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# Total Glycerin™ Kit Testing Protocol

**Overview:** The Total Glycerin™ kit measures glycerin in oil samples.

## **Sample Preparation:**

Take 500ul of the sample and dispense into a 15 ml conical. Add 4.5ml of Standard Prep Reagent. This makes a 1:10 initial dilution. Vortex the sample for 1 minute and warm in heat block at 40° C for 10 minutes. Vortex the sample for another 15 seconds and place the sample back in the heat block. The test will be performed using the 1:10 dilution. Additional dilutions may be needed later. Keep samples in the heat block until all the tests are completed.

## **Test Preparation:**

Label a set of glass 10mm test tubes: RB for the Reagent Blank, C1 through C5 for the three Calibrators; M for Control; and 1, 2, 3, etc. for the samples. NOTE: If running duplicates label 2 tubes.

## **Pipetting Calibrators, Control(s) and Samples:**

Using a positive displacement pipette, transfer 25µl of Reagent Blank, 25µl of each calibrator, 25µl of one or more controls. Wipe the pipette tip prior to dispensing the reagent blank, calibrators, control(s), and samples, and calibrators. Use a new tip for each sample, control, reagent blank, and calibrators. Vortex each sample for 10 seconds and add 25µl of each sample into the designated 10mm test tubes. If running STAT curves just run one control and samples. Run at least one control with each assay. The control will ensure that the assay is being performed correctly.

## **Adding Detectors:**

Dispense out 2-3 aliquots of Reagent A. from the bottle into a waste container. This will ensure that no air bubbles are present in the nozzles. Dispense one aliquot each of Reagent A into each of the reagent blank, calibrators, control(s), and sample tubes. Start the timer for 10 minutes. Cap the tubes and invert 4 times (do not vortex). Place the tubes in the heat block for the remaining 10 minutes.

## **Reading Calibrators and Controls:**

Turn on the SafTest™ Analyzer. Check the 550/690 filter is in place Put in tube adapter.

Begin reading test by choosing RUN or STAT and selecting “TOTGLY”. At the ‘Blank Tube’ prompt, thoroughly wipe then insert a tube containing distilled water. Calibrate the instrument by wiping then inserting reagent blank and then calibrators 1 through 5 as prompted by the SafTest™ Analyzer. If using Stat mode, always check new test by including a control. NOTE: Prior to insertion in the SafTest™ Analyzer, wipe each test tube with a lint-free tissue.

## **Successful Calibration:**

Slope: The slope should be consistent when performing this test if the time and temperature are kept consistent. It will generally be between 2.2-2.6. Calibration must be performed whenever a new kit is opened or whenever there is a problem such as a control is out of established ranges. Correlation must be > 0.99 for test to be acceptable and if it is not rerun test.

## **Reading Samples:**

Following successful calibration, wipe then insert the controls making sure the values of the controls fall within the ranges provided for that lot of controls. If the control is outside the reported or established range or the control exhibits CV's >20%, rerun the assay. If the control(s) value(s) falls within the ranges, wipe then insert the sample tubes and analyze in the SafTest™ Analyzer in the designated order. If duplicate sample CV's are greater than 10% repeat test on sample.

## **Reporting Results:**

The SafTest™ Analyzer will use the calibrators to calculate the total glycerin. These results must be multiplied by the sample dilution.

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 diluted to a higher dilution and retested. If the sample value is less than the value of the lowest calibrator, the instrument will flag the results as ‘LO’.

## **Completing Testing:**

At the end of the day, store calibrators, controls, and reagent bottles with dispensers attached in the refrigerator (2-6°C).