

*Summer school
20.9.2022 in Bratislava*

Pitfalls and advantages of determination of lipoprotein subfractions by the Lipoprint® system

RNDr. PADUCHOVÁ Zuzana, PhD.

Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Comenius University in Bratislava



NEW LIPOPRINT SYSTEM



- We bought the Lipoprint® system

Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry

WHY LIPOPRINT?

- approximately 50% of CAD (*Coronary Artery Disease*) occurs among individuals with normal lipid levels
- up to 50% of people that have suffered from a heart attack had “normal” cholesterol levels at the time of their heart attack
- only 30% of all heart attacks can be clarified on the basis of the measurement of cholesterol itself

LIPOPRINT® SYSTEM

- uses polyacrylamide gel electrophoresis to separate the various lipoprotein subfractions on the basis of size
- uses a lipophilic dye that binds to the cholesterol in the lipoprotein particle prior to electrophoresis
- LDL and HDL fractions and subfractions can be detected
- Quantimetrix Lipoprint System LDL Subfractions kit for LDL panel
 - It identifies up to 12 lipoprotein fractions and subfractions:
VLDL, IDL1-2-3 = MID C-B-A, LDL1-7, and HDL
MID-A and LDL1-2 are non-atherogenic and
MID-C,B and LDL3-7 are atherogenic subfractions

LIPOPRINT® HDL SUBFRACTIONS TEST

- Quantimetrix Lipoprint System HDL Subfractions kit for HDL panel
 - It identifies up to 10 subfractions of HDL, which are classified into three major subclasses:
Large HDL lipoproteins (HDL-L): HDL1-3,
Intermediate HDL lipoproteins (HDL-I): HDL 4-7
Small-dense HDL lipoproteins (HDL-S): HDL 8-10
 - The HDL-L subclass shows an inverse relationship with CVD risk
 - The HDL-S subclass exhibits a direct relationship with CVD risk

LIPOPRINT® SYSTEM COMPONENTS

- ¹Electrophoresis Chamber
- ²Electrophoresis Power Supply
- ³Preparation Rack
- ⁴Preparation Light
- ⁵Digital Scanner
- ⁶iMac computer + software Lipoware
- ⁷Printer



LIPOPRINT® SYSTEM COMPONENTS

- ¹Quantimetrix Lipoprint System LDL Subfractions kit
- ²Quantimetrix Lipoprint System HDL Subfractions kit
- ³Gel tubes for LDL
- ⁴Gel tubes for HDL
- ⁵Loading gel
- ⁶Buffers
- ⁷Control



SAMPLE REQUIREMENTS

- Only fasting (12 hours) serum or EDTA plasma may be used
- Do not use **heparin** as anticoagulant !!!
- Total Cholesterol concentration must be ≥ 100 mg/dl (≥ 2.59 mmol/l)
- Samples are stable for up to 7 days at 2-8°C
- Fresh sample is recommended
- But if the sample needs to be frozen it should be frozen cryogenically (-70°C or less)

PRINCIPLE OF OPERATION

ELECTROPHORESIS

- Lipoproteins are stained with a lipophilic dye and separated using a high resolution, linear polyacrylamide gel.

SCAN

- The gel tubes are placed into the digital scanner to generate a image of the resolved lipoprotein bands.

ANALYSIS

- The digital image is analysed using Lipoware software, which calculates the cholesterol level for each of the lipoprotein fractions and subfractions.

REPORT

- Colour graph of the lipoprotein profile is generated and printed

PROCEDURE

- Prepare the electrolyte buffer solution
- Remove the Gel Tubes from storage buffer
- Remove the storage buffer completely from the top of the gels
- Apply 25 μ L of sample to each tube
- Add 200 /300 μ L of Lipoprint LDL/ HDL Loading Gel to each tube
- Mix the Loading Gel with the specimen by inverting the Preparation Rack several times



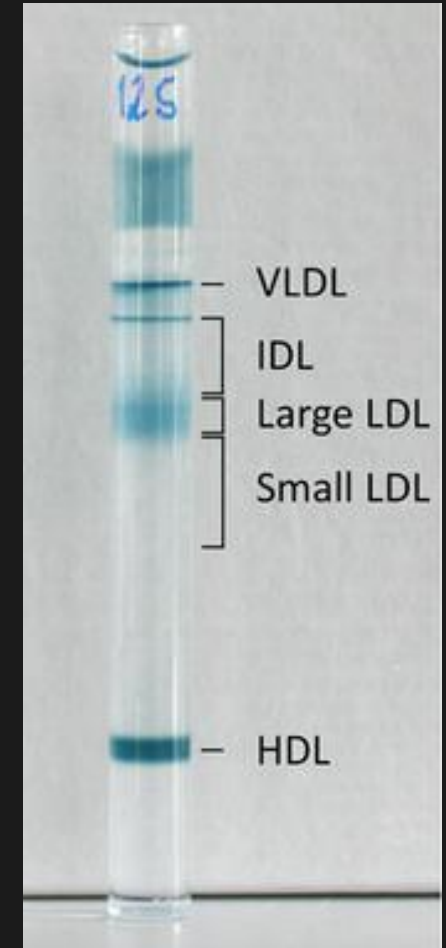
PROCEDURE

- After mixing, place the loaded Preparation Rack against the preparation light. Allow the loading gel to photopolymerize for 30 minutes (but no longer than 40 minutes)
- After the photopolymerization is complete, remove each Gel Tube from the Preparation Rack and carefully insert it into the silicone adapter of the upper chamber
- Place 1000 mL of electrolyte buffer solution in the lower chamber.
The lower buffer may be reused up to five times
- Place 200 mL of electrolyte buffer solution in the upper chamber



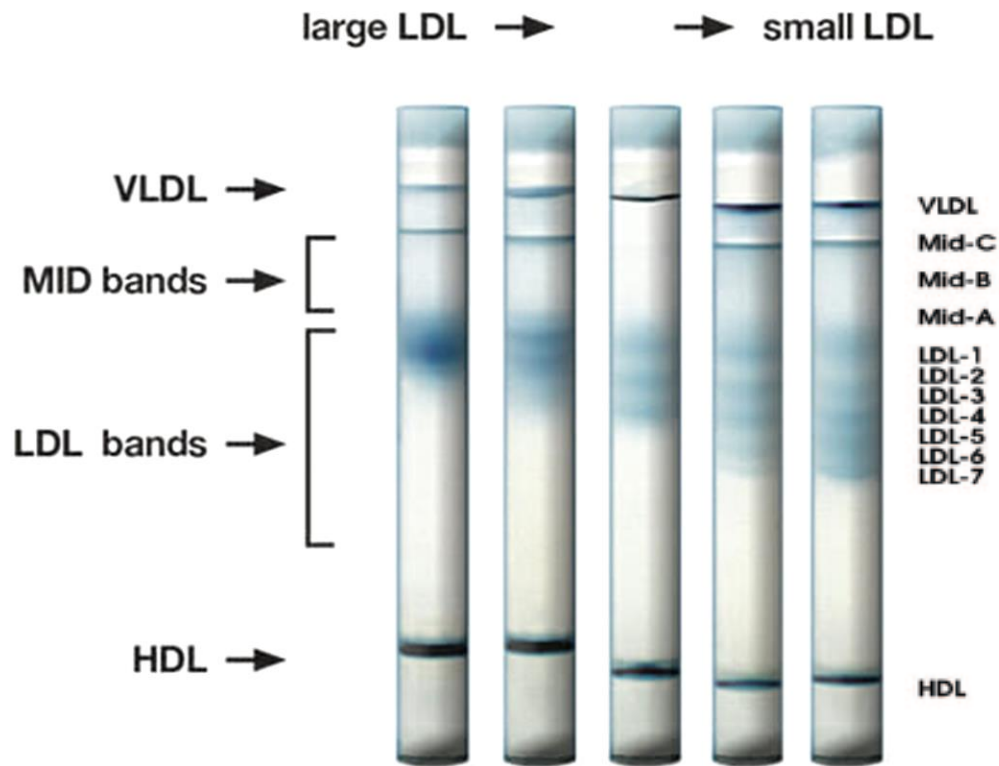
PROCEDURE

- Electrophoretic condition: 3 mA per each Gel Tube, 50 min
- Allow the gel tubes to rest for at least 30 min but no longer than 2 hours before scanning
- The gels are scanned to determine the relative area for each lipoprotein subfraction

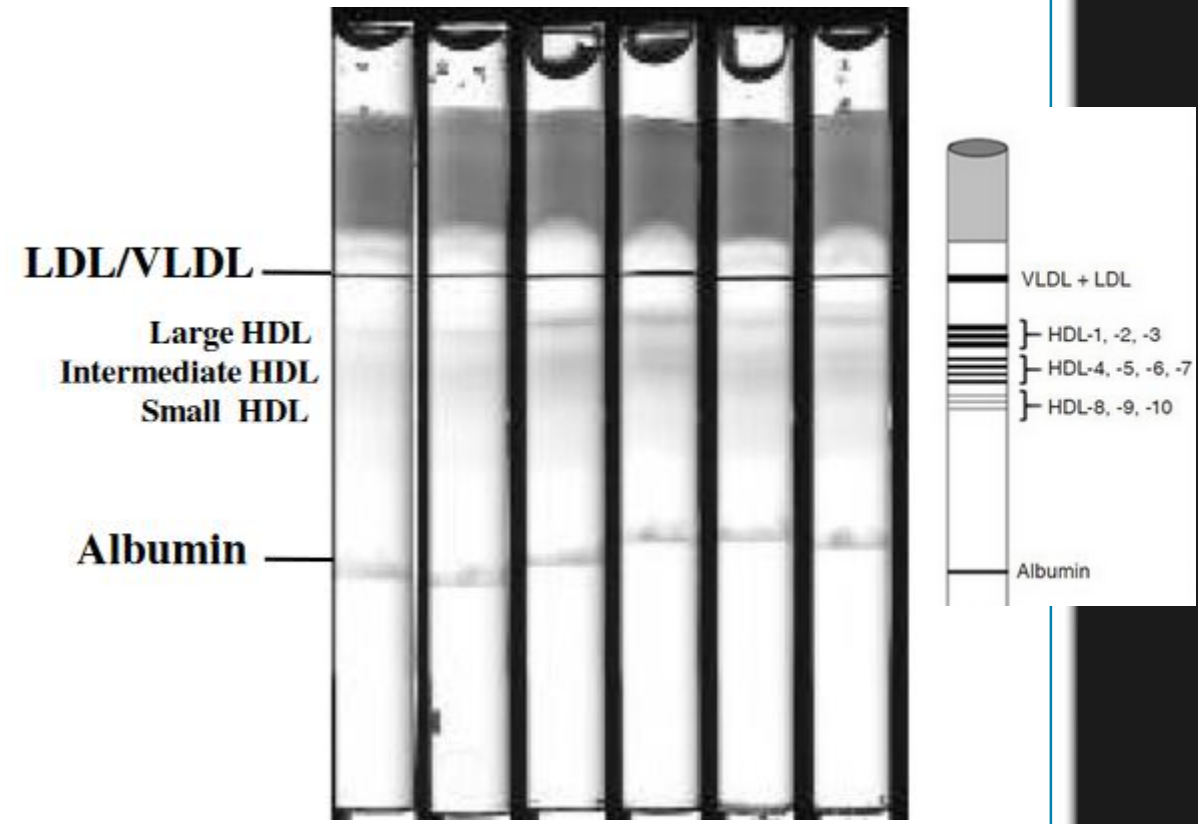


LIPOPRINT® GEL TUBE SCHEMATIC

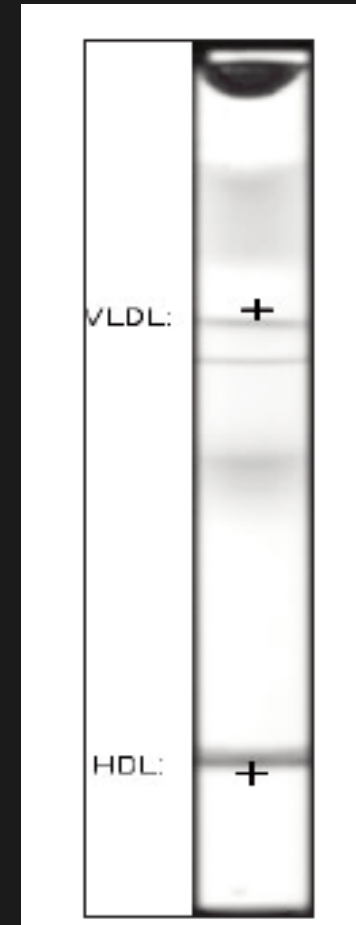
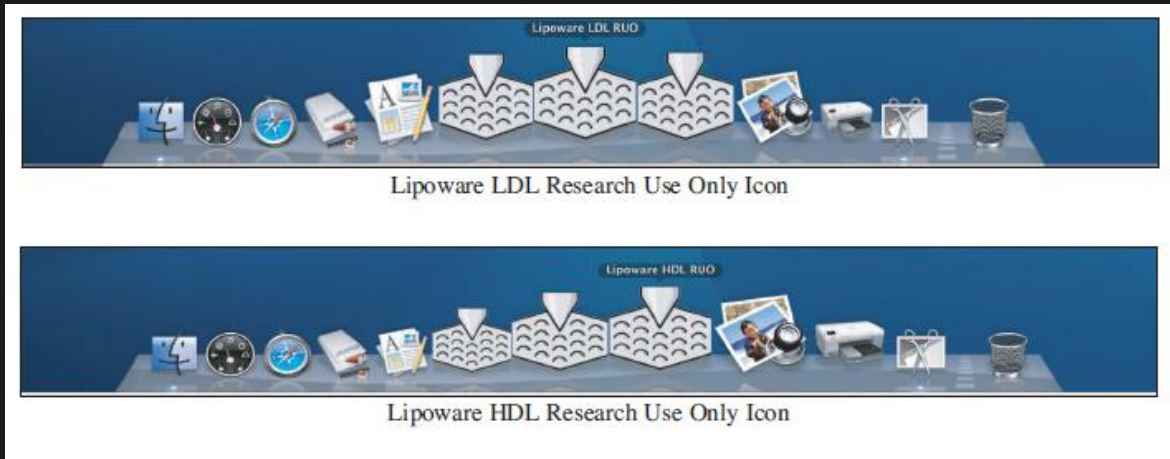
LDL PROFILE



HDL PROFILE

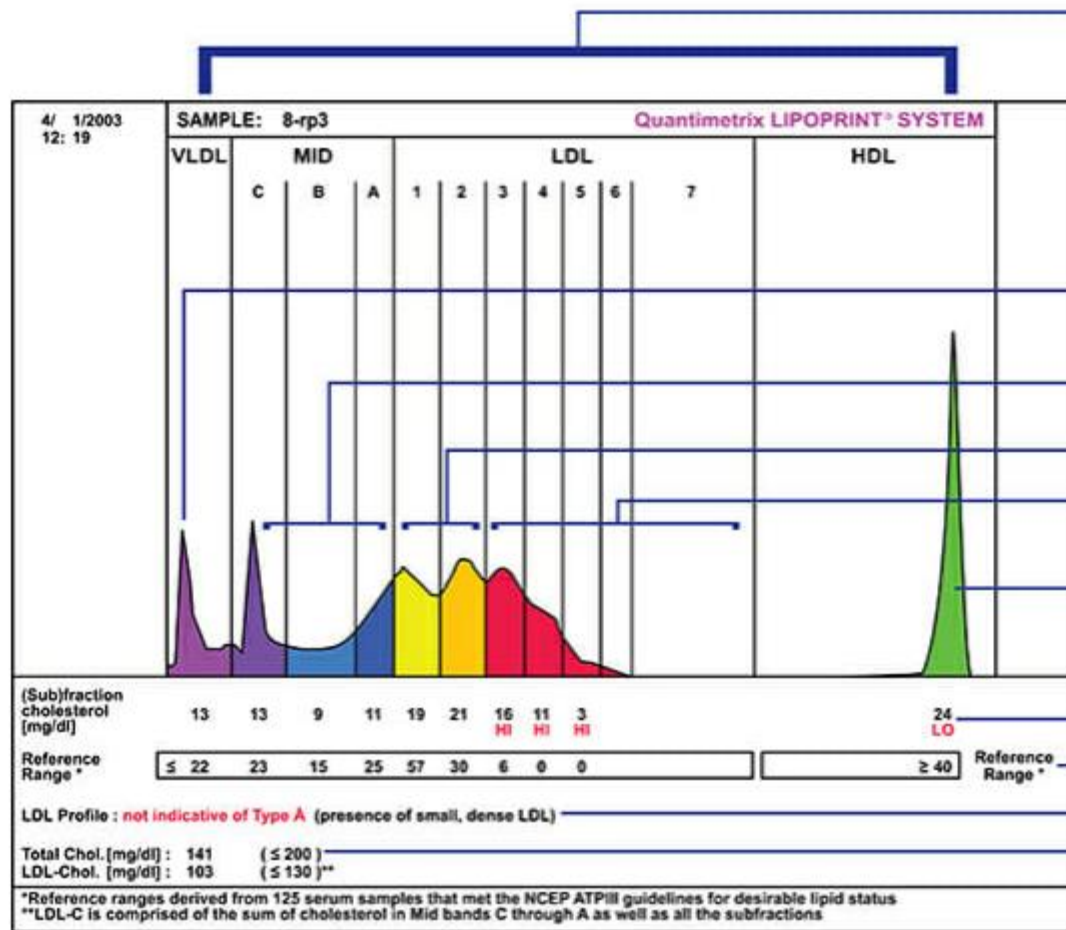


QUANTITATION & IMAGE ANALYSIS



- The scanned gels are analyzed with Lipoware software
- The subfraction cholesterol is calculated by multiplying the relative area of each subfraction by the total cholesterol of the sample
- A color coded profile is generated

COLOUR-CODED LDL PROFILE



Twelve lipoprotein fractions

VLDL increased levels = association with hypertriglyceridemia

Atherogenic subfractions Midbands B and C

Non-atherogenic subfractions Midband A, LDL 1 and 2: not associated with CVD risk

Highly atherogenic subfractions LDL 3 - 7: component of the lipid triad associated with CVD, metabolic syndrome and diabetes

HDL cholesterol – Low HDL is a component of the lipid triad associated with increased CHD risk

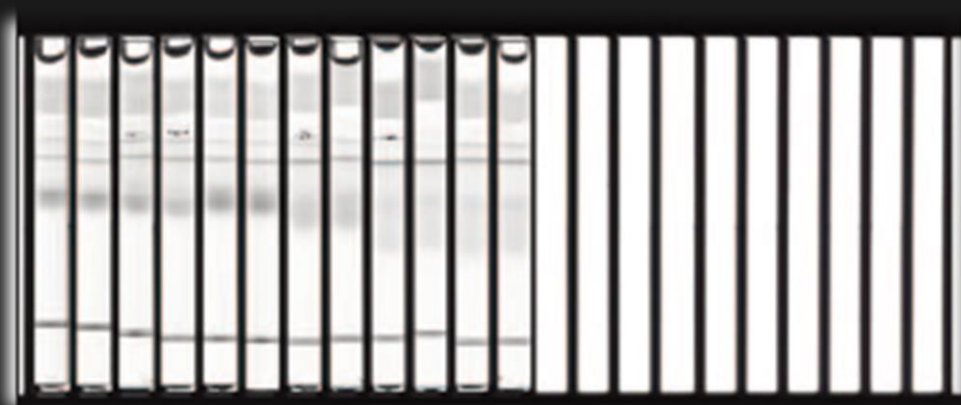
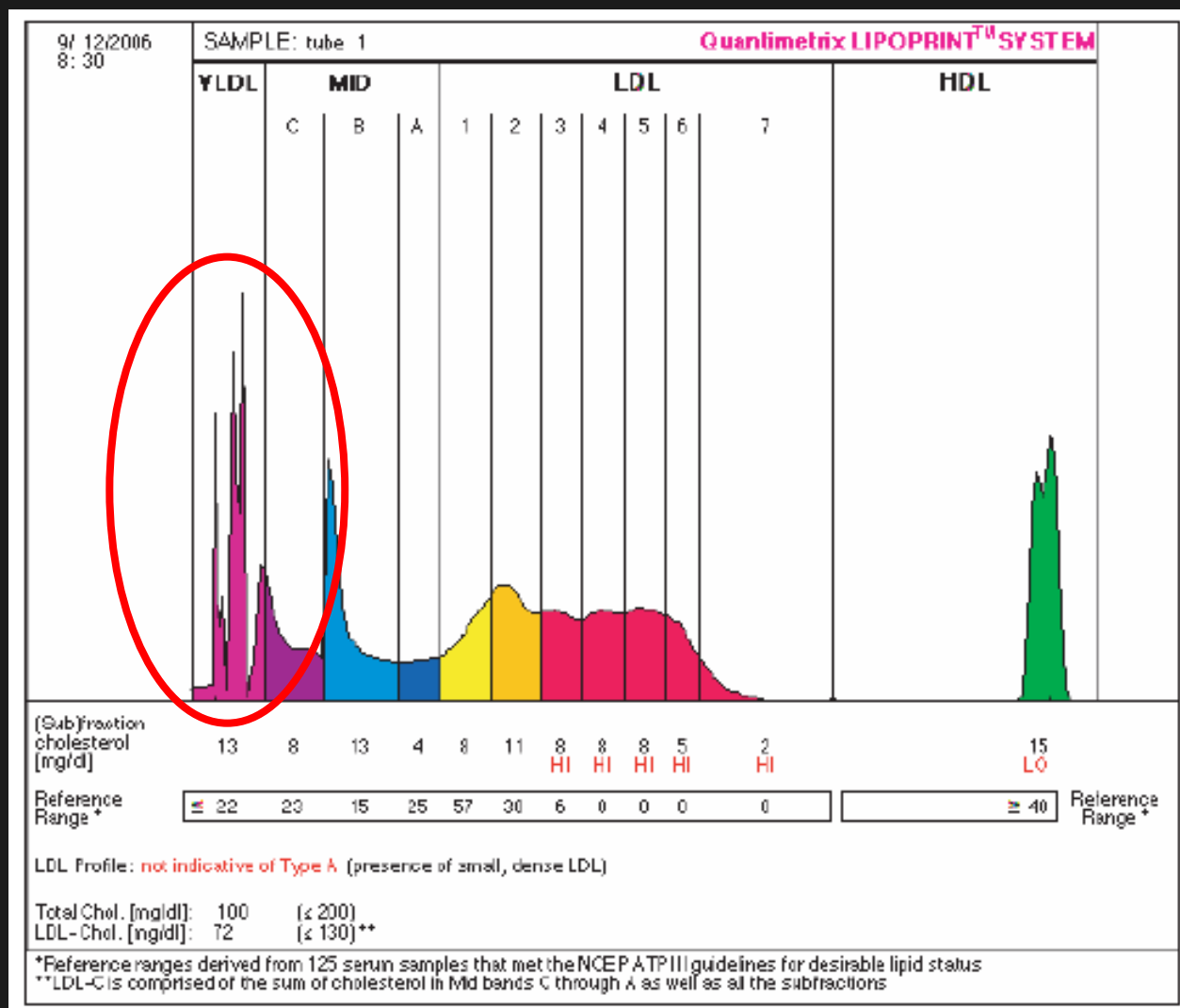
Cholesterol values outside the normal reference range are flagged in red

Normal Reference Range for each subfraction based on NCEP ATP III guidelines

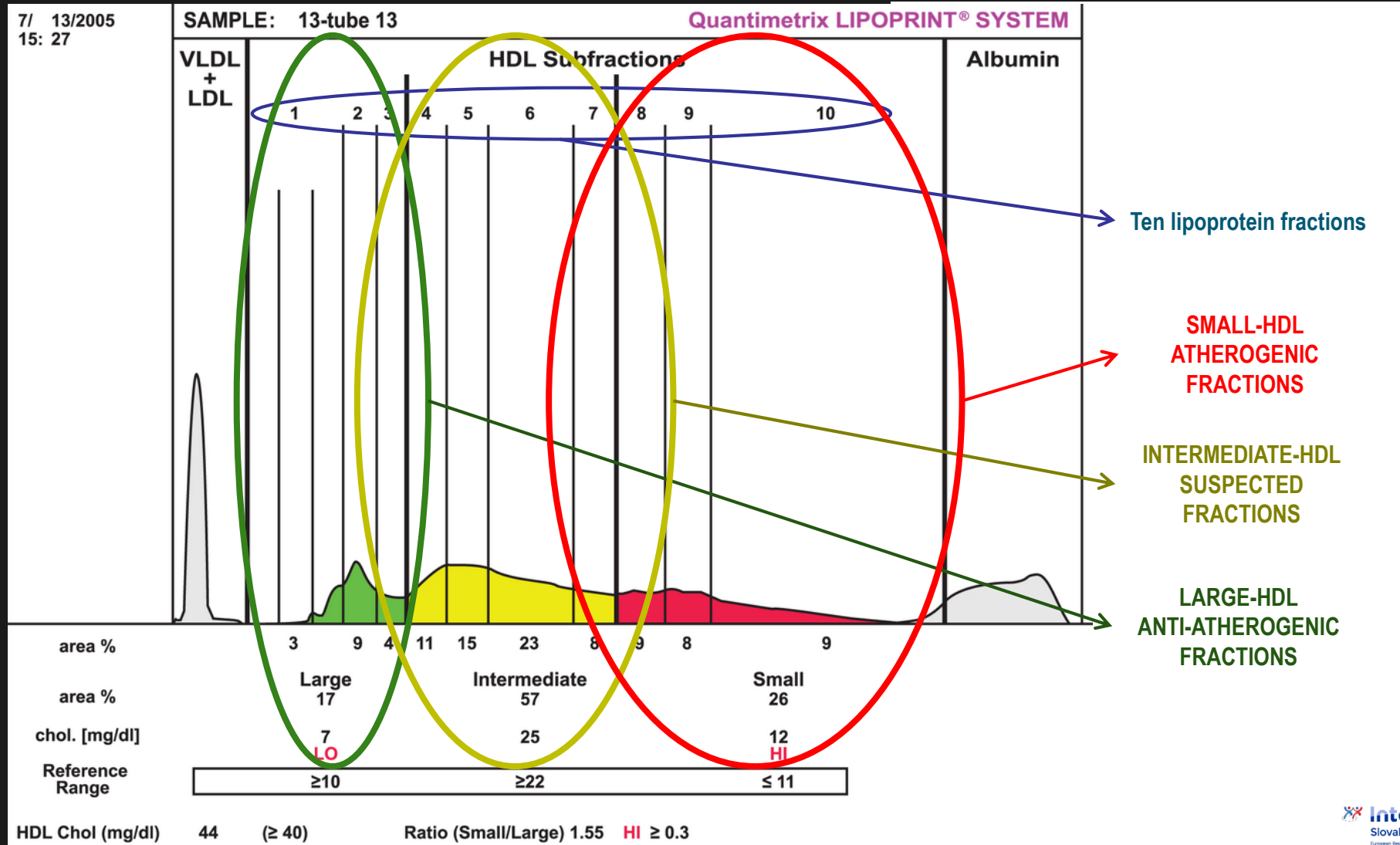
Lipoprotein Profile Classification – predominance of large LDL is classified as Type A and predominance of small dense LDL is classified as Not Indicative of Type A

Total cholesterol level measured independently

WRONG LIPOPROTEIN PROFILE



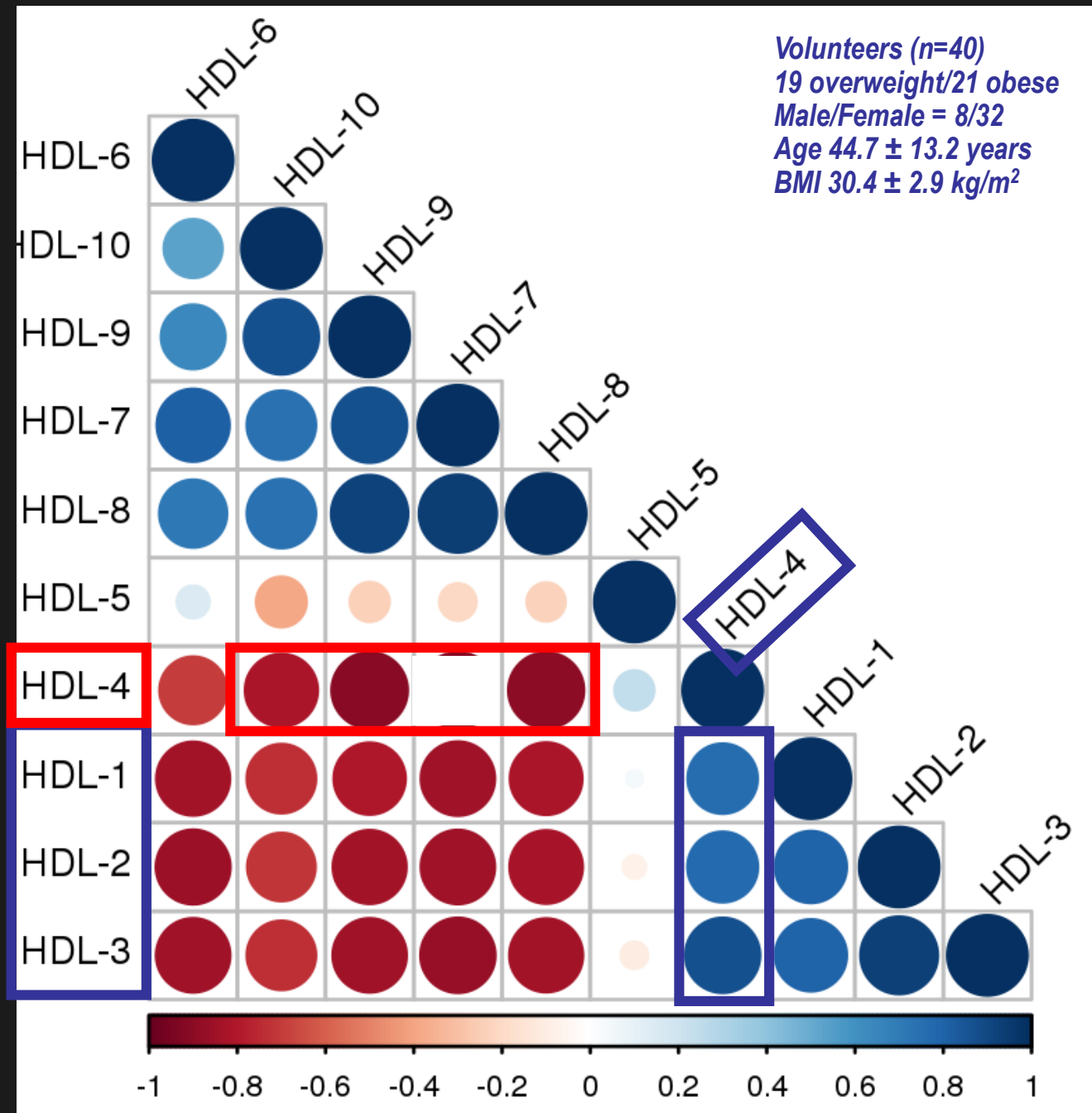
COLOUR-CODED HDL PROFILE



INTERMEDIATE HDL SUBFRACTIONS = SUSPECTED

ATHEROGENIC OR NON-ATHEROGENIC?

The HDL-4 fraction appears to be
ANTI-ATHEROGENIC



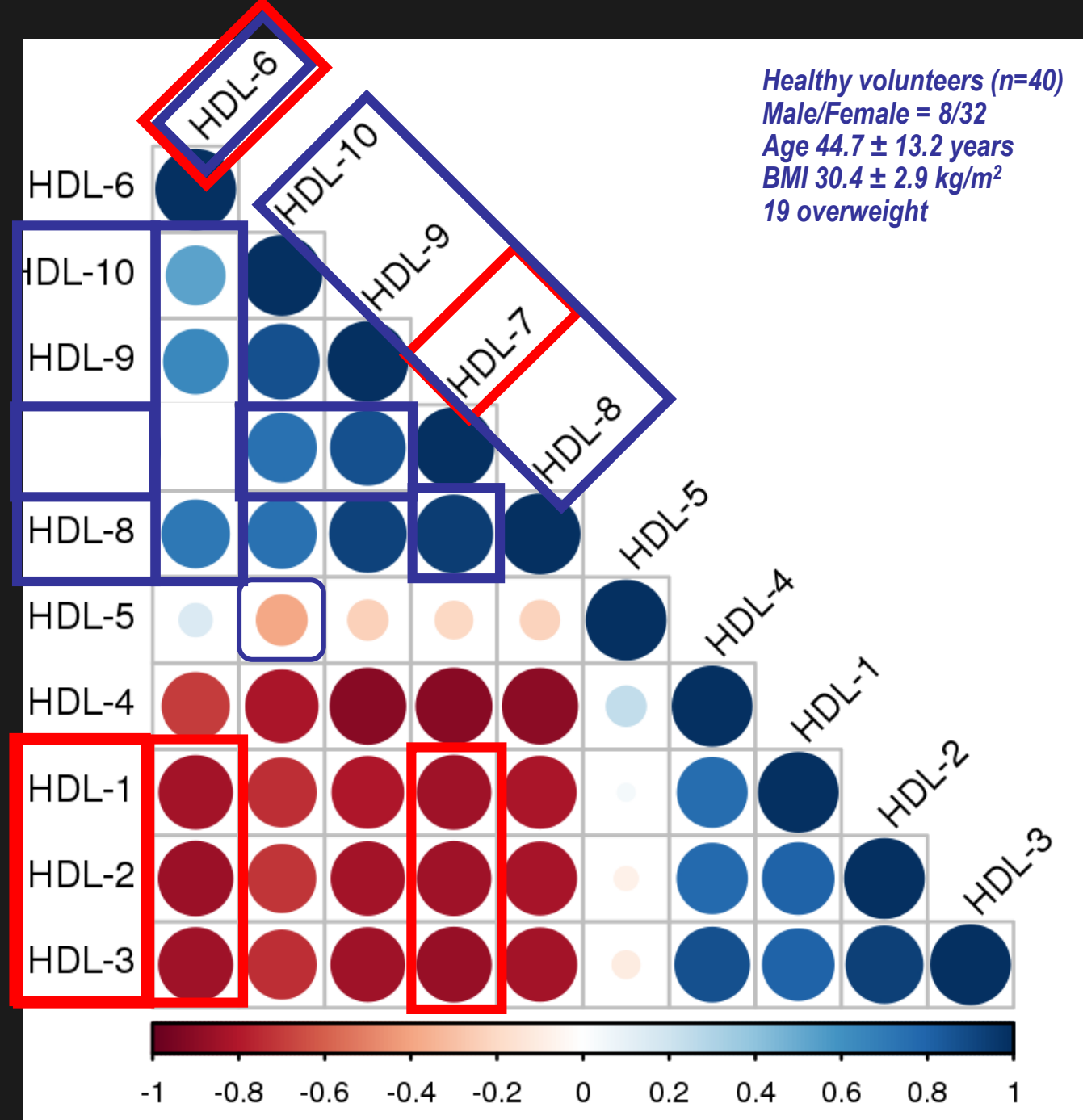
INTERMEDIATE HDL SUBFRACTIONS = SUSPECTED

ATHEROGENIC OR NON-ATHEROGENIC?

The HDL-4 fraction appears to be
ANTI-ATHEROGENIC

The HDL-6 & 7 fractions appear to be
ATHEROGENIC

HDL-5 → Other research are still needed

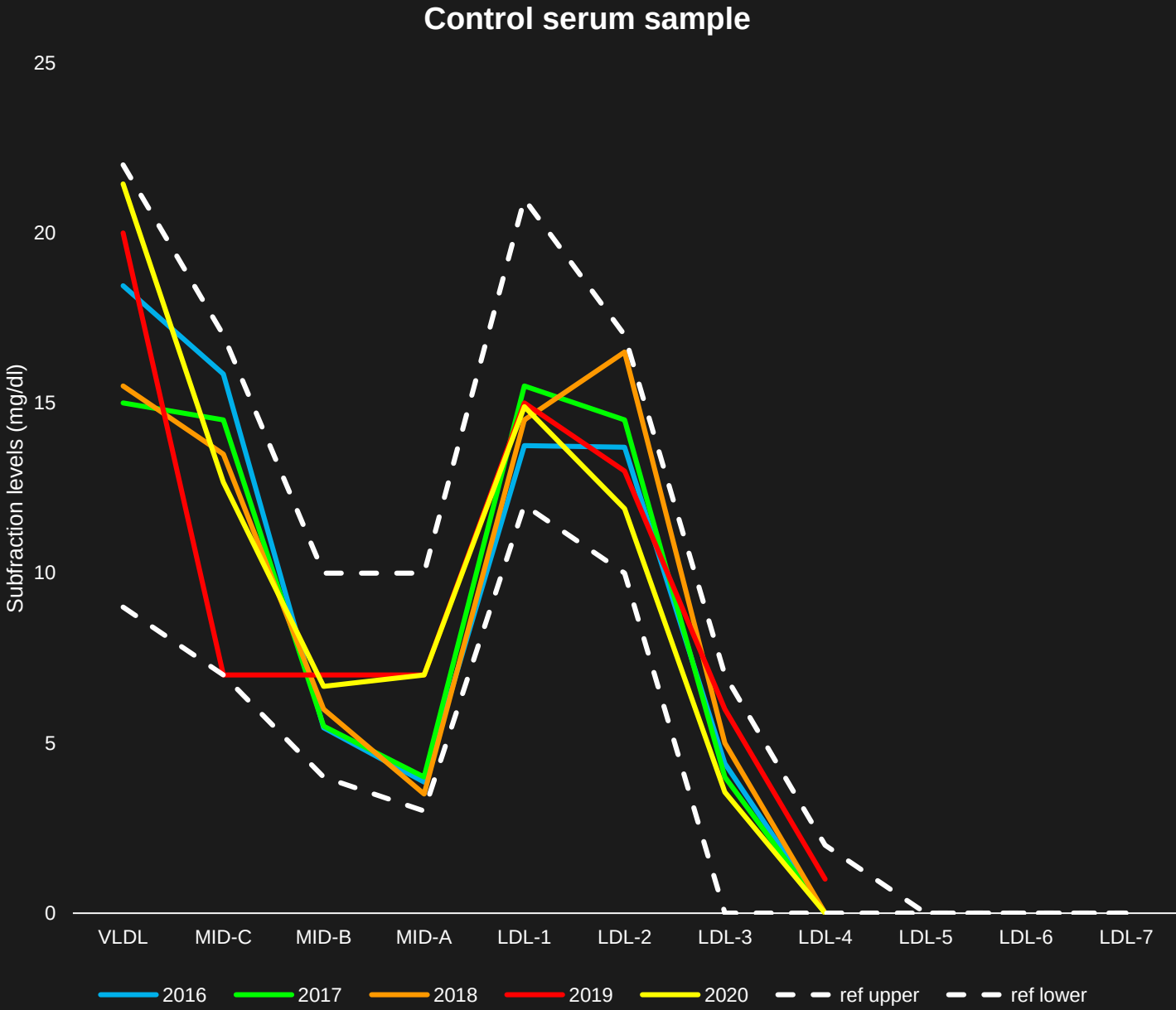


REPRODUCIBILITY OF METHOD

CONTROL SAMPLE

5-years analysis (2016-2020)

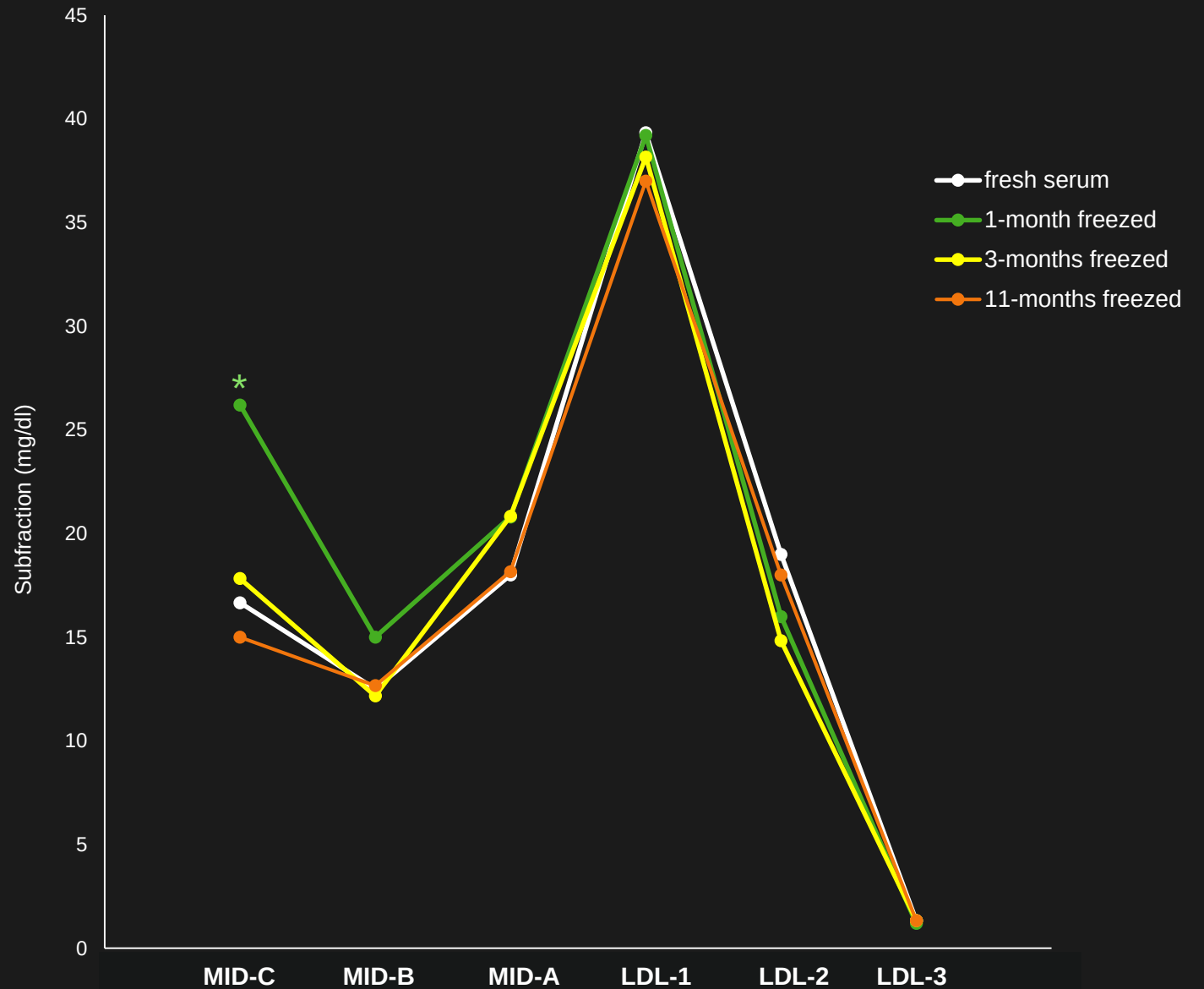
- 2016: n=20 measurements
- 2017: n=2 measurements
- 2018: n=2 measurements
- 2019: n=1 measurement
- 2020: n=9 measurements



CRYOGENIC FREEZING

Effect of cryogenic freezing on LDL subfraction profile

- ✓ No effect of cryogenic freezing on LDL subfractions
- ✓ **1-month freezing** increased only MID-C subfractions
- ✓ **3-** and **11-months** freezing had no effect on MID subfractions



Healthy volunteers (n=6), age 38.8 ± 11.0 years

* $p < 0.05$

ADVANTAGES & PITFALLS

ADVANTAGES	PITFALLS
Normal reference ranges for LDL subfractions	Price per analysis (approx. 34€)
The LDL kit is approved for clinical use & research	Non reference ranges for HDL subfractions
Easy interpretation of colour coded profile	The HDL kit can be used for research purposes only
Results available in less than 3 hours	Small number of samples analysed per 1 run
The HDL test is used in clinical trials and other research studies involving humans and other species (mice, guinea pigs, chimpanzees and pigs)	Small amount of buffer solutions included in kits

Thank you for attention
