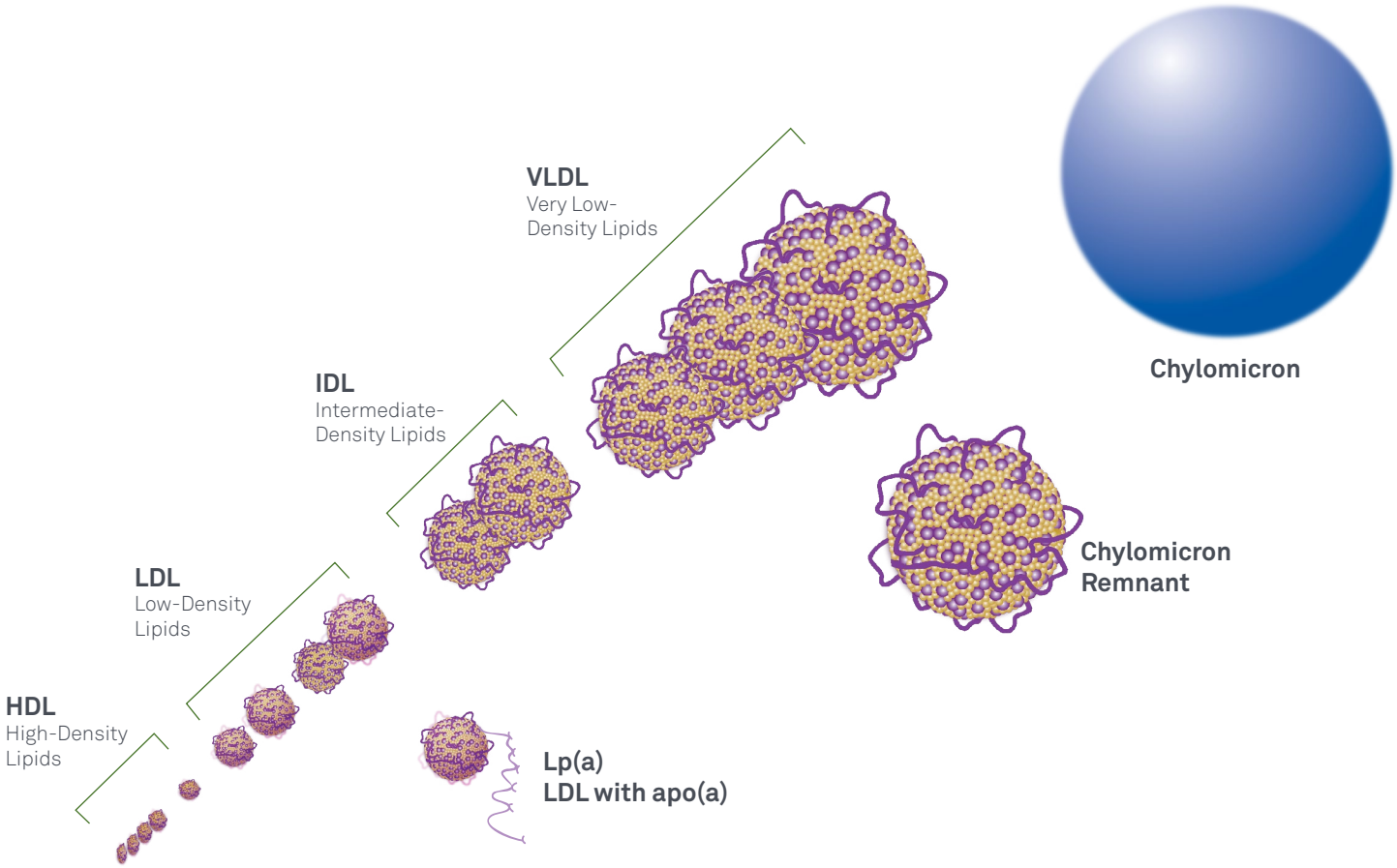
Quest Diagnostics™

Cardio IQ® Ion Mobility

The Latest Evolution in Lipoprotein Fractionation



For Physicians

Evolution of Lipoprotein Subfractionation

Fractionation of lipid subclasses has been used to gain additional insight for management of cardiovascular disease (CVD) in at-risk patients for over 15 years.

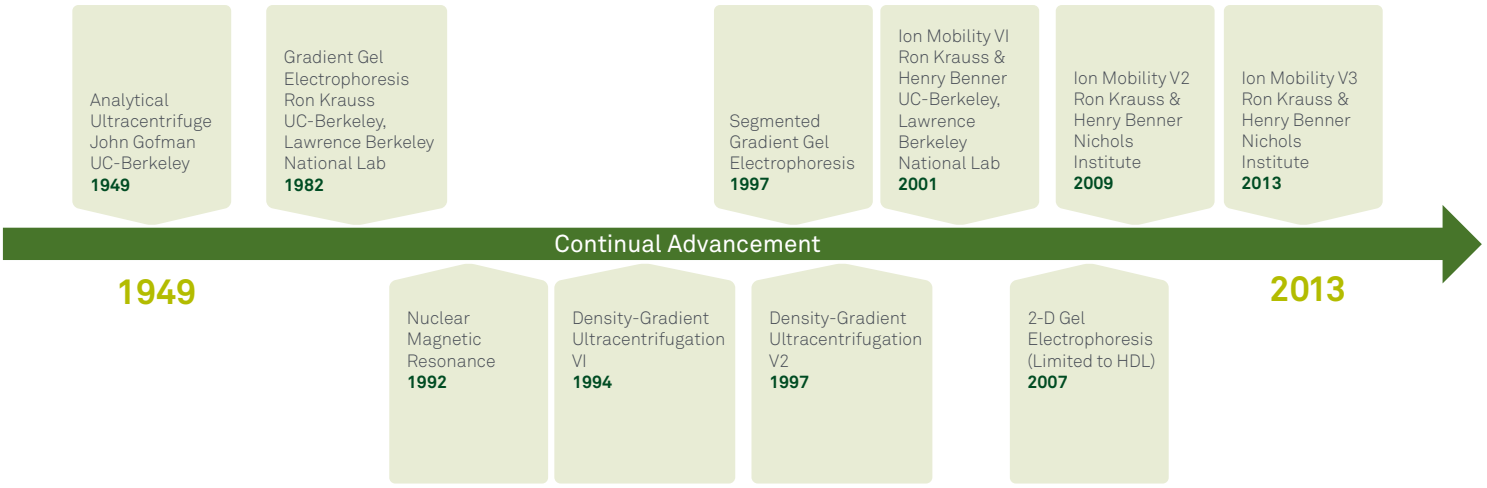
LDL and HDL subclasses have strong scientific literature support,[1-3, 5-15] with a legacy of NIH-funded studies that show lipid subclasses are predictive of short- and long-term CVD risk, atherosclerotic progression, and multiple intervention events.

The insights provided by the lipid subclasses allow for a customized approach to CVD risk management that may ultimately lead to improved patient outcomes.

Since the initial analytical ultracentrifuge characterization of lipoprotein subclasses by Dr. John Gofman at the University of California, Berkeley,[1] a number of lipid fractionation methods have been developed, including density-gradient ultracentrifugation, particle analysis by spectrum, and gel, gradient gel, and 2-D gel electrophoresis.

While these various technologies each had unique strengths, they all represented some degree of compromise between capturing all lipoprotein types, separating the lipid subclasses with high resolution, and delivering direct quantification of the amount of particles within each lipid subclass.

Cardio IQ® Ion Mobility is the latest technology evolution, with a pedigree reaching back to the first lipoprotein characterization work at University of California, Berkeley



The Latest Technology: Cardio IQ® Ion Mobility

Cardio IQ Ion Mobility fractionation is the latest technological evolution in advanced lipid subclass measurement. It combines high-resolution separation of the full spectrum of lipoprotein particles, along with direct quantification of particles in each lipid subclass fraction.

Cardio IQ Ion Mobility separation allows lipoprotein particles to be characterized without any modification of the particles that could potentially impact their apparent size. Ionized lipoprotein particles are electrophoretically separated into gas-phase, distinguishing lipoprotein particles on the basis of size (see Figure 1). Size-selected particles are detected and counted by light scattering.

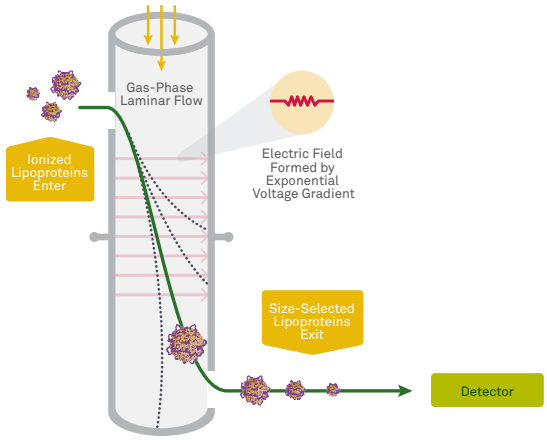


Figure 1. Cardio IQ Ion Mobility separation of lipoprotein
Ionized lipoproteins migrate across a laminar gas-phase flow based on size and electrical field. Only a single size of lipoprotein will exit the field and be isolated (green line) at any point during the voltage gradient; larger and smaller lipoproteins (dotted black lines) are not collected. As the voltage ramps across the gradient, all of the lipoproteins are captured.

Cardio IQ Ion Mobility Advantages

Cardio IQ Ion Mobility represents the future of advanced lipid analysis in clinical practice. By moving beyond the past compromises of other advanced lipid subclass measurements, this tool provides physicians with increased insights to better manage treatment decisions for their patients.

Cardio IQ Ion Mobility is strongly supported by literature and experts in the field[3, 5-15] as the leading method for lipoprotein size assessment. It is being proposed as the new standard in the field. Dr. Ron Krauss, developer of segmented gradient gel technology, developed Cardio IQ Ion Mobility as the next generation in lipid subclass separation.

Cardio IQ Ion Mobility provides:

- Direct, accurate, and reproducible measurement of lipoprotein particles[8]
- Insights that allow customization of therapy for potential improvement in patient outcomes

Clinical Utility

The landmark 2008 Malmö study[3] provided evidence, using Cardio IQ® Ion Mobility subfractionation, that LDL particle number (LDL-P), and Small and Medium LDL particles were associated with higher CVD risk.

Further research published in June and September 2015, again using Ion Mobility technology, provided data that the measurement of LDL-P can identify intermediate-risk patients at risk for CVD events.[4,5] Both clinical studies provide evidence that LDL-P by Ion Mobility provides additional insight over and above standard risk factors for intermediate-risk patients.

Ion Mobility Analysis of Lipoprotein Subfractions Identifies Three Independent Axes of Cardiovascular Risk

Musunuru K, *et al*. *Arterioscler Thomb Vasc Biol*. 2009;29:1975-1980.

The Malmö Diet and Cancer Study[3] indicates that Ion Mobility-determined subclasses have been associated with increased CVD risk. An analysis of 4,594 initially healthy men and women (mean follow-up 12.2 years, 377 incident cardiovascular events, with 206 being coronary events) showed an increase in the number of LDL particles, Small and Medium LDL subclasses from Ion Mobility, along with elevated triglycerides, to be positively associated with increased event risk. This same study revealed Large HDL subclass levels to be inversely associated with event risk, thus supporting the cardioprotective aspect of high HDL-C. This finding is consistent with previous literature defining ALP.

Low-Density Lipoprotein Particle Number Is Associated With Cardiovascular Events Among Those Not Classified Into Statin Benefit Groups

Melander O, *et al*. *J Am Coll Cardiol*. 2015;65(23):2571-2573.

Current guidelines from the American Heart Association/American College of Cardiology define 4 groups of patients who should receive statin therapy.[4]

An analysis of 1,919 participants from the Malmö Diet and Cancer Study[5] not classified into 1 of the 4 statin benefit groups had baseline levels of LDL-P and other lipoprotein subfractions by Ion Mobility measured. The associations of LDL-P and lipoprotein subfraction levels with incident CVD events (myocardial infarction, coronary revascularization, ischemic stroke, or CVD death) were assessed with adjustment for established CVD risk factors, including standard lipid measurements.

After a mean follow-up of 16.2 years, 88 (4.6%) participants had a first CVD event. LDL-P, LDL-small, and LDL-medium were all associated with incident CVD after adjusting for age, sex, LDL-cholesterol, HDL-cholesterol, triglycerides, ApoB, systolic and diastolic blood pressure, antihypertensive medication use, and smoking.

The conclusion from the study showed that lipoprotein subfractions were independently associated with CVD among subjects not classified into 1 of the 4 statin benefit groups, and therefore, LDL-P may be useful for patients and clinicians when deciding on treatment choices.

Atherogenic Lipoprotein Subfractions Determined by Ion Mobility and First Cardiovascular Events After Random Allocation to High-Intensity Statin or Placebo. The Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) Trial

Mora S, *et al*. *Circulation*. 2015;132:2220-2229.

Since CVD events can occur in people with low LDL-C, a clinical trial utilizing the JUPITER[6]* participants was undertaken to determine if Ion Mobility measured lipoproteins or their subfractions predict CVD events in a population with low LDL-C and high hs-CRP. A prospective cohort of 11,186 healthy individuals with LDL-C <130 mg/dL and hs-CRP ≥2 were enrolled; baseline and on-treatment (rosuvastatin) lipoprotein subfractions were measured; and the patients were followed for a mean of 1.9 years (post hoc analysis).

The study revealed in the placebo arm that the measurement of LDL-P by Ion Mobility provides additional insight over and above standard risk factors for intermediate-risk patients. After full adjustment for standard risk factors, including a lipid panel, LDL-P predicted a 16% increase in CVD events per each standard deviation increase of LDL-P (adjusted HR per SD, 1.16, 95% CI 1.02-1.32, P = 0.028). Most LDL subfractions were also shown to be significantly associated with CVD events. In the rosuvastatin treatment arm, on-treatment level of atherogenic particles can contribute to residual risk for CVD and all-cause death during statin therapy, but due to the small number of primary CVD events, the results will need to undergo further evaluation in other studies.

In conclusion, Ion Mobility can identify an at-risk population among low-risk patients with elevated hs-CRP. Further research is required to determine the full relationship between LDL-P and CVD events.

*The JUPITER study is a large placebo-controlled randomized, double-blind clinical trial that enrolled asymptomatic individuals, (women ≥60 years, men ≥50 years) without prior history of CVD and with LDL-C <130 mg/dL and hs-CRP ≥2.0 mg/L. The study was designed to measure rosuvastatin (Crestor) in primary prevention of cardiovascular disease (original publication 2008). Exclusion criteria included triglycerides ≥500 mg/dL, current use of hormone therapy, and previous or current use of lipid-lowering therapy or immunosuppressant agents. Ion Mobility was performed on baseline and month 12 samples (n= 11,186).

Treatment Options†

Treatment via pharmaceutical options, such as statins, niacin, or fibrates, as well as lifestyle changes, has been impactful in correcting ALP,[16] i.e., reducing LDL-particle numbers, changing the distribution of LDL-particles from atherogenic small LDL to larger LDL-particles, and shifting small HDL particles to large HDL particles associated with cardio-protective mechanisms (see Figure 2). The high-resolution subclass separation provided by Cardio IQ Ion Mobility allows healthcare practitioners to follow the change in the lipid profile as the patient responds to therapy. This provides the opportunity to evaluate treatment efficacy and optimize the aggressiveness of therapy in a manner that is personalized to the patient.

†This information is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

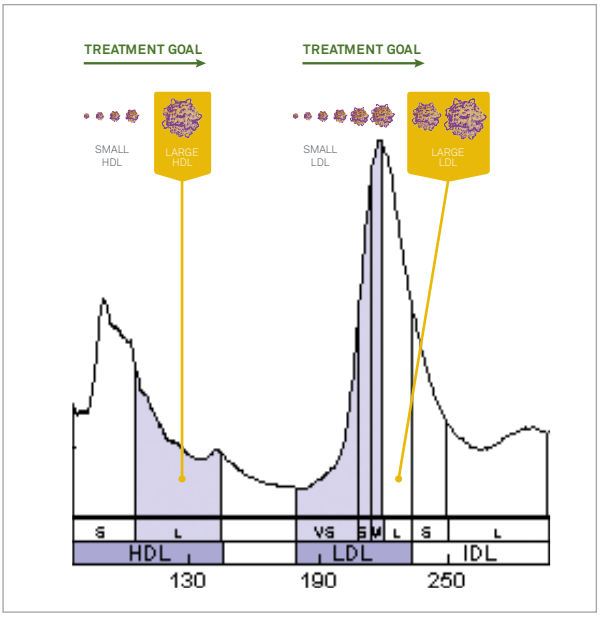


Figure 2. Key clinical management subclasses and treatment strategy

The Cardio IQ® Ion Mobility Report

To provide the data that contribute most to the determination of an individual's cardiovascular risk, Quest Diagnostics provides the Cardio IQ Report (see Figure 3).

The 4 key subclasses from the Cardio IQ Ion Mobility analysis that are most important for clinical management are displayed on the first summary page of the Cardio IQ report in the lipoprotein subfractionation section.

- LDL Particle Number
- LDL Small
- LDL Medium
- HDL Large

Patient Information			Specimen Information	Client Information
Age: 63	Fasting: Y	Gender: M	Collected 04/02/2018	

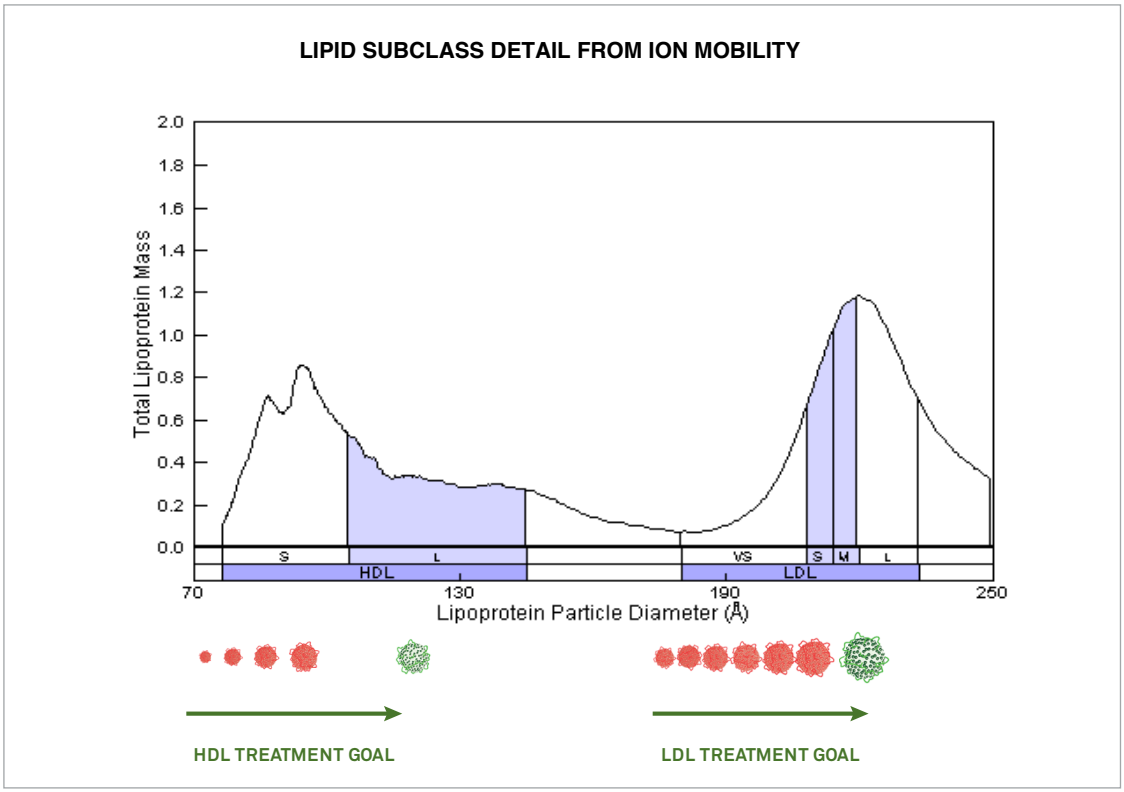
Test Name	Units	Result and Risk Category			Result From	Risk Category Ranges		
		Optimal	Moderate	High		Optimal	Moderate	High
Lipid Panel								Lab: EZ
CHOLESTEROL, TOTAL	mg/dL	166				<200	200-239	>=240
HDL CHOLESTEROL	mg/dL	61				>=40	N/A	<40
TRIGLYCERIDES	mg/dL	81				<150	150-199	>=200
LDL-CHOLESTEROL	mg/dL	89				<100	100-129	>129
CHOL/HDLC RATIO	calc	2.7				<=3.5	3.6-5.0	>5.0
NON-HDL CHOLESTEROL	mg/dL	105				<130	130-159	>159
Lipoprotein Subfractions								Lab: EZ
LDL PARTICLE NUMBER	nmol/L		1203			<1138	1138-1409	>1409
LDL SMALL	nmol/L			236		<142	142-219	>219
LDL MEDIUM	nmol/L			373		<215	215-301	>301
HDL LARGE	nmol/L	9454				>6729	6729-5353	<5353

Figure 3. Summary showing key LDL and HDL clinical management factors

This example represents the resulting report for an order of 91716(X) Lipid Panel and 91604(X) Ion Mobility.

The Cardio IQ® Ion Mobility Report

The detail page graphically depicts the Ion Mobility profile trace and the full spectrum of HDL and LDL lipid subclasses in high resolution. It also reports the LDL pattern and LDL peak size for easier interpretation.



Test Name	Units	Result with Risk Category			Result from	Risk Category Ranges		
		Optimal	Moderate	High		Optimal	Moderate	High
Lipoprotein Subfractions								**Lab: EZ**
LDL PATTERN	Pattern	A				A	N/A	B
LDL PEAK SIZE	Angstrom		222.9			>222.9	222.9-217.4	<217.4

Figure 4. Cardio IQ Ion Mobility detail

High tertile cut points are based on a reference range population. Risk of CVD events is based on a reanalysis (unpublished) of the data presented in Musunuru *et al*. ATVB 2009;29:1975-1980.

Cardio IQ® Ion Mobility: A More Powerful Approach of Lipid Subclass Characterization

By taking into consideration a more powerful risk assessment based on total LDL particles and key lipid subclasses, healthcare practitioners can identify residual risk not revealed by the Lipid Panel or the Lipoprotein Phenotype Pattern B.

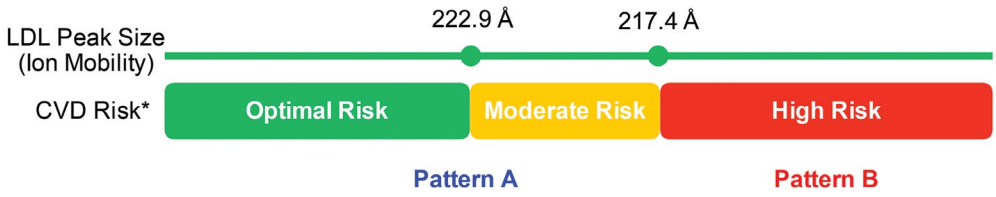


Figure 5. LDL Phenotype versus CVD Risk.

Priorities in Interpretation and Management of Key Clinical Indicators

1

What is the total LDL-particle number? Does it indicate residual risk?

- Consider degree of risk when formulating aggressiveness of therapy
- Follow progressive lowering of particle number to:
  - Gauge patient response to therapy and optimize as needed, and
  - Track progress toward goal

2

What is the quantitative amount of Large HDL subclass within the respective risk category?

- Consider HDL-raising strategy
- Follow progressive increase of particle concentration to:
  - Assess patient response to therapy and optimize as needed, and
  - Assess patient response toward goal

Cardio IQ® Ion Mobility: Lipoprotein Analysis Without Compromise

Cardio IQ Ion Mobility:

- Measures the full spectrum of lipoprotein subfractions and reports those that provide the strongest indicators for cardiovascular risk[3]
- Provides direct, accurate, and reproducible measurement of lipoprotein particles
- Offers insights that allow customization of therapy

Ion Mobility characterization of lipoproteins enables comprehensive insights for physicians to manage treatment decisions for their patients.

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