Chylomicron determination by visual inspection
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Introduction:

Chylomicrons are small, microscopic, lipoprotein (molecule made up of proteins and lipids) particles from the Greek names chylo meaning juice or milky fluid and micron meaning small particle. They have a diameter of approximately 0.5 um. These particles consist of triglycerides (main constituent), phospholipids, cholesterol, proteins, apolipoproteins and fat-soluble vitamins (figure 1).

FIGURE 1: Chylomicron structure and composition (http://gutcritters.com/part-three-dietary-fat-chylomicrons-and-endotoxemia/chylomicrons/)
Chylomicrons form part of a group of lipid transporters called lipoproteins, which includes:

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Abbreviation</th>
<th>Characteristic</th>
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<tbody>
<tr>
<td>Chylomicron</td>
<td>Chylo</td>
<td>Synthesised by enterocytes and deliver energy-rich triacylglycerol</td>
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<tr>
<td>Very low density lipoprotein</td>
<td>VLDL</td>
<td>Synthesised by the liver and deliver energy-rich triacylglycerol (TAG)</td>
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<tr>
<td>Intermediate low density lipoprotein</td>
<td>IDL</td>
<td>Intermediate stage where VLDL is converted into LDL</td>
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<tr>
<td>Low density lipoprotein</td>
<td>LDL (Lethal)</td>
<td>Delivers cholesterol to cells in the body by binding to LDL receptors</td>
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<tr>
<td>High density lipoprotein</td>
<td>HDL (Healthy)</td>
<td>Excess cholesterol is reverse transported back to the liver to be eliminated in bile</td>
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**TABLE 1:** Summary of lipoproteins and their characteristics, arranged in increasing density and decreasing size

Chylomicrons are synthesised in the gastro-intestinal tract and transport dietary or exogenous cholesterol (obtained from outside the body through diet) and triglycerides from the intestines after meals, via the thoracic lymphatic duct into plasma and ultimately to the liver and to other parts of the body, including the adipose, cardiac and skeletal muscle tissue. This process results in chylomicron remnants after the degradation by lipoprotein lipase inside the cells which are then removed by the liver. Lipoprotein lipase hydrolyses triglycerides, releasing free fatty acids which are stored in fat and muscle (figure 2). Under normal circumstances, chylomicron and VLDL half-lives are 10 minutes and 9 hours respectively.
Sufficient levels of lipids, cholesterol and vitamins are essential for normal growth and development. Disturbances in metabolism lead to either increased synthesis of chylomicrons and/or VLDLs or decreased metabolic breakdown causes elevations in triglyceride levels. These disturbances may be dietary indiscretions, genetic mutations of enzymes in the lipid metabolism pathway, including:

- Abnormalities in hepatic VLDL production and chylomicron synthesis
- Dysfunctional lipoprotein lipase-mediated lipolysis (breakdown of lipids)
- Impaired remnant clearance

The clinical significance of abnormal lipid levels in certain lipoproteins may be linked to atherosclerosis. Atherosclerosis is a cardiovascular disease in which lipids plaques and inflammatory cells accumulate on the walls of blood vessels and blocks the arteries.

**Principle:**

The presence of chylomicrons is tested by the qualitative “standing plasma” test also known as the refrigeration test. Specimen is allowed to stand undisturbed for 12-24 hours between 2-8°C. In a refrigerated serum/fluid, chylomicrons are the largest lipoproteins with the least density (figure 3) and rise to form a creamy top layer and the serum clears; very low density
lipoproteins remain dispersed and the serum/fluid becomes turbid. Chylomicrons in this test are visually inspected.

**FIGURE 3:** Density, particle diameter, protein-lipid ratio and size of lipoproteins (http://www.intechopen.com/books/lipoproteins-role-in-health-and-diseases/pathophysiology-of-lipoprotein-oxidation)

**Specimen requirements:**

Pleural fluid (specify type of fluid) and serum SST (serum separator tube)
Transported at atmospheric air temperature (ambience)
Centrifuge upon receipt

**Method:**

When a chylomicron determination is requested in the laboratory the following tests are
usually ordered:
- Serum cholesterol and triglyceride (Optional)
- Fluid Cholesterol and triglyceride
- Fluid appearance
- Fluid supernatant
- Chylomicron electrophoresis

**Results and interpretation:**

The serum cholesterol and triglyceride will be determined on the fluid and on serum if collected/drawn and immediately reported. After completion the fluid sample is labelled with the time it was placed in the fridge to stand undisturbed for the next 24 hours.

**Results after 24 hours:**

The fluid specimen appearance is interpreted 24 hours after the sample was placed in the fridge. Interpretation should always be confirmed by a second person, senior or pathologist and results entered as either:
- Blood-stained
- Clear
- Green turbid
- Milky / turbid

The supernatant is entered as either:
- Absent
- Present

To indicate the presence of a creamy supernatant as seen below (figure 4).
FIGURE 4: Visible chylomicrons in blood or fluid sample (LP-CL-087)

Clinical significance:

Chylothorax:

Pleural fluids with a cloudy/turbid supernatant can be used to test for the presence of chylomicrons to distinguish between pseudochylothorax effusions (chylomicrons absent) and true chylothorax effusions (chylomicrons present). Chylothorax or chyle leak is a type of effusion where lymphatic fluid accumulates in the pleural cavity either by disruption or obstruction of the thoracic duct. Clear supernatant are often associated with the absence of chylomicrons, but confirmation using the standing test is required. Pleural fluid triglyceride determination is also recommended. Pleural fluids with triglycerides values greater than 1, 24 mmol/l can be classified as a chylothorax with high-specificity while triglyceride values less than 0, 56 mmol/l indicate a nonchylous effusion. Cholesterol measurements may be helpful for specimens with equivocal results (neither positive nor negative). Pseudochylothorax
effusions often have high cholesterol levels greater than 6.46 mmol/l while true chylo effusions will have low cholesterol values.

**Chylomicron retention disease:**

Chylomicron retention disease is a rare, hereditary, autosomal recessive disorder that affects the absorption of dietary lipids, cholesterol and certain fat-soluble vitamins, A,D,E and K due to a lack of chylomicron transportation. Mutations in the *SAR1B* gene impair the release of chylomicrons into the blood stream.

The signs and symptoms of chylomicron retention disease appear in the first few months of life and they include:

- Failure of weight-gain and growth
- Diarrhoea
- Vomiting
- Steatorrhea (fatty stools)
- Hypocholesterolemia (low blood cholesterol)

Later in life:

- Impaired nervous system
- Decreased reflexes (hypoflexia)
- Decreased ability to feel vibrations

**Additional testing:**

Chylomicron electrophoresis for confirmation and quantification.
References:

Handling fluid samples for chylomicron determination (LP-CL-087 Autolab SOP)

http://bjhlab.testcatalog.org/show/2168

http://courses.washington.edu/conj/bess/cholesterol/liver.html

http://emedicine.medscape.com/article/126568-overview#aw2aab6b2b2

http://en.wikipedia.org/wiki/Chylomicron


http://ltd.aruplab.com/Tests/Pub/0098457

http://medical-dictionary.thefreedictionary.com/chylomicron

http://www.merckmanuals.com/professional/endocrine_and_metabolic_disorders/lipid_disorders/hypolipidemia.html


http://www.omim.org/entry/246700


http://www.rightdiagnosis.com/c/chylomicron_retention_disease/intro.htm#whatis