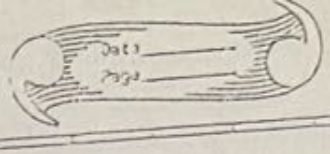
Lipid



* Reference Range CHO :-

→ Higher in male than female
But after menopause reverse

* → Ref. Range for TG (mg/dl) :-

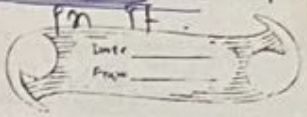
→ measurement
→ result can not given in SI unit.
B/C it is not homogenous
(due to its structure diff in all molecule)

→ Apo A1 & Apo B100 } can be measured.
Lipoprotein (a)

* C/S :-

① defi. in LPL activity :- Type 1 :- familial LPL defi
TG level upto 10,000 mg/dl
② → conc. of VLDL → ↑
apo CII → ↑.
→ expressed in childhood.
→ abdominal pain & ↑ pancreatitis.
Repeated attack
→ Eruptive xanthoma } > 2000 mg/dl
→ lipemia retinalis
↳ milky discolouration of retinal vessels.
due to visualization
↑ level of chylomicron through vessel

Yellow colour of fat due to carotenoid tint in it.



→ do not predispose to atherosclerotic dse

→ Heparinized plasma :

↓

plasma taken into heparinized tube.

→ Postheparinized plasma :

↓

1st heparin injection given to pt

↓

After sometime Blood is taken

↓

due to release of LPL from the surface of endothelium

(LPL is anchored by heparan sulfate
is replaced by heparin

↓

releases the LPL.

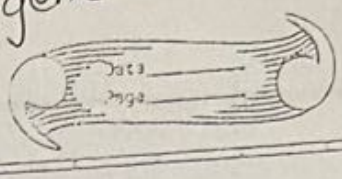
② deficiency of C-II :

→ activator of LPL
→ similar to LPL def.
→ Mildes → B'cz no complete def.
→ only pancreatitis
→ No lipemia Retinalis
→ No eruptive xanthomas
Asis: → (N) LPL activity restored

↓

if C-II is added in postheparinized plasma,

AD codominant :→ 2 diff. dominant gene.



(2) Immunoassay.

↓

- mutated apo-CII may be immunoreactive
- & may be detected.

→ AR

(3) Dysbetalipoproteinaemia :- Type 3

→ defect in remanant removal of LDL & VLDL

→ also k/a "Broad Beta dyslipidemia"

→ defect in apo E. → remanant receptor. → prevent removal from Blood stream.

→ LDL receptor → Binds both apo E as well as apo B100.

density

- Chylo < 0.95] d/t.
- VLDL < 1.006] VLDL
- LDL < 1.019] LDL
- HDL : 1.063 >

c/c:

→ palmar xanthoma : deposition of fat crease on palm

→ Premature atherosclerosis

→ Homozygous E_2 → ob (h) form

AD :- when ab(n) to express require 1 ab(n) allele.
AR : when ab(n) require both ab(n) allele to (phenotypical) phenotypical expression

* In electrophoresis :-
α Band → HDL
pre β → VLDL } some space in Beta & preβ
Beta → LDL } between.

→ **Broad β d'se** :
↓
Beta & pre β → ~~combines~~ continuous Band due to IDL traverse in between.

⇒ Q.

(1) **Familial Hyperchlolesterolemia** : (Type IIa)

→ defect in LDL receptor gene.
↓
- Reduced LDL Binding
- Bind (n) But internalizaⁿ defect → defect in ARH.

→ AD → Heterozygous → 1 in 500 → 500mg/dl
→ AD → Homozygous → 1 in million → 1gm/dl

→ ~~Pcsk9~~ :
PCSK-9 : requir for secretion of cholesterol in ~~to~~ Bile
→ ↑ the LDL-R degradation
→ its enzyme - Protease
→ mutation → lead to gain of function
↓
- So more downregulation of LDL-re
↓
BCz of ↑ degradaⁿ of LDL Receptor

proprotein convertase subtilisin / kexin 9

→ ARH : Adaptor like protein
↓
requires for proper recycling of LDL receptor.

Important for internalization of LDL receptor.

⑤ Familial defective Apolipoprotein B 100 :-
↓
→ mutation of B100
→ ~~subst~~ arginine replaced By glutamin
↓
⇒ ↓ +ve charge
↓
So ↓ LDL receptor Binding of B100

⑥ Hypoalphalipoproteinemia :-
→ mutation apo A1 , apo C-III
→ ↑ incidence of CHD

Tangier d'se :-
↓
- problem in catabolism
- mutation ABCA1 gene
↓
key protein for efflux of cholesterol particulary from macrophage.

→ HDL synthesized from plasma.
↓
apo A 1 from intestine
+
phospholipid complex → kidney
↓
Nascent HDL
↓
From peripheral cholesterol
↓
LCAT
↓
CE
↓
HDL-2

C/F:

* → mutation in gene LCAT → so it will filtered through kidney.
↓
Hypocholesterolipoproteinemia

* LP-X:
→ Int in obstructive jaundice
→ True marker of cholestasis
→ does not discriminate b/w intra & extra hepatic cholestasis.
→ mainly it is a phospholipid bilayer or multilayer structure, vesicle & an aqeous core.
→ mainly not in LCAT deficiency.

multilayer vesicle ⊂ aqueous core

↓

due to insufficient cholesterol esters ⊆ fills core.

* Measurement of lipid, lipoprotein, apolipoprotein:

alkalin → Hydrolysis of ester Bond

↓

CHE ⟶ CH.

→ Lipoprotein : macromolecular complex ⊂ variable composition, size & function

↓

difficult to measure

→ TG : compound made up of glycerol & diff. fatty Acyl group

↓

variable in chain length & degree of saturation

→ CHO : Homogenous molecule.

→ LDL : Consist of 7 subparticles with variable size & composition of lipid

↓

No unique mol. wt.

→ HDL : Heterogenous consist of 12 subclass

designated method : quality 1000s than Ref. method
→ more readily available

→ - 2 major subclass

HDL_2 HDL_3

↓

stronges inverse
ass. of CHD.

Serum → VLDL & chylomicrone removed.

USF → Cholest 200 → β-fraction ↓ LDL + IDL + Lp(a)

→ 15

Mg-prep → chole 45

* Measurement of cholesterol :—

① Enzymatic method :

Cholesteryl esters $\xrightarrow[\text{hydrolase}]{\text{cholesterol}}$ cholesterol + FA

Cholesterol + O_2 $\xrightarrow[\text{oxidase}]{\text{cholesterol}}$ cholest-4-en-3-one + H_2O_2

H_2O_2 + phenol + 4-Aminoantipyrin $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $2H_2O$.

↓

measured at 500 nm.

→ Interference :

- Bilirubin
- Ascorbic acid
- Hb.

} sub. to oxidn c H_2O_2

→ Bilirubin — Interference can be removed By using bilirubin oxidase in reagent → Produce colourless product

→ Hemolysis — dual wavelength reading (Removed)

→ Bilirubin ≥ 5 mg/dl → causes interference

→ plant sterol → very low amount in serum.

→ Use of calibration material with value traceable to CDC ref. method

↓

helps to reduce interlab. variation

→ measurement system for CHO :
indudes — Reagent + calibrator + Instrument from single manfacture

↓

- Results accurate in 1 to 3% of Ref. value
- CV < 2.5 %

→ Free / unesterified CHO can be quantified By removing CHO esterase from reagent.

(2) Reference Method :—

→ given By CDC (Centre for dise control & prevention)

→ 0.5 ml aliquot of serum

+

5 ml alcoholic KOH (KOH dissolved in alcohol)

↓

Hydrolysis of chol. esters

↓

Extraction of total cholesterol from the mixture in 10 ml of hexan for 15 min

↓

Extract is dried in vacumm.

↓

Dry residue treated in 2.2 ml of mixture of ~~acetic acid + acetic anhyd~~ do

Acetic acid + acetic anhydride
+
Sulfuric acid (Liebermann - Burchard reagent)

↓

Colour development

↓

After 30 min

↓

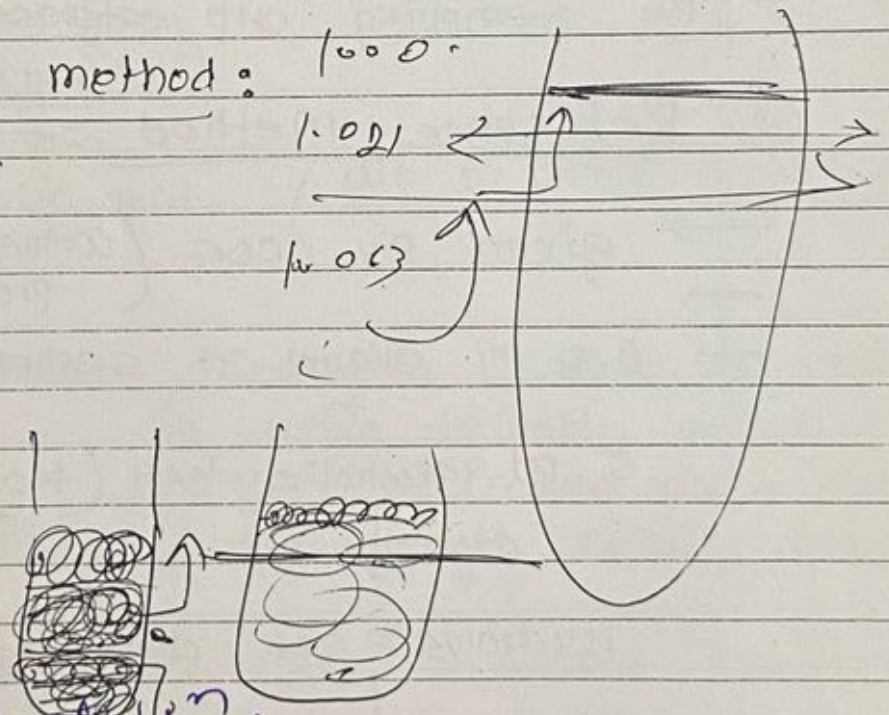
absorbance at 620 nm.

→ Using pure cholesterol is calibrator in this method.

③ → Definative method:

↓

IDMS



HDL separation / quantification.

1st generation / precipitation { Hep+Mn, DS-Mg, Phosphotungstic Mg

2nd gen / facilitated separation → magnetic with / DS-Mg^{2+}

3rd gen / homogenous → ① Ab-4 reagents with ② sulfated α dextran +2 reagent +Mg^{2+}

③ synthetic polymers

④ Immunoinh

* HDL methods :— Adv = Automation / improve precision

2nd homo assay (1) Homogenous method :— (3rd generation)

(a) Sulfated α-cyclodextrin + Mg^{+2}

↓

Selective Block But don't precipitate apo B containing lipoprotein.

↓

Add PEG-linked enzymes (C. esterase and CHOD).

↓

c̄ specifically react c̄ apo A containing lipoprotein & develop colour.

3rd (b) Synthetic polymer + polyanion block apo B lipoprotein

↓

detergent exposes chol. on HDL to enzyme.

↓

Enzymes are added.

4th (c) Immunoinhibition :

↓

Ab added to apo B-100 react c̄ CH / apo B-100 containing CP (VLDL, LDL)

↓

addition on enzymes

5th (d) Enzymes + HDL Blocking reagent

↓

apo B lipoprotein chol. converted into H_2O_2

↓

scavanged (~~destroyed~~) By Catalase

↓

inhibitor of catalase added.
↓
Surfactant disintegrate HDL
↓
H_2O_2 + 4 Aminoantipyrin + phenol → Quinoneimine + H_2O
In some → Not 4-aminophenazon
↓
Some Blue colour compound added.

⟹ Certification can be acquired if assay perform in atleast 1 instrument
↓
& has achieve agreement ∈ Ref. method. for certification By CRMLN.

⟹ Certification for the reagent can not be consider universally applicable to all distributor versions, instrument applica & Lots.

1st human assay
ⓔ Antibody & reagent method :-
contains
1st reagent : PEG
↓
forms aggregation of apo B containing chylomicron, VLDL & LDL
↓
2nd : antibodies : ∈ Blocks the aggregation of Lipoprotein
↓
against apo B100 and apo C → aggregate (blocked) ∈ Ab Lp.

3rd : cho. esterase, oxidase & peroxidase

↓

Acts on unprotected HDL cholesterol.

↓

4th : quowidine salts: solublize aggreate & stops the colour reaction

↓

measurement of colour

② Reference method : (Ultracentrifugation & Polyanion precipitation)

→ By CDC.

→ Ultracentrifugation for removal of VLDL & chylomicron at d = 1.006 gm/ml (Spin for 16.2 hr at 33,700 rpm in Beckman type 50.4 rotor)

↓

VLDL & chymicron accumulate as a floating layer at the top of ultracentrifuge tube

↓

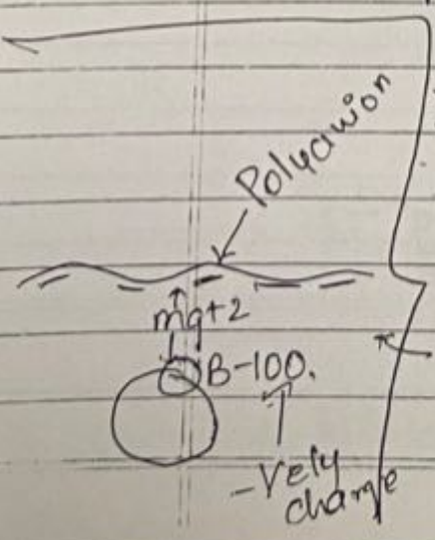
Tube slicing technique to remove VLDL fraction

↓

Infranatant contains IDL, LDL, LP(a), HDL & other serum protein

↓

Add 50 µl of injectable heparin & 100 µl of 1 mol/L manganese chloride, in this infranatant



↓

precipitate form (Precipitate contain apo B 100 containing lipoprotein)

↓

~~remove precipitate By centri~~ Centrifuge

↓

measure HDL cholesterol in supernatant.

⑤ Designated Comparision method :—

→ No ultracentrifugation,

→ Add dextran sulfate + $MgCl_2$

↓

Precipitation

↓

Centrifuge

↓

Supernatant contains HDL

↓

Add reagent in supernatant

↓

measure OD.

④ Precipitation Method :—

① Heparin + ~~Mg~~ Mn^{+2}

② Dextran sulfate + mg^{+2}

③ phosphotungstate + Mg^{+2}

→ Polyanion + diavalent cation (mg^{+2}, Mn^{+2})
(Dextran sulfate, heparin, phosphotungstate)
↓ + sesum
30 min incubation
↓
Precipitation of apo B 100 containing lipoprotein directly from plasma.
↓
~~measure~~ centrifugation
↓
Measure HDL from Supernatant.

→ Interference in Heparin - Mn^{+2} method:
↓
Can not useful for ~~en~~ subsequent enzymatic assay
↓
as residual Mn^{+2} ~~giving~~ interfere ~~c~~ enzyme
↓
give falsely high result.
↓
To avoid this problem
↓
Add EDTA / carbonate to chelate residual Mn^{+2}

→ Drawback of precipitation method:
↓
→ Biological variation in apo B containing Lipoproteins

→ Precipitation of apo B100 containing lipoprotein can be incomplete in samples of high TG

- Inadequate sedimentation of apo B100 containing lipoprotein → overestimation of HDL
- Turbid supernatant due to ↓ in density of LP precipitating reagent compensated by TG → So some complex remain unsedimented.
- V. High TG → precipitate may form a floating layer above the clear supernatant.

To overcome above problem.

→ (a) Ultracentrifugation of sample before precipitation. → TG rich lipoproteins are removed.

→ (b) Centrifuging it for longer time

→ (c) Dilution of sample 2 fold with saline to reduce conc. of TG rich lipoproteins before precipitation

→ (d) Pass the turbid supernatant through 0.45 µm filter to remove unsedimented precipitate.

→ Sample - matrix effect :

↓

① Unusual nature of sample
② Processing effect
③ Addition of anticoagulant / preservative
④

eg : HDL measurement are inaccurate & variable when obtained from lyophilized sample than from fresh sera.

✳ Measurement of Triglyceride :-

① Reference method :- By CDC

TG → 1st extracted quantitatively ā chloroform to remove water soluble interfering substances like glucose, glycerol. from serum

↓

Extract is treated ā silicic acid to remove phospholipids (silicic acid binds & precipitate phospholipid)

↓

Extract containing TG - subjected to

alkaline hydrolysis to produce unesterified FA & glycerol.

↓

TG + KOH ⟶ FA + Glycerol.
(alkalin medium)

Glycerol + Periodate $\xrightarrow{\text{oxidation}}$ formic acid + formaldehyde

Formaldehyde + chromotropic acid ⟶ chromogen

↓

Absorbance at 570 nm.

→ CDC measures → only glycerides → Not free glycerol → By Extraction in chloroform

↓

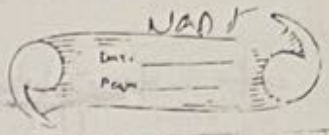
" Triglyceride Blanking "

② Designated Comparision method :

↓

→ given By CRMLN (cholesterol Ref. method Lab. Network)

→ Similar Extraction step as ref. method F/b enzymatic quantitation TG derived glycerol.



(3) Enzymatic methods :→ 1st two steps are common in (a), (b), (c)

GPO-PAP method

(a) ① TG + 3 H_2O —lipase→ Glycerol + FA

② Glycerol + ATP —Glycerokinase→ Glycerophosphate + ADP

↓

P (1) & (2) steps are common.

③ Glycerophosphate + O_2 —Glycerophosphate oxidase→ DHAP + H_2O_2

④ H_2O_2 + 4-Aminophenazone + Phenol —Peroxidase→ Quinemine + 2 H_2O.

(b) (1) & (2) reaction common

③ Glycerophosphate + NAD —Glycerophosphate dehydrogenase→ DHAP + NADH + H^+

NADH measure at 340 nm

④ NADH + Tetrazolium dye —Diphorase→ Formazan + NAD+

↓

measured at 500 nm

(c) ADP form in reaction (2) can be measured

ADP + PEP —Pyruvate Kinase→ Pyruvate + ATP

Pyruvate + NADH + H^+ —LDH→ Lactate + NAD^+

* ~~Triglyceride - Blo~~

→ Enzymatic methods : specific B'cz do not detect glucose & phospholip.id

↓

But glycerol may interfere endogenous.

* TG Blank (correction for endogenous glycerol) :-

→ In healthy subjects freshly collected serum contains glycerol < 5-10 mg/dl

→ It can be highes in sample c high TG : ① pt c DM } → Not significant
② Receiving TPN }
③ hypoglycerolemia → significant

↓

↑ glycerol about 50-100 times (N).

→ 2 approches to remove interference of glycerol during TG measurement:

① Serum Blanking :

② 2. Cuvette Blanking :

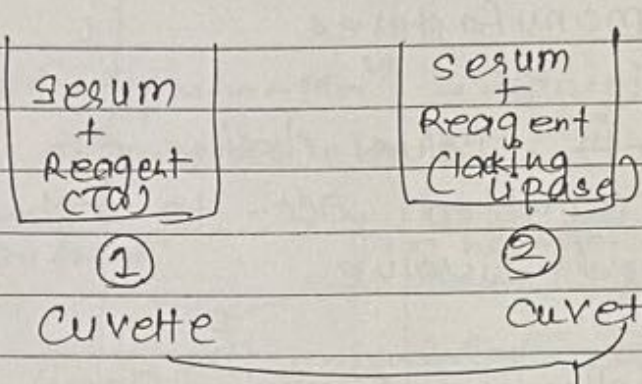
↓

→ Reagent identical to TG estimation reagent is used (lacking lipase)

↓

↓

→ measured Blank value is subtracted from TG measurement



↓

measure absorbance from Both,

↓

→ Real TG absorbance = absorbance from 1st − absorbance from 2nd cuvette

(b) 2-Step approach : In single cuvette

↓

add Serum + Reagent lacking lipase (endogenous)

↓

Consumption of free glycerol to produce colourless product

↓

add lipase containing Reagent

↓

measure absorbance.

② Calibration Blanking :

↓

→ Calibrator ~~value~~ is tested By reference method as well as testing method or manufactures,

↓

~~diff in~~ value ~~desire~~ of calibrator is adjusted acc. to ref. method derived value.

⟹ Traditionally, Fasting sample require to measure TG

↓

to achieve uniform metabolic state.

↓

⟹ But Post prandial collec^{n} / non fasting sample includes remanant lipoproteins that are more atherogenic & Reflective of pt's usual metabdic state.

LDL Cholesterol Estimation :-

chyl ~ 0.95
VLDL - 1.006
LDL - 1.016
HDL - 1.063

Serum → Ultracentrifugation

↓

remove supernatant containing VLDL & chylomicron

↓

Infranatant containing LDL, HDL, IDL and (pCa) + minor VLDL

↓

Add Heparin - Mn^{2+} → precipitate?

measure chol in

↙ in d = 1.006 fraction (VLDL)

↘ in heparin. Mn^{2+} supernatant solution (VLDL+HDL, LDL, IDL)

↓

Diff calculated - LDL

called β quantitation / Bread-cut fraction.

① Reference method : → By CDC

↓

Serum — Ultracentrifugation (d = 1.006)

↓

Remove supernatant containing VLDL & chylomicron → divide in 2 parts

① add reagent in Infranatat to measure cholesterol (HDL + LDLC)

② ~~for~~ add Heparin + Mn^{+2}

↓

Precipitation of apo B100 containing LP.

↓

Centrifugation

↓

Supranatant → contain HDL

Infranatant → contain LDL

↓

add reagent in supranatant

↓

measure HDL

↓

difference or a result is LDL cholesterol.

↓

It is called "Broad cut fraction" ∵ include not only LDL But also IDL, Lp(a)

↓

k/g "β-quantification"

② β-quantification :-

↓

→ Precoussor to Reh. methods develops from HDL & LDL.

→ Usehul when friedwald equation is not reliable.

→ See steps of reh. method for LDL.

→ (VLDL chol) = Total CHO − (d > 1.006 g/mL CHO)

→ (LDL chol) = (d > 1.006 g/ml CHO) − HDL CHO.

③ Homogenous method :-

ⓐ LDL solubdization :-

↓

By using a reagents.

→ 1st reagent : $MgCl_2$, dye, Buffer, α-cyclodextrin sulfate

↓

masking of cholesterol in chylomicron & VLDL

↓

→ 2nd Reagent : cholesterol oxidase, esterase, peroxidase, dye, Buffer, (6.75)

POE-POP

(Polyoxyethylene - Polyoxypropylene)

↓

POE-POP Blocks HDL chol.

↓

now measured LDL-chol ~~int~~ in serum

↓

absorbance measured.

(b) LDL protected / deprotected By surfactants :-

↓

1st reagent : Ascorbic acid oxidase, 4-Amino-antipyrin, detergent, buffer. (6.7)

↓

Solubalization of all non-LDL lipoprotein By detergent

↓

reaction of their chol. By enzymes

↓

Forms colourless product

2nd reagent : N-N'- bic -M -toluidine disod. (DSBMT) salt, Buffer, detergent

↓

detergent specifically release LDL cholesterol

↓

reaction & enzyme produce H_2O_2

↓

H_2O_2 + DSBMT ⟶ colous product + $2H_2O$.

↓

Absorbance measured

(c) LDL protected / catalase :

Reagent 1 : good's Buffer (PH : 6.7), chol. esterase, oxidase, catalase, polyanions, amphoteric surfactant

↓

Amphoteric surfactants selectively protects LDL from enzymatic reaction

↓

Non LDL choles. reacts & enzyme

↓

H_2O_2 consumed By catalase

↓

2nd Reag : good's Buffer, 4-Amido Antipyrin, peroxidase, sod. Azide, .. deprotecting reagent

↓

Sod. Azide : inhibit Catalase

↓

deprotecting reagent removes protecting agent from LDL

↓

reaction By Enzymes on LDL CHO

↓

H_2O_2

↓

produce Blue coloured ~~prodn~~ measures

④ NON - HDL catalase / LDL azide :-

↓

~~1st reagent~~ :

protective agent : to prevent reacⁿ of LDL chol. & enzyme mt in Reagent 1

↓

Non LDL chol. reacts

↓

result in H_2O_2 formaⁿ

↓

Eliminate By Catalase

↓

deprotector & Sod. Azide in 2nd reagent

↓

remove protector from LDL

inhibit Catalase

↓

LDL reacts & enzymes

↓

formation H_2O_2 → colour measure

(h) LDL protected By calixarene / chol. dehydrogenase :—

↓

1st Reagent : Calixarene ∴

↓

LDL ⟶ soluble complex

↓

Other chol. Hydrolyze By esterase

↓

2nd Reagent : deoxycholate

↓

Broke up LDL - calixarene complex

↓

LDL chol. now react ∴ enzyme

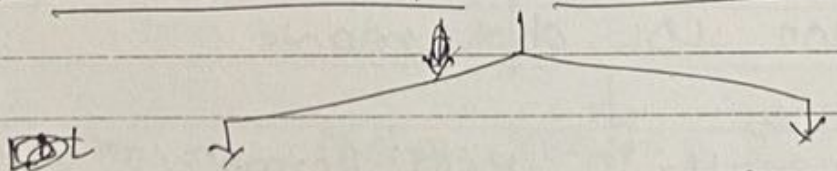
↓

forms β - NADH

↓

measured.

(4) Indirect method : ~~friedwald equation~~ :—



(a) β- quantification (b) Friedwald equation

(b) friedwald equation :— NCEP Recommanded

LDL chol = (Total CHO) − (HDL CHO) − (~~VLDL~~ TG/5)

→ all conc. in mg/dl

→ VLDL = TG/5

→ Variants of friedwald equation :-

(a) LDL CHO = (Total CHO) − (HDL CHO) − (TG × 0.16)
↓
(VLDL)

(b) LDL CHO = (Total CHO) − (HDL CHO) − (TG/6)
↓
VLDL

⟹ Limitations of equation :-

① Samples ∵ TG > 400 mg/dl / ↑ quantity of chylomicrone (non fasting specimen)
↓
At high TG conc. ~~above 400 mg~~
↓
Factor TG/5 for VLDL CHO conc. is not appropriate (↑ in TG doesn't mean ↑ in CHO)
↓
It may contain chylomicron, chylomicron Remnants, VLDL Remnants
↓
all have high TG/CHO ratio
↓
overestimation of VLDL CHO (TG/5)
↓
underestimation of LDL − CHO.

↓

⊗ Friedwold equation range → when TG conc

- < 200 mg/dl → most accurate
- 200-400 mg/dl → Intermediate accuracy
- > 400 mg/dl → not useful → Use β-quantification.

② Type III Hyperlipoproteinaemia (defect in apoE):-

↓

Since or β-VLDL is not (n) int in Blood → (IDL)

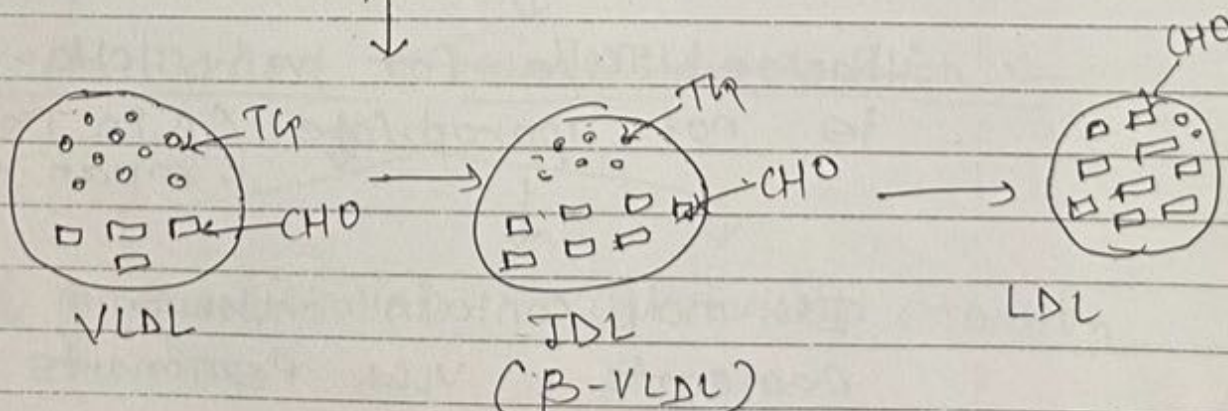
↓

(IDL) β-VLDL is intermediate B/w VLDL & LDL

↓

IDL contains less TG than VLDL & more CHO than VLDL

↓



→ So if more β-VLDL is there → TG/C is less ~~than~~

↓

Underestimation of VLDL CHO

↓

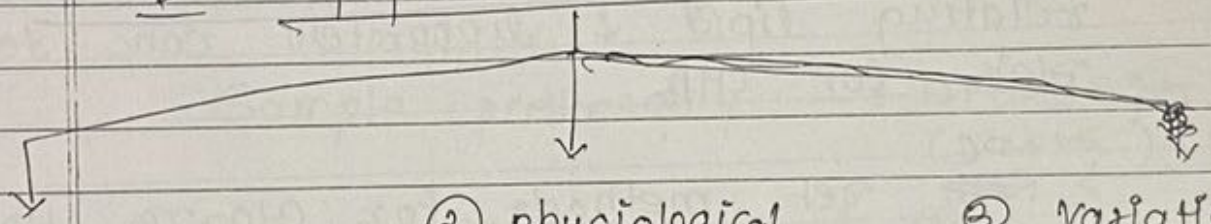
Overestimation of LDL CHO.

⟹ Best method to seperate chylomicron

↓

Sample allow to stand to undisturbed at 4°C overnight.

* Sources of ~~measurement~~ Variation in lipid & Lipoprotein measurement :—



① Analytical variation
- No actual change in conc. ↓ inherent in measurement.
- arises from
→ Sample collection procedure
- Vol. measurement
- instrument function
- Reagent formulation
- Uncertainty in assigning value of calibrators

lead to variation in lipoprotein conc.

② physiological variation
- Actual change in concentration

→ change → redistribution of water in posture:
→ recent food intake
↓
↑ TG upto ≥ 50%
→ Seasonal changes.
(due to change in diet pattern)
↓
Variation tends to occur in Both direction ↑/↓

③ Variation due to specific condition
↓ for wt gain/loss
- Pregnancy
- dietary change
- Acute illness
- ~~drug~~.
- lipid lowering drugs
↓
Changes tends to occur in 1 direction
↓
Not considered as physiological fluctuation.

* NCEP Recommandation for lipid & lipoprotein measurement :—

→ NCEP = National cholesterol Education Programme.

→ CDC = Centre for d'se control & Prevention.

① Database linkage :

↓

→ laboratories that provides lipid & LP measurements should maintain linkage with existing epidemiologic database relating lipid & lipoprotein conc to risk for CHD.

→ CDC ref. methods for CHO, TG, HDL, LDL-C serve as the basis for judging the accuracy of other method.

② Reference method :—

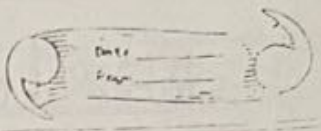
↓

they should provid serum based values.

③ Routine methods :—

↓

Specimen may be serum / plasma.



if EDTA plasma is used for measurement

↓

convert to a serum equivalent value with equation.

Equivalent serum value = plasma value × 1.03

④ Chol. measurement :

↓

→ Sample may be fasting / non fasting.

→ TG, HDL-C, LDL-C

↓

Sample preferably → fasting (12 hrs)

→ for convenience → it may be 9 hr

⑤ Blood Sample :

↓

Should be collected in seated posin

↓

If not possible, then pt should be ~~sample~~ in same posin on each occasion. for sample collection

⑥ Specimen storage :

↓

Serum / plasma seperated from the cell in 3 hrs on collection

↓

- stored upto 3 days at 4°C,
- several wks at −20°C,
  longer period at −70°C

⑦ Serial samples:

↓

To remove the physiological & Analytical variation

↓

mean of several serial measurement for clinical decision should be used

↓

At least 2 serial samples → 1 wk apart

↓

3 serial samples are prefered for TG, HDL, LDL

⑧ Glycerol Blanking:

↓

→ NCEP working group, recommended use of glycerol Blanking for TG measurement

⑨ Goals for analytical performance:

↓

→ NCEP goals in terms of Total error reflects both bias & imprecision
→ CDC standardization criteria

↓

considers it seperately.

High Sensitive C-Reactive Protein

CRMLN:

→ (Cholesterol Reference Method Laboratory Network

↓

(Lipoprotein, TG) measurement

Issues or analytes & matrix effect in in various Reh. material like lyophilized, freeze dried.

↓

lead to conclusion that comparision or Reh. method & other methods can be done with fresh ~~dep~~ pt specimen

↓

So CDC & several other laboratories co-operated to establish "CRMLN".

↓

Each participant laboratory is supplied with fresh serum for measurement of HDL, LDL & TG.

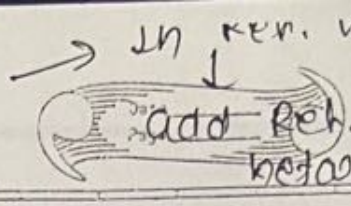
↓

At the same time, this sample is also given to Reh. Laboratory to measure HDL, LDL & TG By Reh. method.

↓

comparsion on all result done

Reference Laboratory



They are usually private, commercial facilities that do high volume routine & speciality testing.

Most of the test perform are referred from physicians, Hospitals & other health care facilities

Ref. labs are used for specialized test that are ordered only occasionally or that requires specialized Instrument.

→ A ~~medicare~~ - ~~carried~~ laboratory that receives a specimen from another referring laboratory for testing & that actually performs test.

→ It performs reference or calibration measurement procedure or assigns reference values to test objects.

→ This reference values are useful for references or sources of traceability of test results

* Referring lab :-
→ A lab. that receives a specimen to be tested & refers the specimen to another laboratory for performance of Lab test.