# What is LipoSEARCH?

LipoSEARCH Lipoprotein Profiling Service is……

is a cutting edge “lipoprotein profiling service” that is applied an explicit measurement method, gel filtering HPLC (High Performance Liquid Chromatography) and patented unique data analysis algorithm.

# New Insight

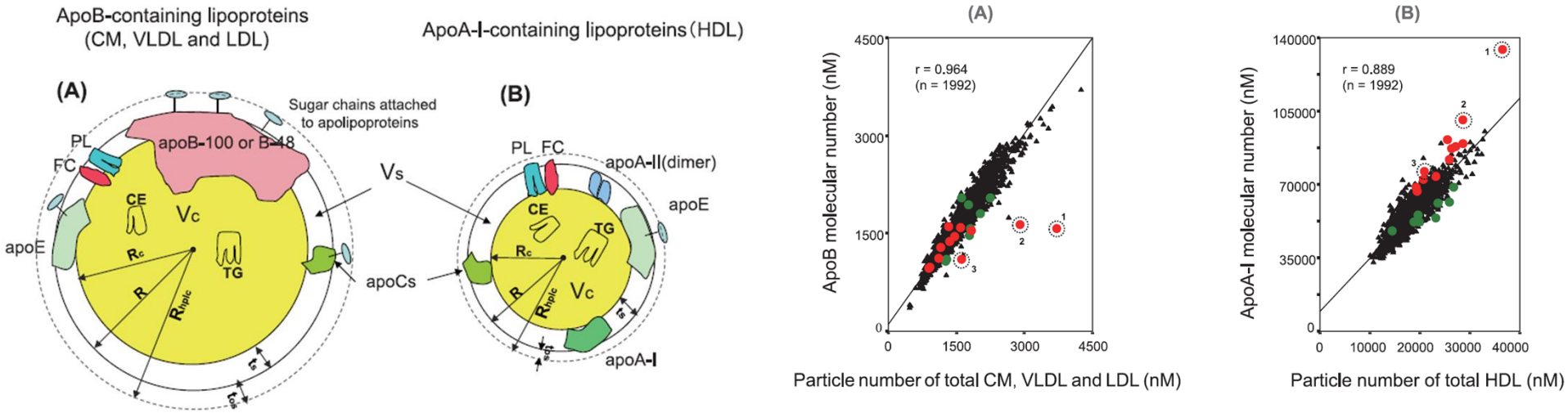
## Theory of Analysis of Lipoprotein Particle Number based on spherical particle model by LipoSEARCH

LipoSEARCH Lipoprotein Profiling Service

A new insight with regard to the theory of analysis of lipoprotein particle number based on spherical particle model by LipoSEARCH has been published by our senior technical advisor, Dr. Okazaki, professor emeritus at Tokyo Medical and Dental University.

Please refer to the following publication for more details.

Recent Advances in Analytical Methods on Lipoprotein Subclasses: Calculation of Particle Numbers from Lipid Levels by Gel Permeation HPLC Using "Spherical Particle Model" Mitsuyo Okazaki and Shizuya Yamashita *J.Oleo Sci. 65, (4) 265-282 (2016)*




Source：J.Oleo Sci. 65, (4) 265-282 (2016)

# 5 Advantages of LipoSEARCH

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1. Provide detailed of lipoprotein profiling data.
   - ◆Cholesterol and triglyceride in 4 major classes (CM, VLDL, LDL, HDL).
   - ◆Cholesterol and triglyceride in 20 sub-classes defined by a particle size.
     - ➡Inclusive quantitative determination of small, dense LDL.
     - *small, dense LDL is considered as a high risk marker of arteriosclerosis.
   - ◆Particle size and particle number.

2. Tiny amount of samples (Human: 45μL* / Animal: 35μL) in blood is only required.
   - ➡Suitable for any research used small animal samples such as mice samples.

* It is not often phenomenon but, reanalysis might be required depending on samples such as a chyle sample. We recommend you to send us ≧100μL (Human) for such requirement if possible. We do not charge for the sample failed to be analyzed due to deficiency of actual sample volume for reanalysis.

3. High reproducibility because explicit measurement method gel filtration ”HPLC” is applied.

4. Any animal samples such as rabbits or monkeys can be applied.

5. Low concentration samples such as medium or CSF can be also applied.

# How LipoSEARCH can be used?

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✔ LipoSEARCH is a useful analyzing tool for
- ◆Evaluating of drug efficacy and mechanism.
- ◆R&D of functional food for dieting.
- ◆Clinical and basic research of clinical condition of metabolic syndrome etc.

# Which research field can be applied for LipoSEARCH?

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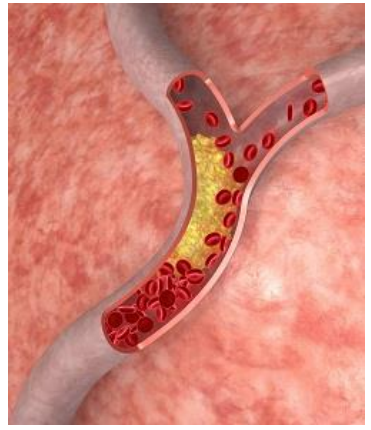
LipoSEARCH can be applied for the following fields.

1. Drug R&D or research in the field of;

✔ hyperlipemia ✔arteriosclerosis ✔ cardio vascular diseases
✔ cerebral stroke ✔ diabetes ✔ metabolic syndrome
✔ NASH (non-alcoholic steatohepatitis)
✔ NAFLD (non-alcoholic fatty liver disease)
✔ hepatitis ✔ depression and Alzheimer's Diseases



2. Functional food R&D
3. Veterinary practice etc.

# Achievement

LipoSEARCH
Lipoprotein Profiling Service

✔LipoSEARCH has been internationally recognized as a reliable lipid profiling service and ≧ 500 references have been published. Search reference. Selected reference list

✔LipoSEARCH has experienced to be applied for

◆Non-clinical test and clinical trial phase II and phase III for new drug development.

◆Evidence of efficacy for functional food development.

# Analyzing items

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| Method | LipoSEARCH (HPLC) |
|---|---|
| Major Classes | ✔ |
| CM | ✔ |
| VLDL | ✔ |
| LDL | ✔ |
| HDL | ✔ |
| Subclasses | ✔ |
| VLDL subclass | ✔ |
| LDL subclass | ✔ |
| HDL subclass | ✔ |
| Particle Size | ✔ |
| Particle Number | ✔* |
| Free Cholesterol | ✔* |
| Phospholipid | ✔* |

* Additional charge will applied.

# HPLC-based system (High performance liquid chromatography、HPLC)

## Mechanism of the profiling system (LipoSEARCH)

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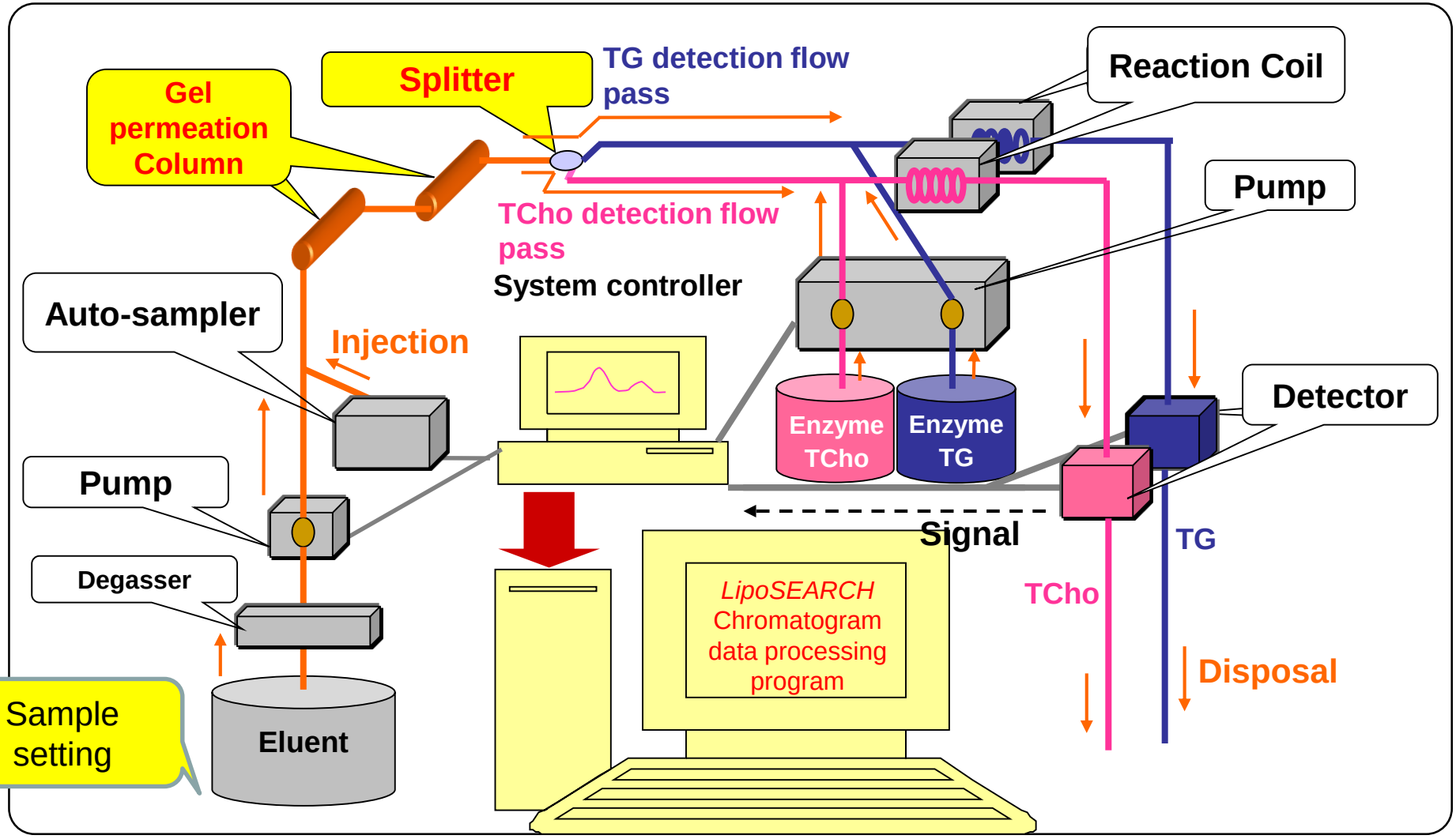
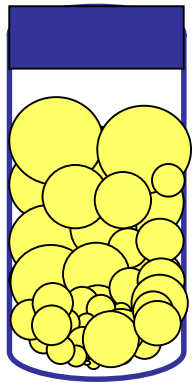


# HPLC-based system (High performance liquid chromatography、HPLC)

# Mechanism of the profiling system (LipoSEARCH)

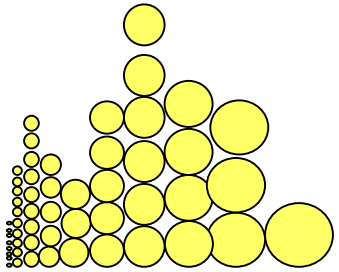
LipoSEARCH Lipoprotein Profiling Service

## Step 1: Sample Separation and Enzymatic reaction



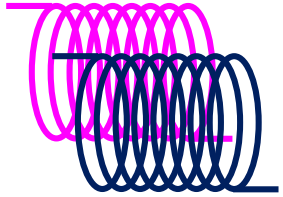
Serum Sample
(10 μL)

Gel
permeation column



Lipoproteins are eluted and aligned by particle sizes.

Aligned lipoproteins are split and drained in two pathways.

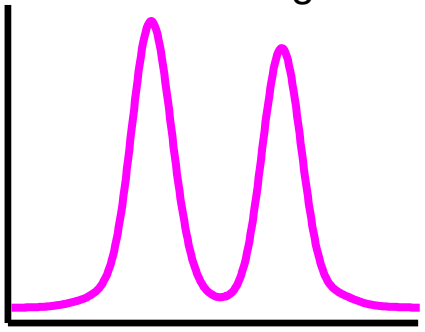


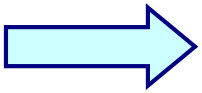
Detect

Detect cholesterol and triglyceride using dedicated enzyme in the coils.

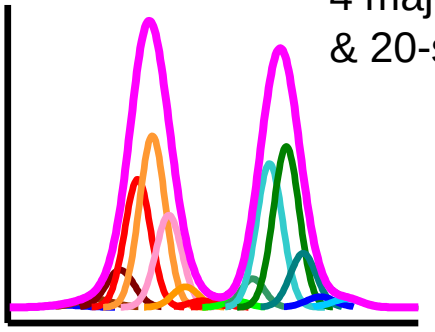
## Step 2: Data Analysis

Chromatogram





Data processing



4 major classes
& 20-subclasses

# Example for data output

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## Total Cho & TG

Unit : mg/dl

| | Total |
|---|---|
| Cho | 237.93 |
| TG | 84.13 |

## Major 4 fractions numeric data

| Class | CM (>80nm) | VLDL (30-80nm) | LDL (16-30nm) | HDL (8-16nm) |
|---|---|---|---|---|
| Cho | 0.05 | 21.36 | 138.21 | 78.31 |
| TG | 0.33 | 46.47 | 24.05 | 13.28 |

## Detailed 20 fractions numeric data

| Peak No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Particle Diameter (nm) | >90 | 75 | 64 | 53.6 | 44.5 | 36.8 | 31.3 | 28.6 | 25.5 | 23 | 20.7 | 18.6 | 16.7 | 15 | 13.5 | 12.1 | 10.9 | 9.8 | 8.8 | 7.6 |
| Sub-Class | | | large VLDL | | | medium VLDL | small VLDL | large LDL | medium LDL | small LDL | very small LDL | | | very large HDL | | large HDL | medium HDL | small HDL | very small HDL | |
| Cho | 0.05 | 0.00 | 0.00 | 0.41 | 2.45 | 6.61 | 11.89 | 38.11 | 52.94 | 31.36 | 10.54 | 4.00 | 1.27 | 1.66 | 2.56 | 19.77 | 26.57 | 16.59 | 6.95 | 4.20 |
| TG | 0.18 | 0.15 | 0.46 | 5.30 | 15.48 | 15.88 | 9.35 | 9.70 | 7.98 | 4.02 | 1.50 | 0.61 | 0.23 | 0.31 | 0.23 | 3.10 | 4.61 | 2.67 | 1.04 | 1.32 |

small, dense LDL

# 20 subclasses

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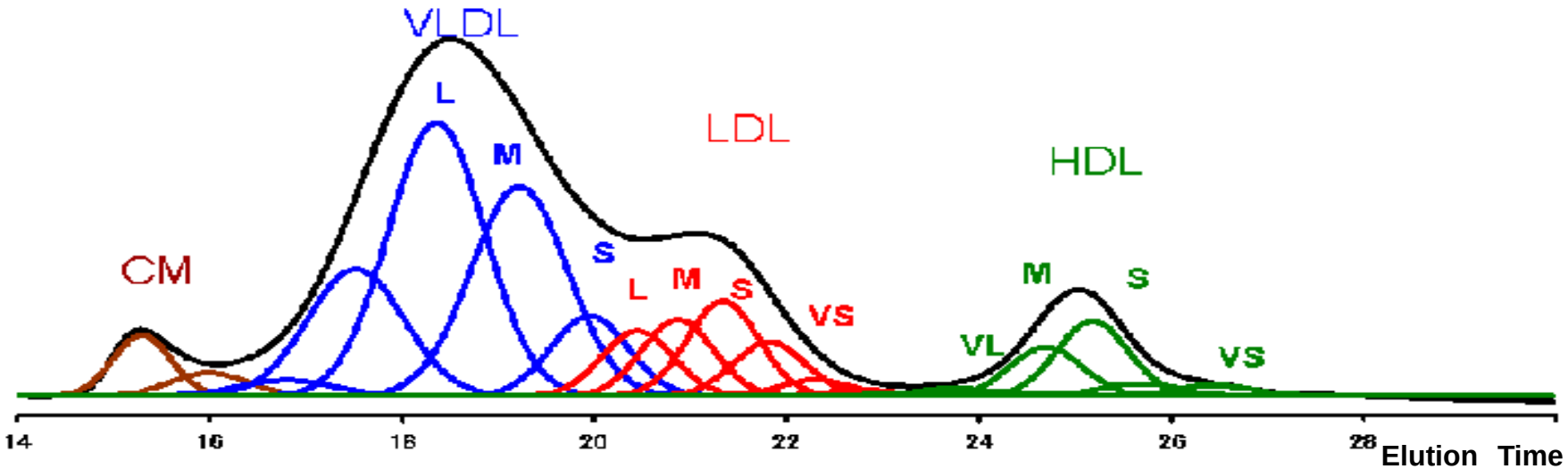


| Major Class | CM >80 nm | | VLDL: 30 - 80 nm | | | | | LDL: 16 - 30 nm | | | | | | HDL: 8 - 16 nm | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Component peak No | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Particle Diameter (nm) | >90 | 75 | 64 | 53.6 | 44.5 | 36.8 | 31.3 | 28.6 | 25.5 | 23.0 | 20.7 | 18.6 | 16.7 | 15.0 | 13.5 | 12.1 | 10.9 | 9.8 | 8.8 | 7.6 |
| Subclass | CM | | L | | | M | S | L | M | S | VS | | | VL | | L | M | S | VS | |

**VL:** Very Large, **L:** Large, **M:** Medium, **S:** Small, **VS:** Very Small

# Clinical meaning may be found in the data.

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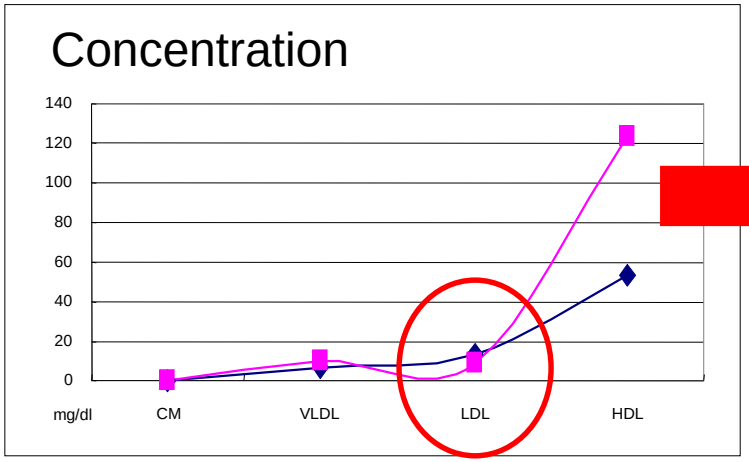
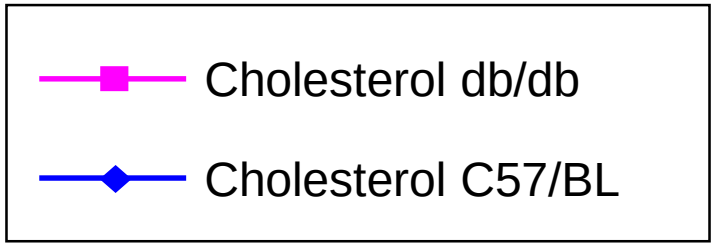
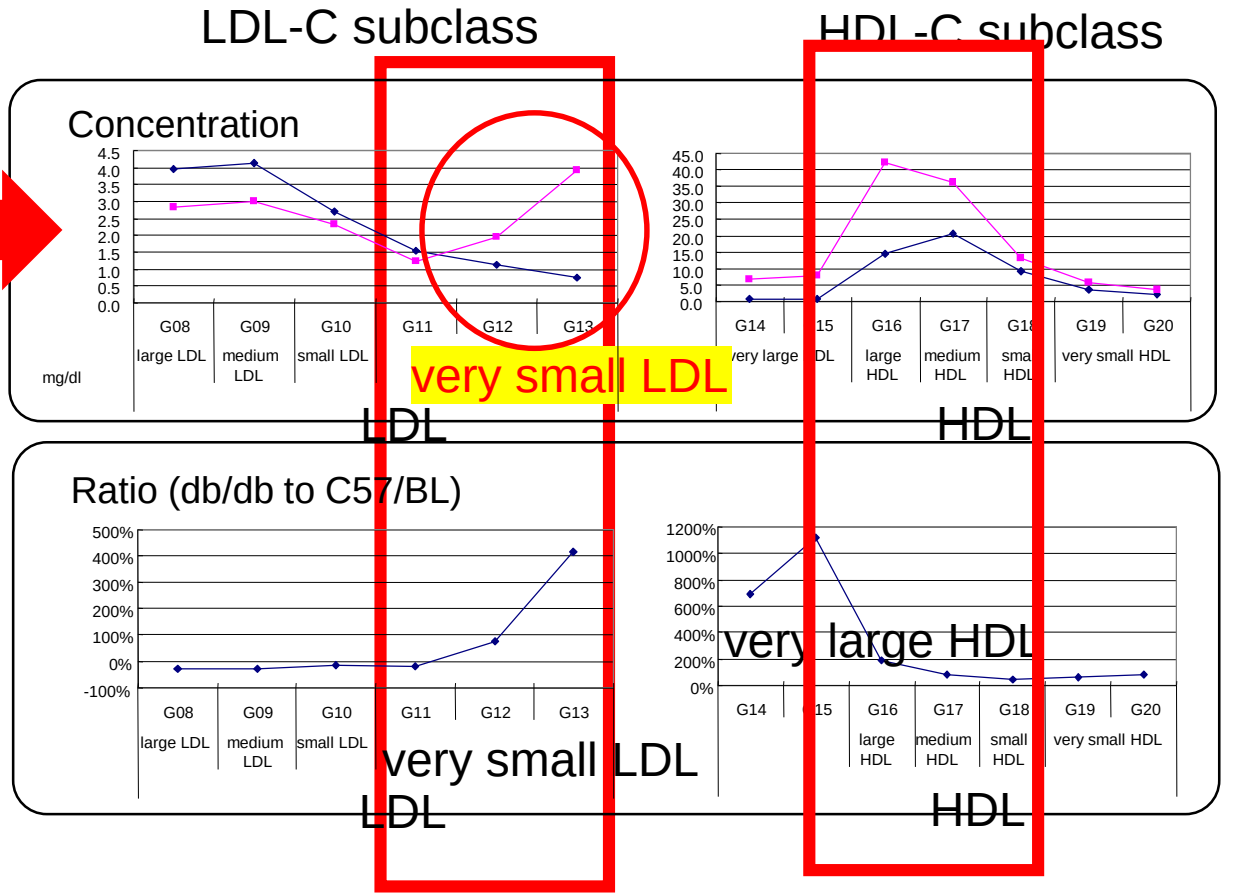
Measurement of 4 major classes

Measurement of cholesterol levels in 20 subclasses (LDL-C and HDL-C region)




IBL Group

# Various samples can be analyzied.

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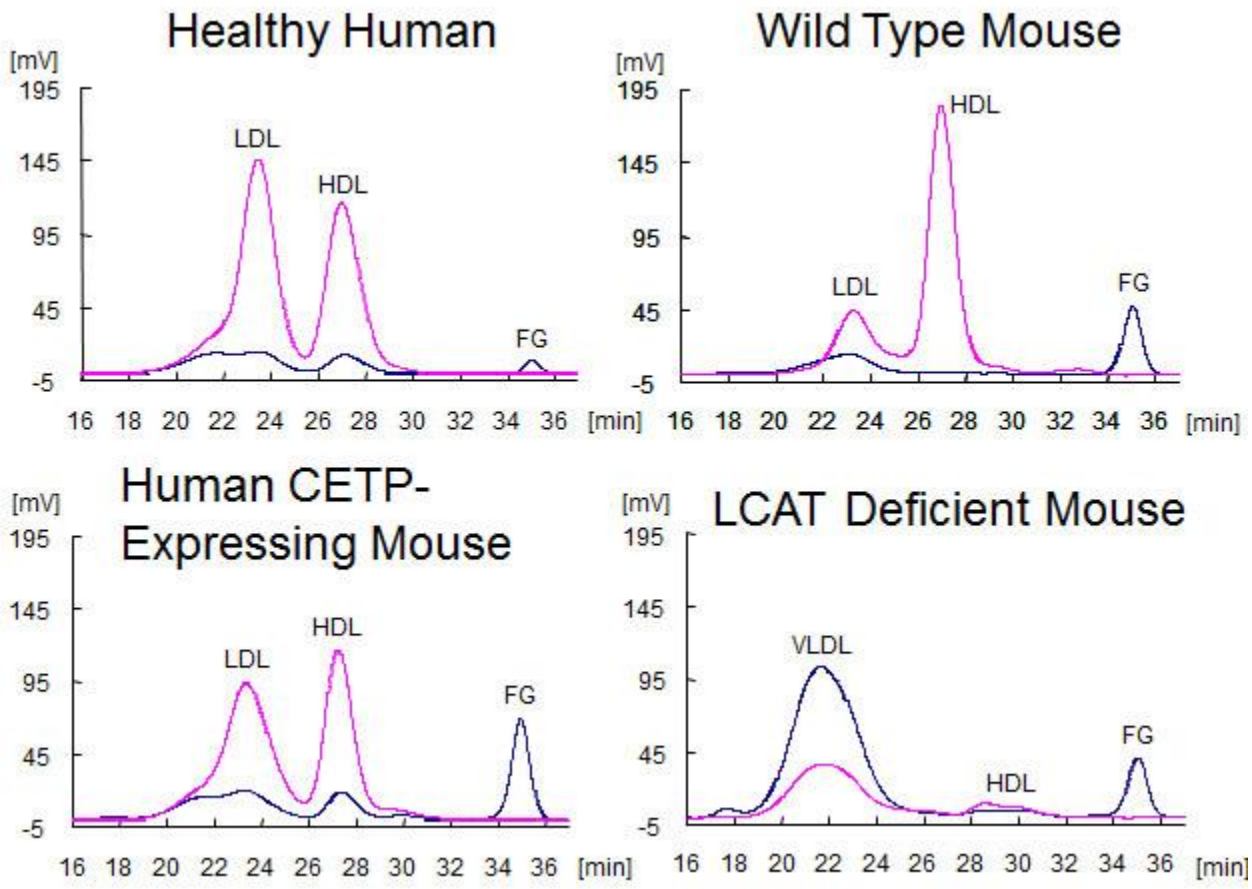
The output result is different from sample to sample.




Source: Wu C., Tsujita M., Okumura-Noji K., Usui S., Kakuuchi H., Okazaki M., Yokoyama S.,
Cholesterol ester transfer protein expressed in lecithin cholesterol acyltransferase-deficient mice, Arterioscler Thromb Vasc Biol. 2002; 22(8): 1347-1353.

# Free Cholesterol and Phospholipids (Option Services)

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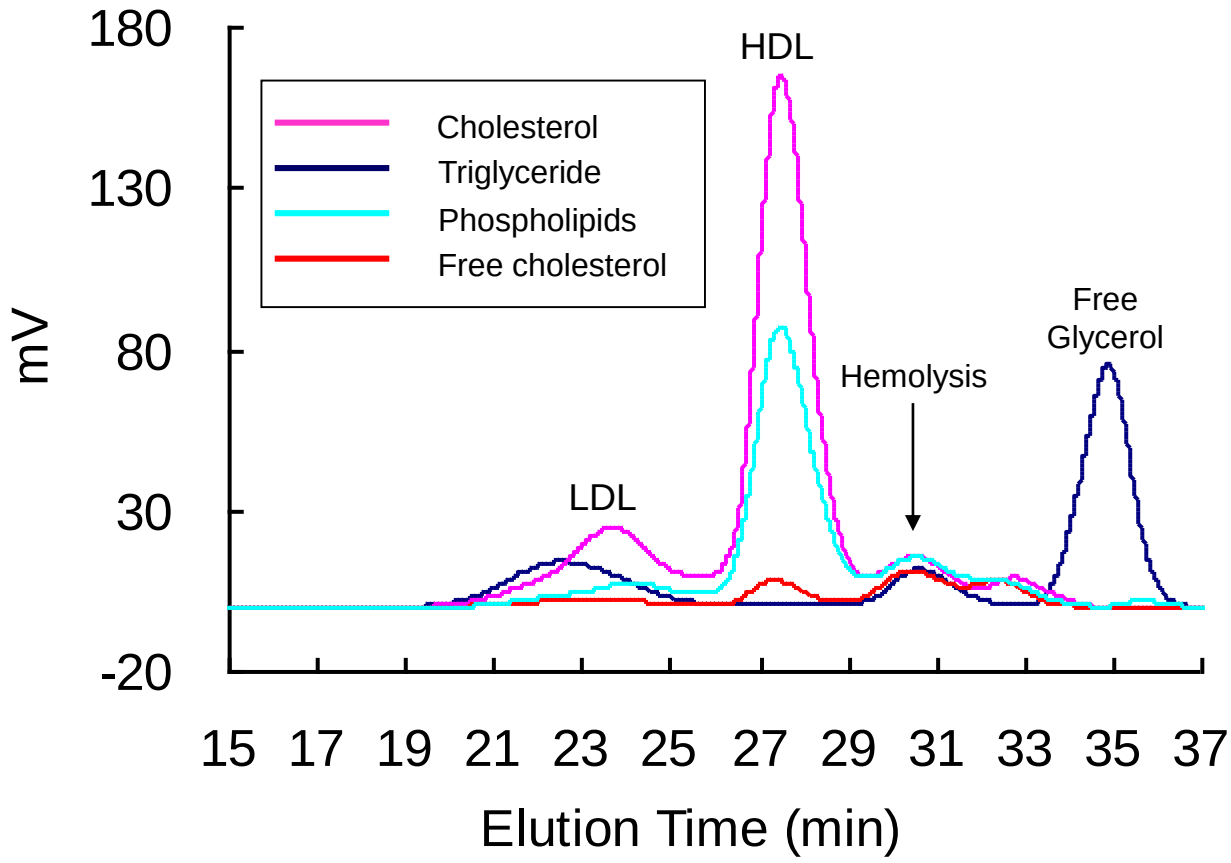
Free Cholesterol and Phospholipids are also measurable with additional charge.



# Preparation of Sample Shipment

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- **Sample volume required**

  - **Serum: Human 45 μL* or more , Animal 35 μL or more (undiluted sample).**

    Leave the sample at room temperature for 30 min after blood collection. Subsequently, after cooling the sample on ice, centrifuge at 3000 rpm for 15 min at 4 ºC. Solidified chylomicrons are often observed in the top separating layer in samples obtained from the fat tolerance test. In such a case, dissolve these thoroughly using a pipette. Use of a blood collection tube with clot activator will facilitate pipetting because the layer of clot activator inserts between the serum and blood clot layer.

  - **Plasma: Human 45 μL* or more , Animal 35 μL or more (undiluted sample).**

    After collection in a plasma separation tube with clotting factor inhibitor, the sample and the inhibitor are mixed by inversion so that they can react sufficiently. After cooling the sample on ice, the plasma is separated by centrifugation at 3000 rpm for 15 min at 4 ºC. For samples obtained from the fat tolerance test, please follow the same procedure as that used with serum samples but use a plasma separation tube containing separating agents.

    * It is not often phenomenon but, reanalysis might be required depending on samples such as a chyle sample. We recommend you to send us ≧100μL (Human) for such requirement if possible. We do not charge for the sample failed to be analyzed due to deficiency of actual sample volume for reanalysis.

  - **For samples of low concentration, such as culture supernatants and cerebrospinal fluid, please contact us before the shipping.**

N.B.

1. **In case that 45 μL(human) or 35 μL(animal) is not available, please enquire us in advance.**
2. Please enquire in advance if you require analysis of samples containing anticoagulant (* Heparin is NOT acceptable as an anticoagulant) or samples that may contain pathogens.
3. We recommend that samples be frozen immediately after the collection and stored at -80 ºC until the shipment. Samples that are repeatedly frozen and thawed are not accepted.
4. Pooled samples should be avoided.

# Preparation of Sample Shipment

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## Packing and shipping

- Supply the sample in a 0.5 to 2.0 mL microtube for centrifugation (e.g., Eppendorf tube) and firmly seal it with Parafilm.
- **Write the ID on each sample tube (e.g., sample name and/or number) using an indelible pen.**
- Pack sufficient cold insulators or dry ice along with your samples.
- If possible, ask the shipping company to keep your samples refrigerated.

N.B.

1. We are NOT responsible for the sample preparation, the arrangement of shipping, any mishandling by the shipping company during the transportation (e.g., spillage during the transportation), and any resultant loss.
2. We will NOT store and/or return your samples.
3. Samples that are repeatedly frozen and thawed are not accepted.

## Consignee/Shipping address

Akita Analysis Center, Immuno-Biological Laboratories Co., Ltd.
100-4, Sunada, Iijima
Akita-shi, Akita 011-0911, Japan
Tel: +81-18-880-5060

- If you use FedEx, we will separately arrange a local chilled transport in Japan. Please note that the above address should be designated as the final destination in your arrangement with FedEx.
- The shipping schedule should be arranged so that the samples can be delivered to the analysis center on weekdays or Saturday. We cannot receive them on Sunday or any national holiday in Japan.

# Preparation of Sample Shipment (Example)

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1

Place shock absorption between sample container and dry ice.

2

Place a cardboard and dry ice on the sample container.

3

Place an additional shock absorption on the top.

4


Close the box with the lid and seal it with a packing tape.

# Effects of freezing and thawing on samples

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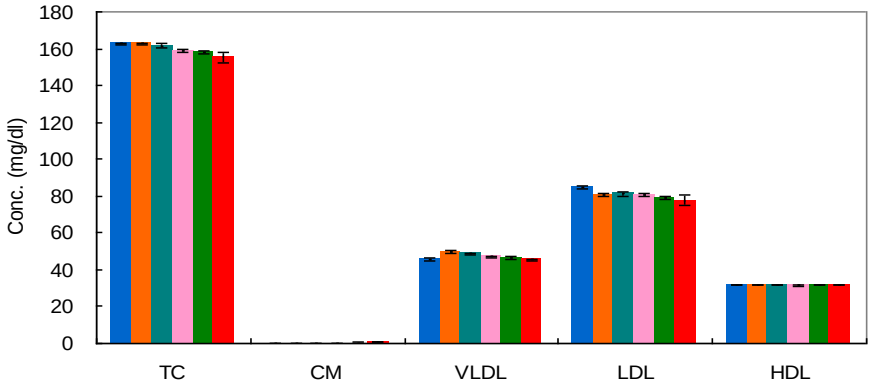
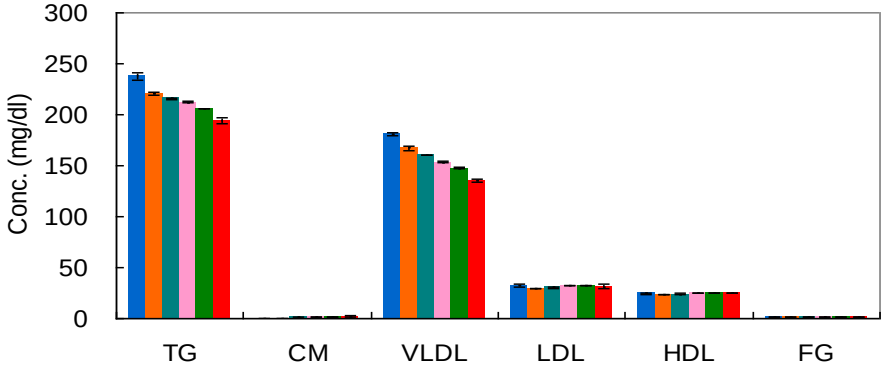
Effects of freezing and thawing on cholesterol level




* Human serum, average of three measurements

| # of times | TC | s.d. | CM | s.d. | VLDL | s.d. | LDL | s.d. | HDL | s.d. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 163 | 0.64 | 0.01 | 0.00 | 45.8 | 0.93 | 84.8 | 1.06 | 32.0 | 0.12 |
| 1 | 163 | 0.47 | 0.07 | 0.01 | 49.8 | 0.77 | 80.7 | 0.78 | 31.9 | 0.19 |
| 2 | 162 | 1.16 | 0.15 | 0.01 | 48.6 | 0.64 | 81.1 | 1.17 | 31.8 | 0.24 |
| 3 | 159 | 0.83 | 0.27 | 0.01 | 46.9 | 0.28 | 80.5 | 0.74 | 31.5 | 0.29 |
| 4 | 158 | 0.83 | 0.40 | 0.02 | 46.5 | 0.51 | 79.3 | 0.74 | 31.6 | 0.08 |
| 5 | 155 | 2.80 | 0.55 | 0.01 | 45.3 | 0.35 | 77.6 | 3.10 | 31.7 | 0.08 |

Effects of freezing and thawing on triglyceride level




* Human serum, average of three measurements

| # of times | TG | s.d. | CM | s.d. | VLDL | s.d. | LDL | s.d. | HDL | s.d. |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 238 | 3.57 | 0 | 0.18 | 181 | 2.03 | 33 | 1.44 | 24 | 0.56 |
| 1 | 220 | 1.9 | 0.4 | 0.16 | 167.0 | 1.66 | 29.4 | 0.50 | 23.6 | 0.27 |
| 2 | 216 | 0.8 | 0.8 | 0.08 | 160.4 | 0.37 | 30.3 | 0.54 | 24.2 | 0.14 |
| 3 | 212 | 1.1 | 1.1 | 0.17 | 153.6 | 0.91 | 32.3 | 0.31 | 25.1 | 0.29 |
| 4 | 206 | 0.4 | 1.6 | 0.26 | 147.5 | 0.98 | 32.2 | 0.27 | 24.8 | 0.46 |
| 5 | 194 | 3.4 | 2.1 | 0.15 | 135.4 | 0.92 | 31.4 | 1.77 | 25.1 | 0.63 |

# FAQ

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Q1: Does the method have any correlation with existing standard method?
A1: The method is correlated with ultracentrifugation method.

Q2: How samples should be stored until the shipping?
A2: Please keep samples at -80C. Although one freeze-thaw cycle is acceptable, the repeated cycles will affect the data.

Q3: Does hemolysis affect the results?
A3: A clear peak appears for hemolysis after HDL and the peak usually does not affect the calculation of HDL data.

Q4: Is there any requirement for feeding animals or subjects?
A4: The method has no particular requirement for the way of feeding. It depends on the study.

Q5: Is there any validation data such as CV data and reproducibility?
A5: Various validation data are available.

/http://www.lipo-search.com/eng/faq/

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## Support Information

# What is Lipoprotein?

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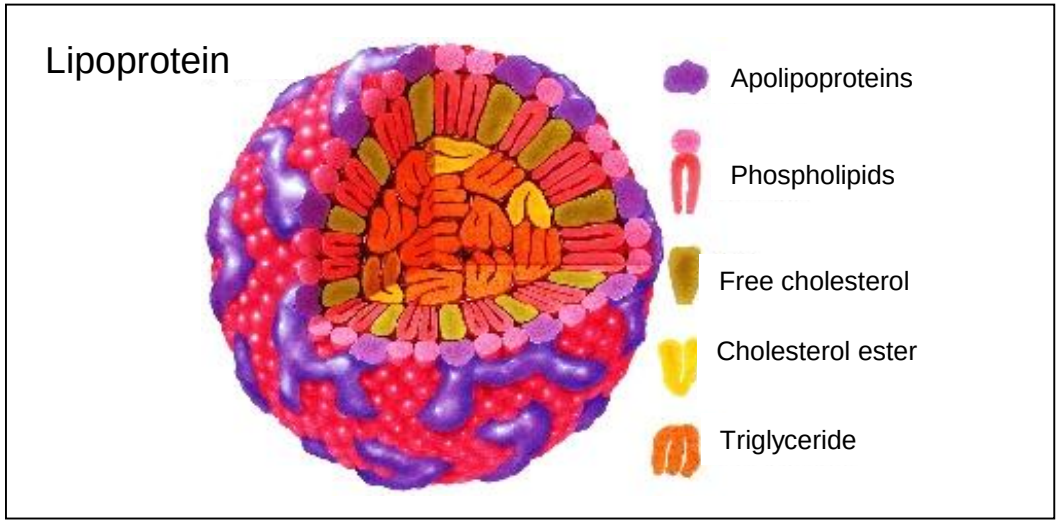


Quoted from http://www.gik.gr.jp/~skj/HL/hyperlipemia.php3

Cholesterol and triglyceride can NOT be flowed in blood steam themselves because they are hydrophobic. They need to have a carrier.

Lipoprotein is hydrophilic carrier of cholesterol and triglyceride to flow in blood stream.

# Classifications of Lipoprotein

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| 4 Classification of Lipoprotein | | CM | VLDL | LDL | HDL | |
|---|---|---|---|---|---|---|
| | | | | Bad Cholesterol | Good Cholesterol | |
| | | Chylomicron | Very Low Density Lipoprotein | Low Density Lipoprotein | High Density Lipoprotein | |
| | | | | | HDL2 | HDL3 |
| Density | | < 0.96 | 0.96 – 1.006 | 1.019 – 1.068 | 1.068 – 1.125 | 1.125 – 1.21 |
| Lipid compositions | TG | 85% | 55% | 10% | 5% | 4% |
| | CE | 5% | 12% | 37% | 18% | 12% |
| | FC | 2% | 7% | 8% | 6% | 3% |
| | PL | 6% | 18% | 22% | 29% | 23% |
| Proteins contained | | 2% | 9% | 23% | 42% | 58% |
| Apolipoprotein compositions | | Apo-I, ApoB-48, ApoC | ApoB-100, ApoC, ApoE | ApoB-100 | Apo-I, Apo-II, ApoC, ApoE | Apo-I, Apo-II, ApoC |

Size become smaller

Density become higher

**TG**: Triglyceride / **CE**: Cholesterol Ester / **FC**: Free Cholesterol / **PL**: Phospholipid

# Lipoprotein Metabolism

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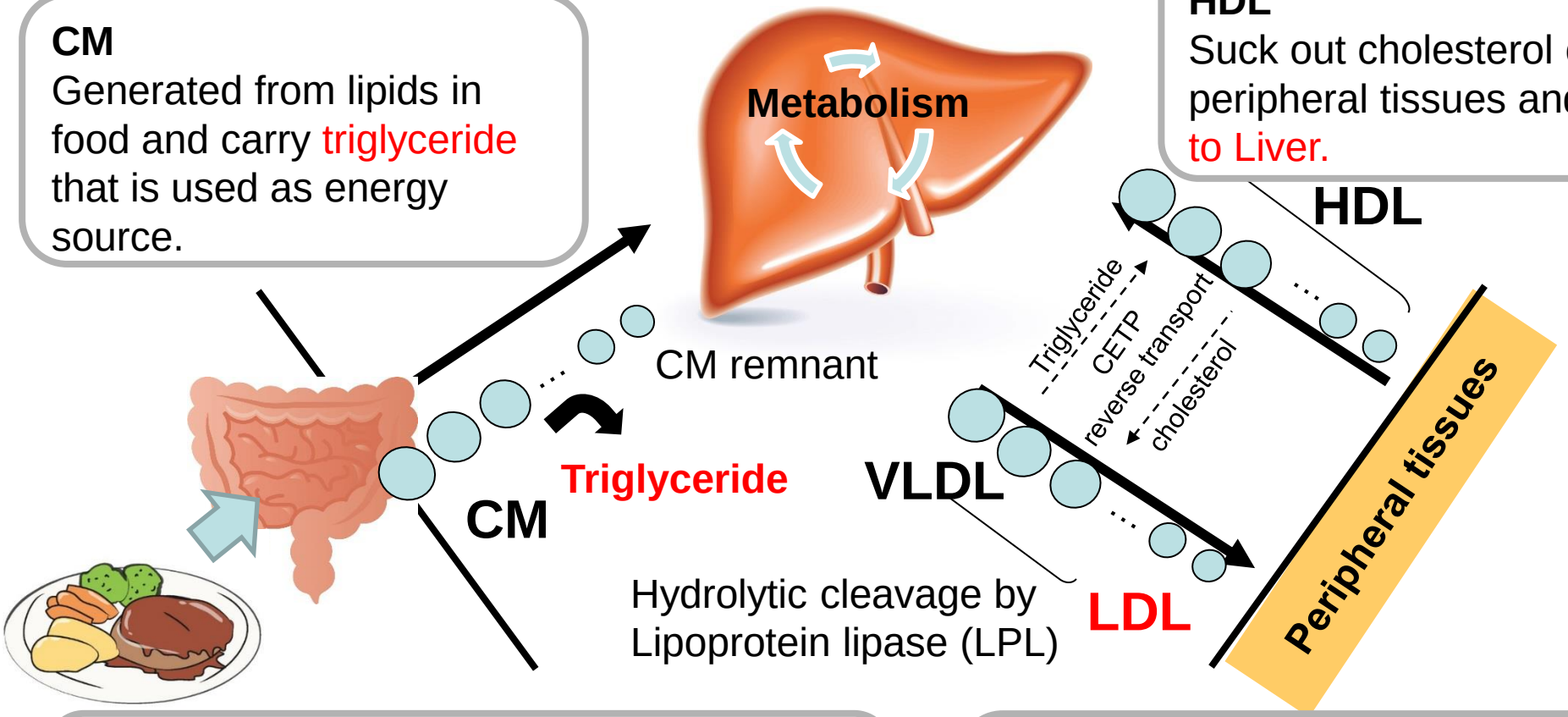
**CM**
Generated from lipids in food and carry triglyceride that is used as energy source.

**HDL**
Suck out cholesterol of the peripheral tissues and return to Liver.




**VLDL**
Synthesized in liver and rich in triglyceride. Lose triglyceride in the course of metabolism and thus becoming LDL.

**LDL**
Generated from VLDL in the course of metabolism and carry cholesterol through the peripheral tissues.

# What is the highest risk factor for cardiovascular event?

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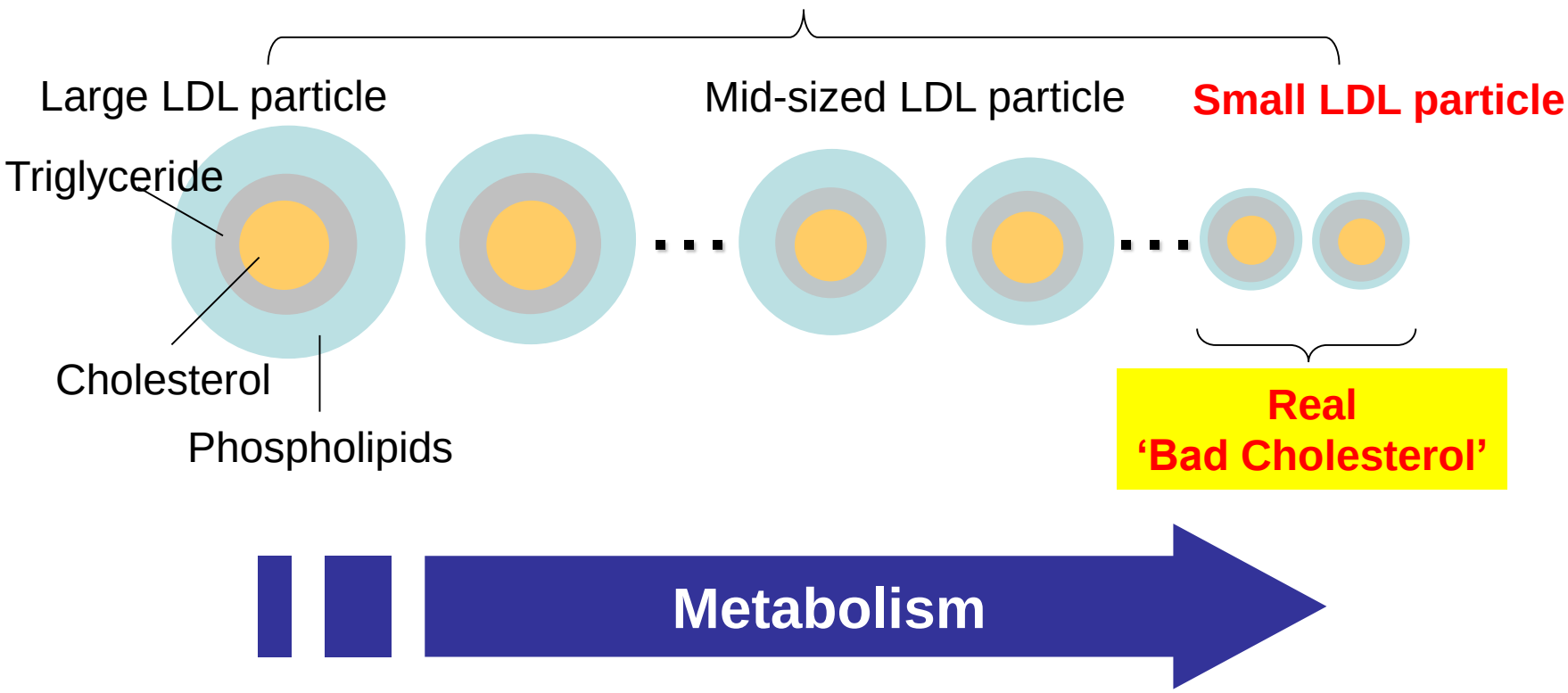
It is suggested that small, dense LDL is the most atherogenic.




Large LDL is metabolized to LDL and it is suggested that the smaller LDL is the most "Bad Cholesterol" because it is believed that it is particularly atherogenic in recent study.

# Research Trend

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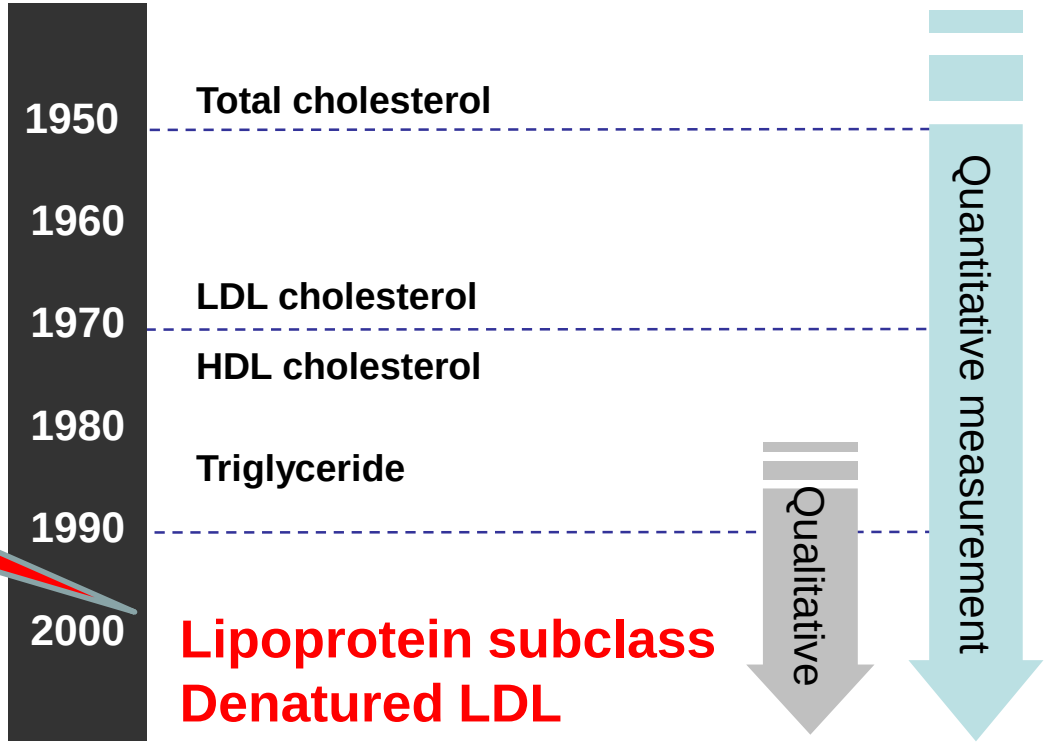
Detailed analysis of Lipoprotein become more important for exploring what is the real risks of cardiovascular diseases.

Need to carefully look into details of LDL (Bad Cholesterol).

LipoSEARCH Lipoprotein Profiling Service

LipoSEARCH has the best profiling system in the world!



# Information about LipoSEARCH

# Contact

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Contact Us
Immuno-Biological Laboratories Co., Ltd.
Email: do-lipo@ibl-japan.co.jp

Note: SLB will be completely merged with its parent company Immuno-Biological Laboratories Co., Ltd (IBL-Japan) as of 1st November 2021. All services provided by SLB will be taken over by IBL. Could you please contact us : E-mail: do-lipo@ibl-japna.co.jp