PROTOCOL

Free Fatty Acid Test Kit

Cat. No. 07KTFA2000
Revision Date: 2020-01

1 Prepare Reagents

1. Remove Free Fatty Acid Reagents A, B, Calibrators, Control and Preparation Reagent from the refrigerator and allow them to reach room temperature (18–25°C). This should take approximately 2 hours.
2. Power **ON** the SafTest™ analyzer by pressing the **[I]** marked portion of the power switch, which is located on the rear panel of the SafTest™ analyzer. Allow 5 minutes to warm up.
3. Preheat the heat block (12.75 mm) to 37–44°C. Use temperature setting **LOW** and dial #4. This is an approximate setting, check with thermometer to verify. Adjust dial (0–10) to correct for the appropriate temperature.

2 Set up the Calibration Curve

NOTE: *When you receive a new kit you must first run a calibration curve*

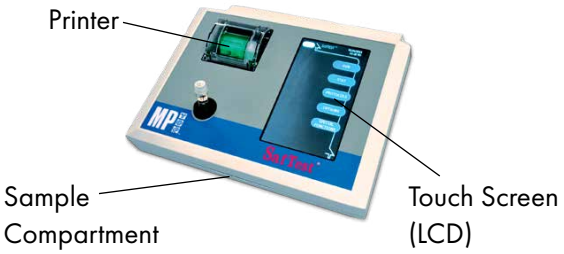
1. Label 5 new 12 mm glass test tubes to correspond to the Calibrator 1, 2, 3, 4 and 5; and one new 12 mm tube for the Control.
2. Mix amber bottles containing the Calibrators and Control to ensure you have a homogenous solution.
3. Use a positive displacement pipette to transfer 100 µL of the Calibrators into the corresponding labeled test tube.
4. Use a positive displacement pipette to transfer 100 µL of the Control into the corresponding labeled test tube.

NOTE: *Use a new tip for each Calibrator and Control. The same tip can be used if running a duplicate. Must remove all air bubbles from tip before drawing final sample*

3 Add Reagents & Vortex

1. Before dispensing reagents, gently swirl the contents of each reagent bottle.
2. To eliminate air bubbles and oxidized reagent, dispense 4–5 aliquots of each reagent into a waste container immediately before use.
3. Dispense 1 aliquot of Free Fatty Acid Reagent A into every calibrator and control test tube.
4. Dispense 1 aliquot of Free Fatty Acid Reagent B into every calibrator and control test tube.
5. Once you have aliquoted Free Fatty Acid Reagent B into the last test tube start the timer for 10 minutes.
6. Cap the test tubes and vortex them at the fastest dial setting for 30 seconds.
7. Place the test tubes in the heat block at 37–44°C for the remaining time.

NOTE: *Once time has ended, IMMEDIATELY begin reading your samples. If samples are not read immediately, the tubes must be discarded and test should be rerun*



4 SafTest™ Analyzer Set Up with Calibration [RUN]

1. Press **[RUN]** icon on the touch screen.
2. Press **[ACIMSA / FFA]** icon; when highlighted yellow, press **[NEXT]** icon.

3. At the "Blank Tube" prompt, insert a test tube filled half way with distilled water in the sample compartment. Do not remove tube until prompted or after beep.
4. At the "Cal #_ of 5" prompts, insert Calibrators 1–5 in the sample compartment.

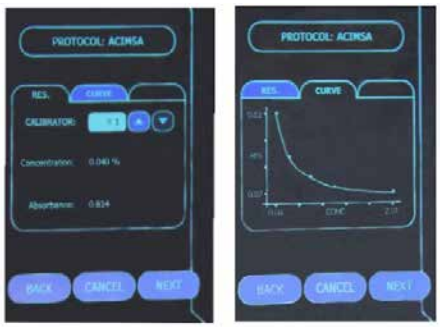


Figure 1.
ACIMSA / FFA
calibration screen shots

5. At the "DO YOU WANT TO STORE THE CALIBRATORS"prompt, select **YES** (Figure 2).

Figure 2.
ACIMSA / FFA store
calibrators screen shot

6. Select the number of replicates to be analyzed (1–5). Use the "+" or "- "arrows to select a number (normally duplicates, i.e. 2 samples should be analyzed to ensure results are accurate and consistent).
7. Press the **[NEXT]** icon.
8. At the "Smpl #_ Rep #_, Insert Tube" prompt, insert the Control tube into the sample compartment.
9. SafTest™ analyzer will continue asking for samples until the user quits the program by pressing **[STOP]** and then **[DONE]**.

NOTE: *The SafTest Analyzer arbitrarily numbers each sample 1–99. Be sure to re-label the sample you are running*

10. Verify that the control falls within the Free Fatty Acid Control range which can be found on the Free Fatty Acid Control Package Insert. Your test value for the Control should approximate this ranges. If it does not, rerun the test.

5 Prepare Samples

1. Samples should be prepared according to **Sample Preparation Quick Card**.
2. Label one new 12 mm glass test tube per sample or two new glass test tubes if preparing duplicates.
3. Read each test tube twice when prompted for a duplicate. When analyzing duplicate samples, ensure each test tube is read a total of two times. A sample, when run in duplicate, should have four total readings (two each for each test tube).
4. Use a positive displacement pipette to transfer 100 µL of each prepared sample into the corresponding labeled test tube.
5. Dispense 1 aliquot of Free Fatty Acid Reagent A into every sample test tube.
6. Dispense 1 aliquot of Free Fatty Acid Reagent B into every sample test tube.
7. Once you have aliquoted Free Fatty Acid Reagent B into the last test tube start the timer for 10 minutes.
8. Cap the test tubes and vortex them at the fastest dial setting for 30 seconds.
9. Place the test tubes in the heat block at 37–44°C for the remaining time.

6 Analyze Samples

1. Press **[STAT]** icon (Figure 3).

Figure 3.
Home Screen Shot

2. Press **[ACIMSA / FFA]** icon, when highlighted yellow press **[NEXT]** icon.
3. At the "Blank Tube" prompt, insert a test tube of distilled water in the sample compartment.
4. Select the number of replicates to be analyzed. Use the "+" or "-" arrows to select a number (Normally two replicates should be analyzed to ensure that results are consistent).
5. Press **[NEXT]** icon.
6. At the "Smpl #_ Rep #_, Insert Tube" prompt, insert control or sample tubes in the sample compartment.
7. SafTest™ analyzer will continue asking for samples until the user quits the program by pressing **[STOP]** and then **[DONE]**.

7 Calculate Your Results

1. The SafTest™ analyzer will use the Calibrators to calculate the acid content as percent (%) oleic acid in the sample.
2. Adjust instrument results by taking into account the dilution factor. For example:

Dilution Factor	SafTest™ Results	Dilution x Results	Final Results
1:4	0.19%	4 x 0.19%	0.76%

3. The final result can be calculated to % oleic acid on a fat basis by taking the percent fat into consideration when performing your calculation. For example, with a sample that is 15% fat and yields a final result of 0.76%:

Final Result	Result/(%Fat/100)	Final Result
0.76%	0.76/(15/100)	5.07%

8 Storing Equipment

1. At the end of the day, store the Calibrators, Control, and reagent bottles (with dispensers attached) at 2–8°C.

2. To maximize lamp life, turn **OFF** the SafTest™ analyzer when not in use. Power **OFF** the SafTest™ analyzer by pressing the **[O]** marked portion of the power switch, which is located on the rear panel of the SafTest™ analyzer.

Troubleshooting

If the sample value is greater than the value of the highest calibrator, the instrument will flag the results as "**HI**." The sample must be prepared at a higher dilution and retested; see **Additional Dilutions QuickCard**.

If the sample value is less than the value of the lowest calibrator, the instrument will flag the results as "**LO**". Values that are flagged "**LO**" should be reported as "< value of the lowest calibrator."

Check the instrument printout for flags or error messages before reporting results. The coefficient of variation (%CV) should be less than 10%. Higher variations will be flagged, in which case you should repeat the test.

EXCEPTION: A large %CV is expected with samples that are measured at the low end of the calibration curve. For such measurements, do not repeat the test.

The range for the Control is found on the Free Fatty Acid Control Package Insert. Your test value for the Control should approximate this range. If they do not, rerun the test.

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