73 °F (23 °C) are OK. At lower temperatures, the rate of the Biopressure reaction slows down, and the pressure change also is lower, just like car tires lose pressure in cold temperatures. Therefore the assay is less sensitive at lower temperatures. However, it can still be run at temperatures down to about 65 °F.

At higher temperatures, the opposite effect occurs: the reaction will go faster and generate higher values. In principle this is not bad *per se* – the assay becomes slightly more sensitive with the higher pressures generated. There is nothing wrong with using a higher temperature up to about 90°F (32°C), with two cautions: 1. the higher pressure resulting from higher temperatures may exceed the guidelines we provide here – therefore you will have to pay attention to concentration calculations; 2. most users' environments aren't set up to control higher temperatures that well, so increased variability may result.

Appendix B. More information on quantitative mode

You can run quantitative mode with various standards if you wish to get a more detailed picture of the assay response. For example, when MLF is just starting, or prior to starting, you may want to run a higher concentration standard. Even better, *multiple* standards can enhance the accuracy of the method.

Making other standards. You can make a standard of any concentration by diluting either the 0.4 g/L standard, or the 1 M Malic standard (which is 134 g/L). Two recommended standards are 1.0 g/L and 0.10 g/L.

To make a 1.0 g/L standard: Add 30 µL (0.040 mL) of the 1 M Malic acid to 4.0 mL of the 0.0 Malic Acid standard and mix well.

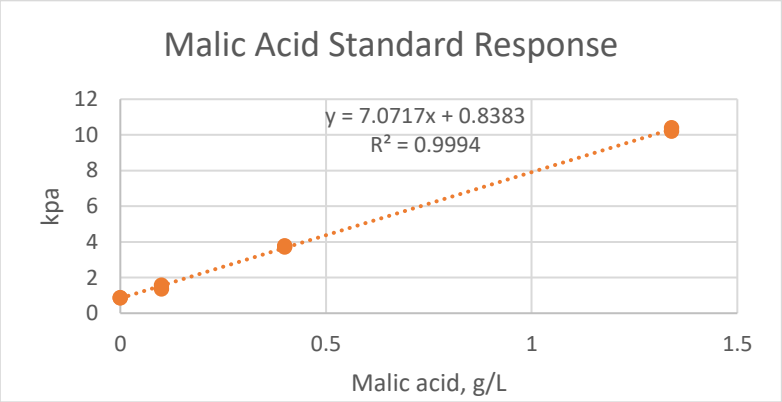
To make a 0.10 g/L Malic Acid standard: add 1 mL of the 0.40 g/L malic acid standard to 3.0 mL of the 0.00 g/L solution and mix well.

To make other standards: You can make any concentration you want using the above examples as a guide (we recommend not going above about 1.4 g/L , because you may exceed the top range of the SC-55). You can even use your own source of pure malic acid powder to prepare any standard. However, in that case be aware: 1) always use the 0.00 standard to make final dilutions; 2) malic acid is very acidic; any standard prepared from powder should be adjusted up to pH 5.0 ± 0.2 with sodium hydroxide of appropriate concentration before final dilution into the 0.00 standard.

Any concentration standard can then be used in the quantitative mode calculation (page 8, eq.1). Note the value of S needs to be the g/L level of your standard.

Quantitation via multiple standards and calibration curve. If you want, set up a calibration curve and plot the results in Excel or similar programs to get the linear fit parameters; use these to calculate your answer. The example data in the table below is plotted as x vs. y, i.e., g/L malic acid vs. kPa response. Here we have run duplicates of three malic acid standards and the 0.00 standard.

Malic Acid, g/L	kpa
0.00	0.87
0.00	0.86
0.10	1.36
0.10	1.57
0.40	3.70
0.40	3.77
1.34	10.4
1.34	10.2



A plot of the results gives a linear response: y (i.e., kpa) = 7.0717 * x (i.e., g/L) + 0.8383. So for a sample, it's g/L = (kpa-0.8383)/7.0717; e.g. if we ran a wine sample in this test and got a response of 3.43 kpa, we calculate

g/L malic acid = (3.43 – 0.8383)/7.0717 = 0.36 g/L in the test.

Now we have to multiply by 2 to account for the dilution of the wine in step 6 of the procedure:

0.36 * 2 = 0.72 g/L in the wine sample

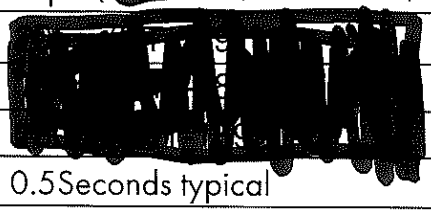
Appendix C. SC-55 Manometer: additional information

Operation

1.ON / OFF button:
to turn on or off the power
Press the ON / OFF button to turn on the power of manometer. When turned on, the screen will be displayed for about two seconds and then will display the normal measurement mode.
2.Clearance button:
When turned on, if there is no input differential pressure, instrument will not be cleared by itself. Necessarily need to be manually cleared, press the HOLD button for about 5 seconds,
The screen will show —it will be cleared later.
3.UNITS button:
intermittently press the UNITS button there will revolve various units at the bottom of the screen. These units are as follows: bar, mmHg, Ozin2, Kgcm2, psi, inH2O, Kpa, ftH2O, inHg, cmH2O, mbar.
4.DIF button:
the pressure value when pressing the DIF button subtracting the pressure value before pressing.
5.HOLD button:
pressing the HOLD button will keep the current measured result. Pressing once more will lift the hold function.
6.REC button:
this button is on the side of the instrument, intermittently press this button will display the maximum, minimum, average result in process of recording measuring. Meanwhile the other function buttons are locked and can not operated. Long pressing the REC button for about 5 seconds and it will quit recording mode.

7.Backlit button:
pressing the backlight button and the backlight will turn on. In dark environment can use this function. Press the backlight button again to turn off the backlight.
8.Automatic power-off function:
it will automatically power off if instrument not work for more than 25 minutes. This can extend the battery life. If do not want to turn off the power, press hold button first , then press the ON / OFF button to start.
LCD will show "ON" to close the function of automatic power-off.

Specifications

Display	Dual LCD
Accuracy	±0.3%FSO(25°C)
Repeatability	±0.2%(Max+/-0.5%FSO)
Linearity/Hysteresis	±0.29% FSO
Maximum Pressure	10psi ([redacted] 
Response Time	0.5Seconds typical
Low Battery Indicator	Yes
Over Range Indicator	Err.1
UnderRange Indicator	Err.2
Operating Conditions	32°F to 122°F(0 to 50°C)
Storage Conditions	14°F to 140F°(-1 to 60°C)
Power Supply	1X 9V Battery (included) or External 9VDC

Error Codes

An error message will appear on the display if the meter fails an internal diagnostic test.

1. Err. 1: Pressure value is over the range.
2. Err. 2: Pressure value is below the range.

Battery Replacement

When the battery power falls low, "**BAT**" will appear on the LCD. Replace the 9V battery.

Technical assistance: info@vinmetrica.com tel. 760-494-0597