

ANALYTICAL APPROACH IN LIPIDS ABNORMALITIES

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LABORATORY RISK FACTORS

- **Common Lipid Markers** Including TC, HDL-C, LDL-C & TG
- **Uncommon Lipid Markers** Including Lp(a), beta-VLDL, Apo A-I & Apo B-100

ATP III *(Adult Treatment Panel III)*

CLASSIFICATION IN ADULTS

LDL Cholesterol

- <100 *Optimal*
- 100-129 *Near Optimal*
- 130-159 *Borderline high*
- 160-189 *High*
- ≥ 190 *Very high*

HDL Cholesterol

- <40 *Low*
- ≥ 60 *High*

Total Cholesterol

- <200 *Desirable*
- 200-239 *Borderline high*
- ≥ 240 *High*

Triglycerides

- <150 *Normal*
- 150-199 *Borderline high*
- 200-499 *High*
- ≥ 500 *Very high*

NCEP *(National Cholesterol Education Program)*

CLASSIFICATION IN CHILDREN AND ADOLESCENTS

LDL Cholesterol

- <110 *Desirable*
- $110-120$ *Borderline*
- ≥ 130 *High*

Total Cholesterol

- <170 *Desirable*
- $170-199$ *Borderline*
- ≥ 200 *High*

FACTORS AFFECTING LIPID & LIPOPROTEIN DETERMINATION

- Factors Related to Patients
- Factors Related to Specimen
- Factors Due to Analysis

FACTORS RELATED TO PATIENTS

- **Biological variation**
 - 1) **Physiological variation**
 - 2) **Age**
 - 3) **Sex**
 - 4) **Season**
 - 5) **Diet**
 - 6) **Fasting**
 - 7) **Lifestyle**
- **Disease**
- **Drugs**

Inter-individual & Intra-individual Biological Variations

Analyte	Inter-Individual CV%	Intra-Individual CV%	Method CV%
Triglyceride	56.8	28.8	4.7
Cholesterol	22.3	8.2	2.3
HDL-C	28.3	12.4	2.5
Apo B	27.6	9.5	2.7

Physiological Variation

- **The NCEP guidelines recommended averaging at least two successive measurements to reduce the effects of both preanalytic and analytic sources**

Age

- **Cholesterol concentration increases by aging in both sex with beginning from adulthood**

Sex

- **After childhood and up to age 50, cholesterol concentration is lower in women than men**

Season

- **Cholesterol concentration is slightly more in winter**

Recent Feeding

- Chylomicron uptake requires 6-9h
- So , 9 hours fasting is sufficient, but usually 12 hours fasting is recommended
- Fasting is not necessary for TC and HDL-C

Diet

- **Saturated Fats and cholesterol result in considerable increase in cholesterol concentration**
- **Diet affecting on lipid profile requires several weeks**
- **So, patient should have a usual diet for 2 weeks and no weight change**

Diseases

- Patient should not have MI or shock during previous month
- Fever, trauma, surgery increase TG and lower TC and HDL-C
- Weight loss results in decreasing TG and transient increasing TC and LDL-C
- Chronic disease results in severe decrease in LDL-C and HDL-C
- Diabetes, Thyroid disease, liver disease, and renal disease result in secondary dyslipidemias

Drugs

- Different drugs affect on patient lipid profile, e.g. **OCP** increases VLDL and **anabolic steroids** increase VLDL and decrease HDL
- Abnormal results should be repeat 2-4 weeks later

FACTORS RELATED TO SPECIMEN

- Posture during sample collection
- Prolonged venous occlusion
- Venous vs capillary samples
- Plasma vs serum
- Storage

NCEP Guidelines for Acceptable Measurement Error

Analyte	Bias	CV	Total Error
Triglyceride	$\leq 5\%$	$\leq 5\%$	$\leq 15\%$
Cholesterol	$\leq 3\%$	$\leq 3\%$	$\leq 9\%$
HDL-C	$\leq 5\%$	$\leq 4\%$	$\leq 13\%$
LDL-C	$\leq 4\%$	$\leq 4\%$	$\leq 12\%$

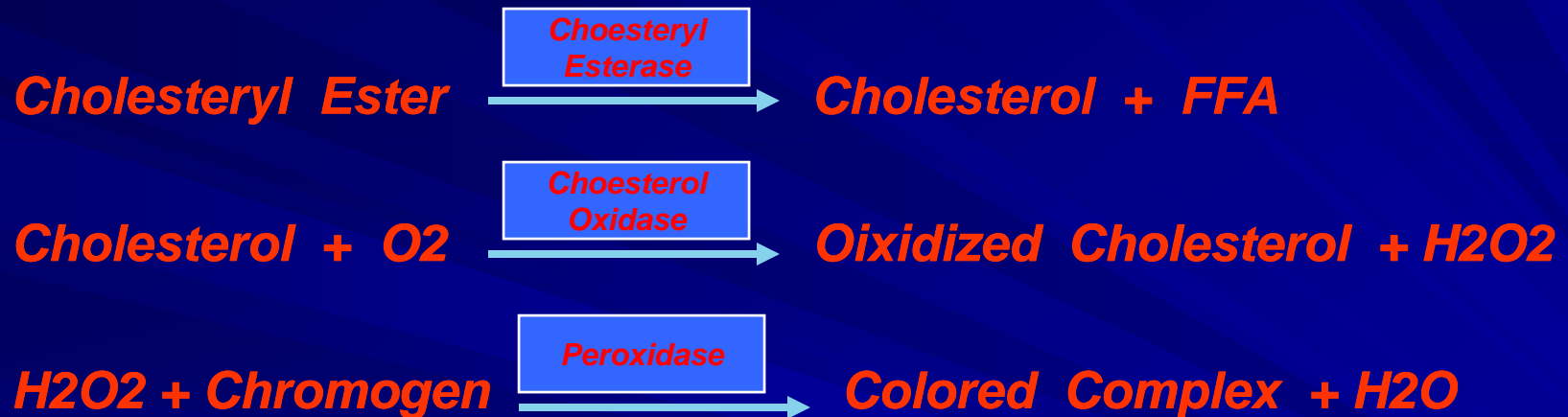
CHOLESTEROL DETERMINATION

Chemical

- *Liberman-Burchard*
- *Schoenheimer-Sperry*
- *Abell-Kendal*

CHOLESTEROL DETERMINATION

■ Enzymatic



CHOLESTEROL DETERMINATION

Sample

- *Fasting Is Not Necessary*
- *Effect of Posture & Venous Stasis*
- *Increase during pregnancy*
- *Interference due to sitosterol*
- *Is not affected by activity, alcohol, and OCP*
- *Stable for 4 d, 3 m, and Some years at 4°C, -20°C and -70°C Respectively*

TRIGLYCERIDE DETERMINATION

Chemical

- *Extraction*
- *Hydrolysis*
- *Glycerol Determination*

TRIGLYCEROIDE DETERMINATION

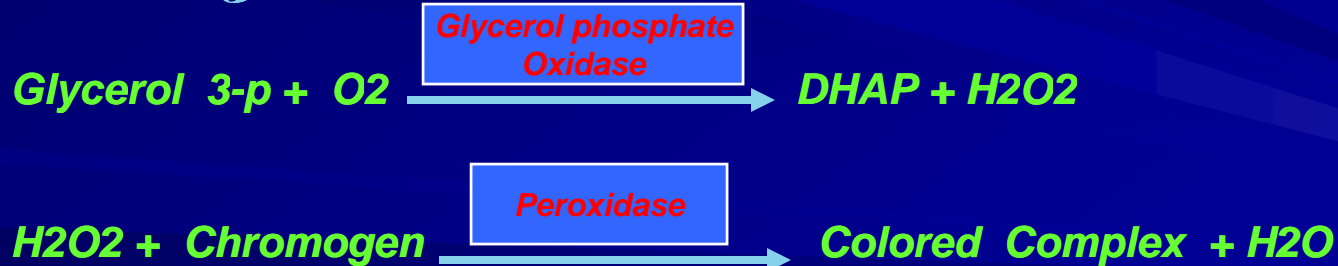
■ Enzymatic



UV Spectrophotometry



Colorimetry



TRIGLYCERIDE DETERMINATION

Glycerol Interference

- *Severe Activity*
- *Some uncontrolled diabetics*
- *Very high TG concentration*
- *Long storage*
- *Hyperglycerolemia*
- *Medication*
- *Contamination*

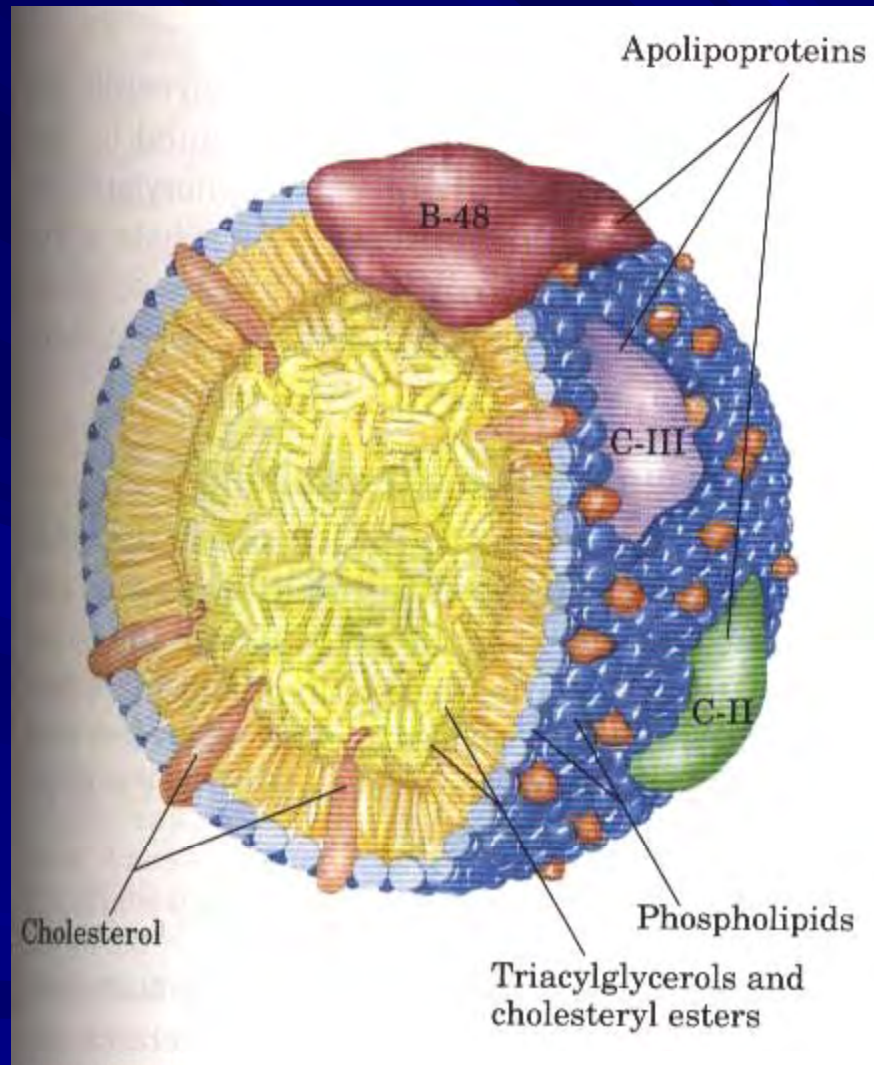
TRIGLYCERIDE DETERMINATION

Specimen

- *Fasting Is Necessary*
- *Affected by Posture, Venous Stasis, Activity, alcohol, OCP*
- *Increases during pregnancy*
- *Oxidants & Reductants*
- *Testing in the Same Day*
- *If Necessary, Storage at 4°C for a few days, -20°C for 3 m and -70°C for Years*

LIPOPROTEIN ANALYSIS

- *Ultracentrifuge*
- *Electrophoresis*
- *Serum Appearance*
- *Precipitation Methods*
- *Calculation*
- *Apolipoprotein Determination*

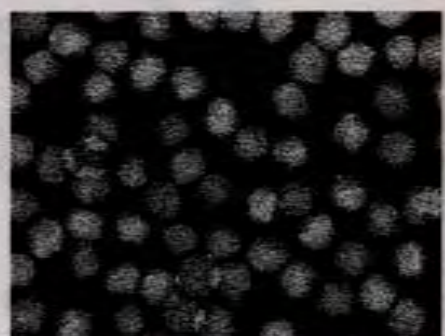
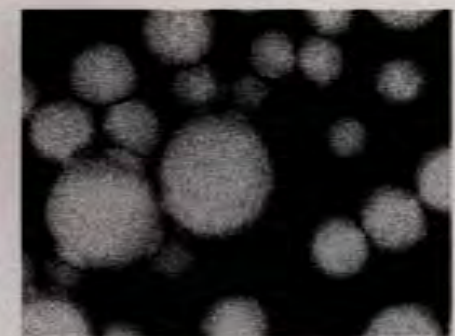
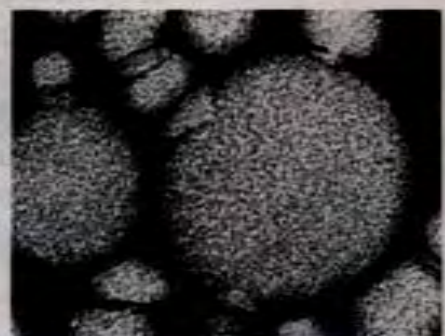
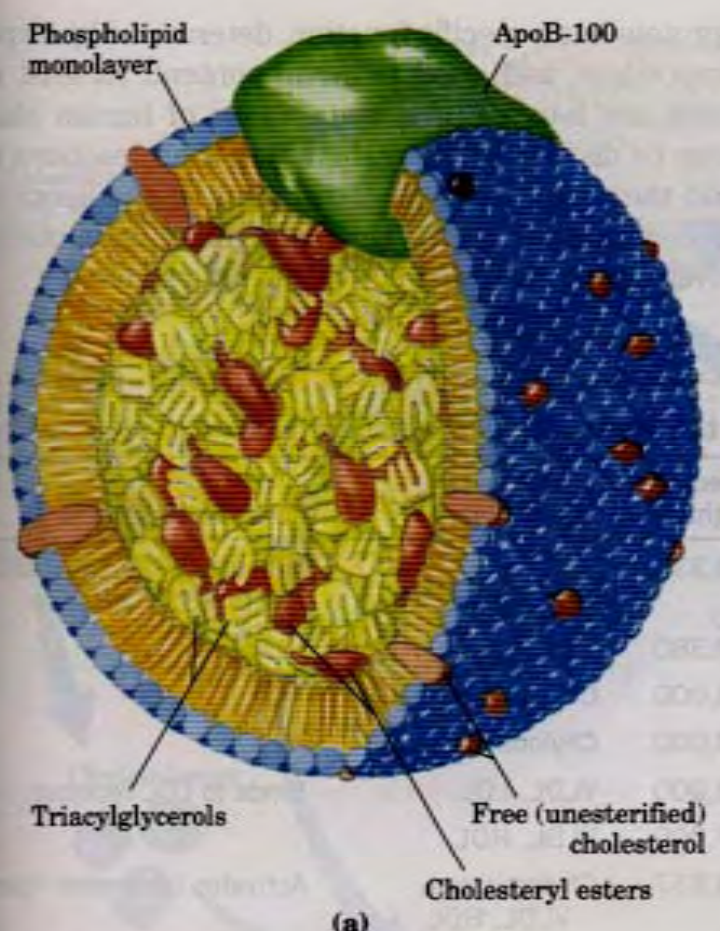


MAJOR LIPOPROTEINS

- *CHYLOMICRON*
- *VERY LOW DENSITY LIPOPROTEINS (VLDL)*
- *LOW DENSITY LIPOPROTEINS (LDL)*
- *HIGH DENSITY LIPOPROTEINS (HDL)*

MAJOR LIPOPROTEINS

LIPOPROTEINS	DENSITY (g/ml)	DIAMETER (nm)	ELECTROPHORESIS	PROTEIN (%)	TRIGLYCERIDE (%)	CHOLESTEROL (%)	PHOSPHOLIPID (%)
<i>Chylomicron</i>	<i><0.950</i>	<i>75-1200</i>	<i>Origin</i>	<i>1-2</i>	<i>86</i>	<i>4</i>	<i>8</i>
<i>VLDL</i>	<i>0.950-1.006</i>	<i>25-75</i>	<i>Pre-β</i>	<i>10</i>	<i>50</i>	<i>20</i>	<i>20</i>
<i>LDL</i>	<i>1.019-1.063</i>	<i>20-25</i>	<i>β</i>	<i>20</i>	<i>11</i>	<i>46</i>	<i>22</i>
<i>HDL</i>	<i>1.063-1.210</i>	<i>7.5-20</i>	<i>α</i>	<i>50</i>	<i>3</i>	<i>27</i>	<i>30</i>



(b)

MINOR LIPOPROTEINS

- *Chylomicron Remnants*
- *VLDL Remnants (IDL)*
- **Beta-VLDL (Floating β Lipoprotein)**
- **lipoprotein (a) or Lp(a)**

SERUM APPEARANCE

- Increased Chylomcron → *Turbidity, Creamy Layer*
- Increased VLDL → *Turbidity*
- Increased LDL → *Clear*
- Increased HDL → *Clear*

HDL-C DETERMINATION

- Precipitation of Apo B Containing Lipoproteins (VLDL, IDL, LDL) by
- Polyanions & Bivalent Cations
 - Heparine Sulfate & Mn^{2+}**
 - Dextran Sulfate & Mg^{2+}**
 - Sodium Tungstate & Mg^{2+}**

LDL-C DETERMINATION

- Ultracentrifugation
- Immunochemical
- Calculation with *Friedwald Equation*

$$\text{Total-C} = \text{HDL-C} + \text{LDL-C} + \text{VLDL-C}$$

$$\text{LDL-C} = \text{Total-C} - \left(\text{HDL-C} + \frac{\text{TG}}{5} \right)$$

Friedewald Equation Assumptions

- *All plasma TGs are carried in VLDL*
- *TG/Chol ratio of VLDL is invariant*

Friedewald Equation Limitations

- *Factor that gives the best estimate of VLDL-C varies among populations and depends on the triglyceride method used*
- *There must be no chylomicron, chylomicron remnant, IDL, β -VLDL and Lp(a)*

APOLIPOPROTEINS DETERMINATION

- *Apo AI*
- *Apo B*
- *Lp(a)*

