



TOTAL LIPID REAGENT SET (COLORIMETRIC METHOD)

INTENDED USE

For the quantitative determination of the total lipid index in serum using the sulfo-phospho-vanillin colorimetric method.

INTRODUCTION

In blood, at least 95% of the lipids exist in combination with protein. These lipoproteins can be quantitated by disrupting this complex. This test is used as a screening method for hyperlipidemia.¹ The sulfo-phospho-vanillin (SPV) method for the colorimetric determination of the serum total lipids was described by Charbrol et al² and modified by several investigators^{3,4} by omitting phosphoric acid, shortening reaction time, and increasing reagent stability. Our reagent is based on all of these modifications.

PRINCIPLE

Lipids react with sulfuric acid to form carbonium ions, which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically.

REAGENT COMPOSITION

1. *Total lipid color reagent*: vanillin, potassium monobasic phosphate, preservative.
2. *Total lipid standard* (600 mg/dl): potassium oleate equivalent to 600 mg /dl of total lipids when run according to the instructions with this procedure.

WARNINGS AND PRECAUTIONS:

Exercise the normal precautions required for handling of all laboratory reagents. Pipetting by mouth is not recommended for any laboratory reagent. Concentrated sulfuric acid causes severe burns. Exercise extreme care in handling this chemical.

STORAGE AND STABILITY

1. Store all reagents at room temperature.
2. All reagents are stable until the expiration date indicated on the individual bottle.

REAGENT DETERIORATION

1. *Physical appearance*
Slight brown color appearing in the color reagent, appearance of turbidity or crystals that will not readily dissolve are signs of reagent deterioration.
2. *Control assays*
Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

SPECIMEN COLLECTION

1. Collect whole blood by venipuncture and allow clotting. Centrifuge and remove serum. Samples should be collected with patient fasting for 12 hours.
2. Serum may be kept at room temperature for four hours and at refrigerator temperature (2 - 8°C) for 48 hours. Serum should not be frozen.
3. Hemolysis must be avoided.

MATERIALS REQUIRED BUT NOT PROVIDED

Concentrated sulfuric acid, sample and reagent, pipettes, test vials or cuvettes, timer, 100°C heating bath. control serum, spectrophotometer.

**Note: Concentrated sulfuric acid must be ACS, Reagent Grade that is non-yellowed.*

PROCEDURE (MANUAL)

1. Label test tubes: blank, standard, control, patient, etc.
2. Transfer 10 ul (0.010 ml) of sample to its respective vial, discharging sample into bottom of vial.
3. Carefully transfer 1.0 ml of concentrated sulfuric acid to each vial and mix thoroughly so that the entire sample is dissolved.
4. Place all vials in 100°C heating bath for 20 minutes.
5. Remove all vials and place all vials in cold water for 3-5 minutes
6. Add 2.0 ml of total lipid color reagent down the side of each vial, mix by swirling, and place in cold-water bath for 15 minutes. (NOTE: Mixing concentrated sulfuric acid with an aqueous solution generates a large amount of heat, therefore exercise appropriate care.)
7. Set wavelength of spectrophotometer at 530nm and zero the instrument with the blank. Read and record the absorbance of all vials. (Wavelength range: 500-550nm).

PROCEDURE NOTES

1. The Final color is stable for at least 60 minutes.
2. Samples with values above 1,300 mg /dl should be diluted 1:1 with saline, re-assayed, and the results multiplied by two.
3. Hemolyzed serum samples must be avoided, as they will cause falsely elevated results.
4. Thorough mixing is crucial in this method because of the viscosity of the concentrated sulfuric acid employed in the reaction.

PROCEDURE LIMITATIONS

Heptane, ethyl benzene, tetrahydrofuran, detergents and soaps are known to interfere with the sulfo-phospho-vanillin method. Alcohols with a carbon chain greater than C2 will interfere. The reaction is specific only for unsaturated compounds. Saturated fatty acids do not react. For these reasons, Knight et al⁵ have suggested that it would be more appropriate to refer to the results as an "index of total lipids" when the method is utilized.

CALCULATIONS

Use the absorbance readings of the STANDARD and UNKNOWN(S) to calculate total lipid values as follows:

A = Absorbance

$$\frac{A(\text{UNKNOWN}) \times \text{Conc. of STD}}{A(\text{STANDARD})} = \frac{\text{Total Lipid in UNKNOWN}}{\text{(mg/dl)}}$$

Example:

$$A(\text{patient}) = 0.45$$

$$A(\text{standard}) = 0.50$$

$$\text{Concentration of standard} = 600 \text{ mg /dl}$$

$$\frac{0.45}{0.50} \times 600 \text{ mg/dl} = 540 \text{ mg/dl}$$

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established total lipid values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES⁶

400 - 800 mg/dl

PERFORMANCE CHARACTERISTICS

1. **Linearity:** 1300 mg/dl
2. **Sensitivity:** Based on an instrument of A = 0.001, this procedure has a sensitivity of 3 mg /dl.
3. **Comparison Study:** A study performed between this procedure and an identical procedure resulted in a coefficient of correlation of 0.98 with a regression equation of $y = 1.0x - 2.76$.

	With in Run		
	Mean (mg./dl)	S.D.	C.V. (%)
Normal	650	27	4.1%
Abnormal	1130	60	5.3

Run to Run

	Mean (mg/dl)	S.D.	C.V. (%)
Normal	634	38	5.9%
Abnormal	1138	74	6.5%

REFERENCES

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