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High and low density lipoprotein cholesterols reference materials different dissociation degrees stability influence research

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ABSTRACT

Serum high density lipoprotein cholesterol reducing and low density lipoprotein cholesterol rising are clearly cardiovascular disease independent risk factors. Therefore, demands on HDL-C and LDL-C detection system stability are constantly increasing. The paper just discusses on HDL-C and LDL-C detected methods, gets that current most universal applied and highest measurement precise method is homogenous method, on this basis, the paper designs A(HDL)/A(LDL)as specific evaluation indicators different reference materials represented HDL and LDL dissociation degrees experiments in automatic analyzer. By experiment result data, it is clear that:NUST-9 selectivity is not ideal, it cannot arrive at certain experiment demand ;NUST-6to HDL selective dissociation efficiency is not ideal; surfactant B-66 to HDL selective dissociation is good, and its suppression effects on LDL is good, when B-66 concentration is 6g/L, its suppression dissociation efficiency on LDL and dissociation on HDL are the best;With A-90 concentration increasing, dissociation difference almost doesn't change, and dissociation extent changes for HDL is very small, suppression dissociation efficiency on HDL is very well, but dissociation degree on LDL gets bigger.

KEYWORDS

Homogenous method; Dissociation degree; Detection system; Stability; Specific evaluation indicator.

INTRODUCTION

Atherosclerosis and disorder of lipid metabolism, especially for concentration of cholesterol of plasma rising, they are closely linked, clinically detected serum cholesterol contents as indicators to diagnose atherosclerosis and disorder of lipid metabolism. The paper on this basis, targeted at HDL-C and LDL-C detected methods, it makes discussion, the purpose is to explore different detection systems stability of synchronization, in the hope of providing references for relevant clinical symptoms diagnosis and prevention and curing.

For high-low density lipoprotein cholesterols detection methods research, many people have made efforts. Among them, Tang Ji-Bin etc. (2014)pointed out that high density lipoprotein cholesterol was one of regular blood lipid detection important items, basically it adopted direct method to detect in full automatic analyzer, and direct detection method mainly contained immune-separation、polyanionic surfactant method 、PEG enzymatic modification and clearance, every method had its own advantages and disadvantages.[1] Dong Jun etc.(2006)established a kind of new anticipation to be used as reference method of high density lipoprotein cholesterol and low density lipoprotein cholesterol correct detected method, and established serum HDL-C and LDL-C correct detected methods, which could be used as HDL-C and LDL-C detection reference methods.[2] Guo Wei etc.(2013)on the basis of reviewing whole cholesterol detection development history, detailed introduce cholesterol detection standardization, put forward that he hoped there were more clinical laboratories to participate in cholesterol standardization works, and be referenced in other clinical laboratories detection items standardization.[3]

The paper on the basis of formers research, discusses HDL-C and LDL-C detected methods, focuses on analyzing homogenous method, designs a kind of experiment that uses NUST-9、NUST-6、B-66 and A-90 four kinds of reference material to affect HDL and LDL dissociation degrees in automatic analyzer, in the hope of exploring different detection methods stability of synchronization from experiment data.

HIGH, LOW DENSITY LIPOPROTEIN CHOLESTEROL DIAGNOSTIC REAGENT DEVELOPMENT OVERVIEW

Yan Sheng-Kai (2002)pointed out that atherosclerosis (AS)、coronary heart disease(CHD)occurrence rate was in negative correlation with serum high density lipoprotein cholesterol (HDL-C)level, and was in positive correlation with low density lipoprotein cholesterol (LDC-C)level[4]. Research thought that HDL-C(<40mg/dL)was main risk factor of CHD, and high HDL-C(>60mg/dL)was regarded as negative risk factor, which still took LDL-C level reduction as primary goal of CHD prevention and curing[5]. Therefore, it is clear that checking serum HDL-C and LDC-C levels had very big significances in clinical diagnosis. In the following, it overviews HDL-C detected method and LDL-C detected method, in the hope of providing theoretical references for CHD prevention and curing as well as diagnosis.

HDL-C detected method

Detecting HDL-C methods are various, Chinese current clinical and laboratory directly detecting HDL-C methods can be classified according to first generation, second generation and third generation, below overview and explain three generations' detected methods :

First generation :It is chemical precipitation method, its concrete principle is that precipitator can selectively aggregate lipoprotein with positive charge and contains apoB, HDL is dissolved in solution, use centrifugation method, it separates non-HDL agglutinates, analyze supernate cholesterol then can detect HDL-C. HM method and PTA-Mg^{2+} method together with polyethylene glycol enzymatic modification method have successively been clinical applied in America, Europe and others to certain extent. Michel R etc.(2006)pointed out Chinese society of laboratory medicine recommend to use precipitating with phosphotungstic acid-mg as high density lipoprotein cholesterol detected method, and

as lipoprotein and apolipoprotein detection reference standards[6], but detection result had certain errors, which mainly because HDL according to whether contained apoE element or not, it could divide into two types, HDL that contained apoE element generated HDL-C occupied 10% of total HDL-C, precipitation method could precipitate HDL that contained apoE element, but HDL that didn't contain apoE element would not be precipitated, so it reduced HDL-C concentration, and meanwhile it couldn't fully precipitate apoB lipoprotein so that supernate had certain turbidity and affected result accuracy. The first generation detection method specimen needs to make centrifugation in advance, it cannot implement whole detection process fully automatization, so it limits the method application to certain extent.

The second generation :It is magnetic bead separation method, add reagent (including 250mmol/L Mg^{2+} and magnetic bead and so on)in serum, after mixing vibration in spiral type mixer, transfer to test tube that has magnetic property, and put in Hitachi analyzer, DS will combine with apoB particle and are attracted by magnetism in magnetic field, and are separated in one minutes, for patients that suffer hypertriglyceridemia, the process needs 30 minutes, HDL-C will rest in supernate that can detect by using enzymatic solution. The method for lower concentration cholesterol, increasing detection serum volumes then can promote its accuracy, but when HDL-C concentration is larger than 5g/L, VLDL-triglyceride fatty acid concentration is around 5g/L, magnetic bead method is prone to suffer interference and then affect its detection result accuracy[7]. Though magnetic bead separation method omits centrifugation steps, it needs special fixtures, the invention and reagent are not suitable to popularize, and accuracy is not ideal, therefore its adaptation range is limited.

The third generation :It is homogenous method, overseas directly detecting HDL-C homogenous methods without precipitation, usage of specimen is little, it doesn't need precipitation treatment, and can used to automatic biochemistry analyzer detection, due to its simplicity, fast, reducing pre-processing will reduce cost, and easy to automatization, it has already attracted domestic and overseas attentions and widely applied in clinical regular analyses, used to clinical automatic detection, in the aspect of accuracy and precise aspect, it basically arrives at NCEP analysis target, therefore the third generation detected method has been widely used at present.

Homogenous method or direct detected method could be used in automatic analyzer without carrying on pre-processing, is on the basis of chemistry and immunology, non –HDL particle doesn't need to precipitate, after proper vibration, enzymatic reagent will selectively dissociate serum samples' cholesterol, most of automatic analyzer can be fulfilled in a small test tube. The first homogenous method detecting HDL-C reagent was firstly developed by Japanese, the method was considerable novelty and proposed in 1995, which used α-SCD and Mg^{2+} selective suppress CM and VLDL, PEG modified cholesterol esterase and cholesterol oxidase had stronger selectivity on HDL-C and big LDL particles' cholesterol. Jonas A etc. (2002) pointed out, other all kinds of novelty methods used liquid chromatography, gradient electrophoresis method, two-dimensional electrophoresis method, plasma focusing method, constant speed electrophoresis, capillary electrophoresis method and others to separate and make quantization on lipoprotein and apolipoprotein elements, but its disadvantage is it still needs to detect other elements[8].

LDL-C detected method

In human blood circulation, cholesterol is mainly transferred by low density lipoprotein. Clinical laboratory detecting low density lipoprotein cholesterol most common method is Friedewald calculation method. The method is according to patients' empty stomach state's serum total cholesterol (TC)、HDL-C and triglyceride (TG), it makes calculation and gets LDL-C concentration, though the method has been widely used in clinical detection, but it is not suggest to use the method to detect non-empty stomach state samples 、high TC blood stasis(TG>4.52mmol/L)patients samples and III type hyperlipoproteinemia samples.[9]

From LDL-C detection method evolution process, except for Friedewald calculation method, there are also ultracentrifugation method, electrophoresis method, precipitation method, immune-separation method and homogenous method, in the following, it overviews them respectively :

Ultracentrifugation method:the method dissociates lipoprotein is making dissociation by utilizing balance valve and velocity method according to floating or precipitation parts density and water density differences after adjusting samples densities by adding sodium bromide or potassium bromide and other salt in samples. Lipoprotein preparative separation can ultra-centrifuge through putting serum or plasma in non-protein solution, letting very-low density lipoprotein(VLDL) that contains TG and chylomicron to float, and using liquid-moving machine or sucker to recycle. 1.006kg/L ultracentrifugation lower level part can adjust density to 1.063kg/L by adding potassium bromide and other salt to carry out ultracentrifugation so as to let LDL to partial float, from which cholesterol contents are detection result of LDL[10]. Wang Bei-Li etc. (2013) pointed out that as a kind of separation technique, ultracentrifugation method not only had tedious steps and consumed time, and in case that separation could not get stable lipoprotein than may change due to slat concentration and centrifugal force and other factors, in addition, due to ultracentrifugation method needed lots of different types of instruments and test tubes, different detection batches experiment environment had certain differences, separation result quality and height depended on technicists' practical operation and other causes, which caused ultracentrifugation method repeatability being bad, cross contamination being more, and technical requirements were high, which was hard to develop in clinical experiment, but ultracentrifugation method still could be used as foundation of reference methods[11].

Electrophoresis method:The method cholesterol detection linear can arrive at 4000mg/L, every strip lowest limit of detection is 42mg/L, and compare to BQ method detection result, LDL-C average deviation is 2.9%, total error is 7.8%, for TG samples in 2000~4000mg/L and>4000mg/L, their bias respectively rise to 5.5% and 6.9%[12]. Electrophoresis method not only can make quantitative detection on main lipoprotein, but also can directly observe different lipoproteins changes, the method is detecting III type of hyperlipoproteinemia patients characteristic wide β strip confirmed test. Compare to using enzymatic method and immunochemistry method detecting lipoprotein automatic instrument, electrophoresis method detecting lipoprotein needs more workers and operation techniques requirement is higher, so it is not suitable to regular laboratory that samples quantity are larger, while relative adapts to some special professional laboratory.

Precipitation method:Before NCEP suggesting to use direct method to detect LDL-C, many reagent companies have already started developing LDL-C detection reagent, by adding into specific reagent, it lets LDL particles to form into specific precipitation. By centrifugation, it lets LDL precipitation part to precipitate in the bottom of test tube, after that, detects serum TC and upper level suspension liquid cholesterol, calculates the two difference then can get precipitation part LDL-C contents, in addition, it can also get LDL-C result by directly detecting precipitate after solution. Early period precipitation method detecting LDL-C cannot replace convenient Friedewald calculation method, there is also no researches show that compare to calculation method, and it has better precise, correctness and specificity.

Immune-separation method:Direct LDLTM utilized multi colony anti-person apo A-I and apoE anti-body that can combine with polystyrene rubber latex, designed to remove chyle particles、HDL、VLDL and IDL particles, and then directly detected LDL-C parts[13]. Multiple items research finds that immune-separation method between-run CV is2.0%~5.2%, total error average value is 13.8%(11.8%~15.1%), samples can store 3 weeks at most on the condition of 4^{o}C, but samples freeze thawing still will affect detection result, in addition, there is also immune-separation method that uses simple magnetic bead precipitation method, it can promote samples handling efficiency, but it still needs larger samples absorption quantity and specific anticipation, and specificity is still to be further promoted[14].

Homogenous method:Different detergents and other chemical elements, by blocking, dissolving different types of lipoproteins, it acquires LDL-C detection specificity, LDL-C detects in the same

sample cup by enzymatic method, all reagents suppliers all provide dual reagent, it can fit for most automatic chemistry analyzer, development of homogenous method detecting LDL-C, it finds the method imprecision can arrive at NCEP required standards (<=4%), lowest limit of detection is 2mg/L, detection linear can arrive at 4100mg/L[15].

Foreign countries divides clinical regular laboratory directly separation detecting LDL-C method into three generations, the first generation is chemical precipitation, the method main drawback is when TG level is higher, sometimes result is lower that is caused by LDL precipitation incompletion[16];the second generation method has two kinds, they are respectively immune-separation method and magnetic bead heparin separation method, the method doesn't need to centrifuge, operation is simple, precise is high, correlation with Friedewald formula method is good, disadvantage is it needs specialized separation tube, reagent cost is higher, it is hard to carry out automatization, and is not suitable to freeze or freeze-drying specimen detection[17];the third generation is cloud-phase detection method, specimen dosage is little, it doesn't need to do precipitation treatment, it can be used to automatic analyzer detection, in the aspect of accuracy and precise aspects, both can arrive at NCEP analysis target.

HOMOGENOUS METHOD THEORETICAL BASIS

Homogenous method mainly has PEG enzymatic modification method、immune-separation method、selective inhibition method and clearance four kinds, which are also adopted four kinds of regular detection methods, in the following, it makes theoretical statement on the four methods.

Apply polyethylene glycol (PEG)to modify cholesterol esterase and cholesterol oxidase, modification enzyme has selectivity on lipoprotein structure, the order is LDL<VLDL<CM<HDL;make sulfation on α-cyclodextrin, form into sulfuric acid α- cyclodextrin salt molecule cavity outside as hydrophilic group, it can wrap compound, and meanwhile cyclodextrin has many first grade and second grade hydroxy group than can be exchanged and replaced by sulphate group, and increase sulfuric acid α- cyclodextrin salt molecule biochemistry attributes and inclusive attributes ;Mg^{2+} and glucose(DS) combined action can go together with CM、VLDL、LDL to form into precipitation, in case sulfuric acid α- cyclodextrin salt exist, wrap glucose, Mg^{2+} and non-high density lipoprotein formed precipitate, and then avoid modification enzyme effects on non-high density lipoprotein, from which $Mg^{2+}$and α-cyclodextrin salt can reduce cholesterol activity, especially for chylomicron and very-low density lipoprotein, therefore it will not be so troublesome as precipitation method that needs to precipitate such lipoprotein element, therefore in the effect of Mg^{2+}, DS, α-SCD and modification enzyme, it can directly detect HDL-C.

Main response equation is as following shows:

α-SCD + 4-AA + DS + Mg^{2+} + non-HDL → solubility compound

HDL + CHER (modification) + CHOD(PEG modification) → △4-cholestenone + H_2O_2

H_2O_2 + POD + HDAOS →coloration

In immune-separation method, IRC method first step is sample vibrating with polyethylene glycol PEG4000, letting PEG and LDL、VLDL and CM to form into compound, in the second step, add apoB and apoC-III vibration, apoB and apo--III as well as LDL、VLDL and CM affinity is strong, form into antigen-antibody compound, after gathering, then add into enzyme reagent and 4-AA, now enzyme and non-accumulated HDL will react, then add into guanidine acid vibration to let polymerase chain reaction to stop and dissolve in the first step reaction formed precipitation, otherwise formed precipitation will disturb coloration. α-SCD

IRC method main reaction method is as following shows:

Non-HDL + anti-apoB/C-III + PEG4000 →solubility compound

HDL + CHER + CHOD → △4- cholestenone + H_2O_2

H_2O_2 + 4-AA + POD → coloration

Guanidine hydrochloride ends enzyme reaction and dissolves reaction solubility compound

In immune-separation method, AB method main response equation is as following shows:

Non-HDL + apoB anti-body →solubility compound

HDL + CHER + CHOD → △4- cholestenone + H_2O_2

H_2O_2 + 4-AA + FDAOS → coloration

Selective inhibition method is PDD method, use a kind of surfactant and polyanion as well as enriched apoB lipoprotein to form into polymer, the other kind of specific surfactant selective dissolution HDL, it dissociates HDL-C reaction in enzyme reagent, directly precipitates HDL-C. The method detects through adopting kit, it mainly relies on different surfactants that have different dissociation abilities on different lipoproteins, in reagent-I, it contains α-SCD, DS, Mg2+ and dispersing surfactant, DS and Mg2+ as well as CM、VLDL、LDL forms into precipitation, under effects of α-SCD, it is wrapped into its cavity, reaction inhibitor and α-SCD wrapped compound mutual effects, and forms into solubility compound, inhibits CM、VLDL、LDL being reacted by enzymatic reagent dissociation. In reagent -II, it contains reaction inhibitor and HDL forms into solubility compound, which can selectively dissociate HDL and enzymatic reagent to react coloration, according to absorbance changes, it can detect HDL-C contents, PDD method main response equation is as following shows:

Non-HDL + polyanion + dispersing type surfactant(SAA_1) →solubility compound

HDL + CHER + CHOD + reaction accelerant(SAA_2)→ △4- cholestenone + H_2O_2

H_2O_2 + POD + HDAOS → coloration

Clearance method mainly has reaction accelerant –peroxidase clearance method that is (SPD method) and catalase clearance method(CAT method) two kinds. Among them, the former mainly utilizes surfactant selectivity differences on HDL、LDL、VLDL and CM, adopts kit to detect, its main response equation is as following shows:

Non-HDL + SAA_1 →solubility compound

Free cholesterol + CHOD → H_2O_2

$2H_2O_2$ + POD → $2H_2O + O_2$

HDL + CHER + CHOD + SAA_2 → △4- cholestenone + H_2O_2

H_2O_2 + 4-AA + DSBmT + POD → coloration

CAT method principle is reagent I special surfactant 1 selectivity acting on serum LDL、VLDL and CM, lets them to dissociate from non- HDL cholesterol, under effects of cholesterol esterase

(CHER) and cholesterol oxidase(CHOD), reacts and generates hydrogen peroxide, hydrogen peroxide enzyme acts on hydrogen peroxide to dissolve it into water and oxygen, and then is cleared by hydrogen peroxide, in reagent II sodium azide will inhibit hydrogen peroxide enzyme activity, surfactant 2 dissociates HDL released high density lipoprotein cholesterol, acting with enzymatic reagent includes cholesterol esterase, cholesterol oxidase 、peroxidase, on the conditions of color developing agent storing, it occurs to coloration response, according to coloration extent then it can detect HDL-C, the method main response equation is as following shows :

Non-HDL + SAA_1 + CHER+ POD → △4- cholestenone + H_2O_2

$2H_2O_2$ + POD → H_2O_2 + O_2

HDL + NaN_3 + SAA_2 + CHER + CHOD → △4- cholestenone + H_2O_2

H_2O_2 + POD + HDAOS → coloration

DISSOCIATION DEGREE STABILITY RESULT ANALYSIS

In clinical detecting serum, HDL-C uses automatic analyzer, detection is fully automatic, it doesn't needs manual operation, and the paper researched content is HDL-C and LDL-C reference materials stability in regular detection system, in order to explore different reference materials stability of synchronization in its detection process, the paper selects A(HDL)/A(LDL)as specific evaluation indicator, the indicator reflects HDL and LDL dissociation degree difference, the indicator is yields and excellent indicator, that is to say, the indicator value is larger, then stability of synchronization will be better.

Kit attempted recipe is as following shows:

α-SCD(1.1g/L)

Anhydrous $MgSO_4$(1.2g/L)

TOOS(0.15g/L)

CHOD(2U/mL)

DS(0.7g/L)

SAA_1(0.01g/L~80g/L) is dissolved in MOPS buffer solution(0.1M, pH6.5)

As TABLE 1 shows, it has inhibition effects on LDL, and selective dissociation effects on HDL is relative good, and completed response surfactant HLB value five minutes after adding corresponding reagents.

TABLE 1 : Kit's different surfactants HLB values

Type	NUST-9	NUST-6	B-66	A-90
HLB	12.7	13	13.2	14.5

As TABLE 2, it shows different concentrations NUST-9 impacts on pure HDL and pure LDL dissociation extents.

TABLE 2 : Different concentrations NUST-9 to pure HDL and pure LDL dissociation extent impacts result

Concentration	Type	Dissociation difference	Dissociation ratio	Concentration	Type	Dissociation difference	Dissociation ratio
0.01g/L	HDL	0.005	0.940	5.00g/L	HDL	0.006	1.170
	LDL	0.008			LDL	0.008	
0.05g/L	HDL	0.006	1.088	10.00g/L	HDL	0.007	1.090
	LDL	0.008			LDL	0.010	
0.10g/L	HDL	0.006	1.120	20.00g/L	HDL	0.006	1.150
	LDL	0.008			LDL	0.008	
0.50g/L	HDL	0.007	1.178	40.00g/L	HDL	0.010	0.950
	LDL	0.009			LDL	0.016	
1.00g/L	HDL	0.005	1.273	80.00g/L	HDL	0.012	0.908
	LDL	0.006			LDL	0.020	

By TABLE 2 data, it can get following showed conclusions:

From dissociation difference data changes, it is clear that dissociation difference, with NUST-9 concentration increasing, its dissociation ability on HDL and LDL sterling is gradually strengthening.

From dissociation ratio data changes, it is clear that NUST-9 to LDL selective inhibition effects are firstly increasing and then decreasing, when NUST-9 concentration is 1g/L, it assigns maximum value, now dissociation degree on LDL is also the minimum, and under the concentration inhibiting HDL dissociation efficiency is also the strongest, which is bad for its dissociation.

NUST-9 selectivity is not ideal; it cannot arrive at certain experiment requirements.

As TABLE 3, it shows different concentrations NUST-6 impacts on pure HDL and pure LDL dissociation extents.

TABLE 3 : Different concentrations NUST-9 to pure HDL and pure LDL dissociation extents impacts results table

Concentration	Type	Dissociation difference	Dissociation ratio	Concentration	Type	Dissociation difference	Dissociation ratio
0.01g/L	HDL	0.003	0.563	5.00g/L	HDL	0.005	1.010
	LDL	0.008			LDL	0.007	
0.05g/L	HDL	0.004	0.758	10.00g/L	HDL	0.005	1.262
	LDL	0.008			LDL	0.006	
0.10g/L	HDL	0.005	0.854	20.00g/L	HDL	0.010	1.212
	LDL	0.009			LDL	0.010	
0.50g/L	HDL	0.005	0.946	40.00g/L	HDL	0.004	0.909
	LDL	0.008			LDL	0.005	
1.00g/L	HDL	0.005	0.947	80.00g/L	HDL	0.003	0.759
	LDL	0.009			LDL	0.006	

jBy TABLE 3 data, it can get following showed conclusion:

With NUST-6 concentration increases, dissociation degree and selectivity on HDL and LDL presents first increase and then decrease trend, in the moment 10g/L, dissociation ratio arrives at maximum value that now inhibition and dissociation efficiency on LDL is the best.

From dissociation difference data, it is clear that when NUST-6 is in 0.1g/L~10g/L, dissociation degree on HDL remains unchanged, therefore it also inhibits HDL dissociation to certain extent.

On a whole, NUST-6to HDL selective dissociation efficiency is not ideal.

As TABLE 4, it shows different concentrations B-66 to pure HDL and pure LDL dissociation degree impacts.

TABLE 4 : Different concentrations B-66 to pure HDL and pure LDL dissociation degree impacts result table

Concentration	Type	Dissociation difference	Dissociation ratio	Concentration	Type	Dissociation difference	Dissociation ratio
0.01g/L	HDL	0.002	0.544	5.00g/L	HDL	0.012	4.545
	LDL	0.010			LDL	0.004	
0.05g/L	HDL	0.003	0.505	10.00g/L	HDL	0.020	4.428
	LDL	0.009			LDL	0.007	
0.10g/L	HDL	0.004	0.677	20.00g/L	HDL	0.009	2.727
	LDL	0.009			LDL	0.005	
0.50g/L	HDL	0.004	0.758	40.00g/L	HDL	0.018	1.704
	LDL	0.008			LDL	0.012	
1.00g/L	HDL	0.005	1.515	80.00g/L	HDL	0.032	1.672
	LDL	0.009			LDL	0.029	

By TABLE 4 data, it can get conclusion as following show:

Firstly with B-66 concentration increases, selectivity on HDL sterling strengthens, inhibition effects on LDL strengthens, especially when concentration is 5g/L, efficiency is good, with concentration gradually increases, dissociation efficiency on LDL strengthens, inhibition effect weakens.

Surfactant B-66 selectivity dissociation on HDL is good, and inhibition effect on LDL is good. To further define concentration when B-66to LDL inhibition ability is strongest, according to data tendency, it judges and mainly tries B-66 concentration in 5g/L nearby to LDL inhibition effects, its concrete experiment data is as TABLE 5 shows.

TABLE 5 : Concentration 5g/L nearby B-66 to pure HDL and pure LDL dissociation extent impacts result

Concentration	Type	A0	A1	A1-A0	$\frac{A(HDL)}{A(LDL)}$
1.00g/L	HDL	0.001	0.006	0.005	1.515
	LDL	0.002	0.006	0.004	
4.00g/L	HDL	0.002	0.007	0.005	1.894
	LDL	0.002	0.006	0.004	
5.00g/L	HDL	0.002	0.017	0.015	4.545
	LDL	0.003	0.008	0.005	
6.00g/L	HDL	0.001	0.017	0.016	4.564
	LDL	0.002	0.007	0.005	
8.00g/L	HDL	0.001	0.017	0.016	3.463
	LDL	0.002	0.009	0.007	
10.00g/L	HDL	0.003	0.023	0.020	4.428
	LDL	0.003	0.010	0.007	
12.00g/L	HDL	0.002	0.013	0.011	2.381
	LDL	0.002	0.009	0.007	

By TABLE 5 data, it is clear that when B-66 concentration is 6g/L, the inhibition effects on LDL and dissociation on HDL are the best.

As TABLE 6, it shows different concentrations A-90 to pure HDL and pure LDL dissociation degree impacts.

TABLE 6 : Different concentrations A-90 to pure HDL and pure LDL dissociation degree impacts result

Concentration	Type	Dissociation difference	Dissociation ratio	Concentration	Type	Dissociation difference	Dissociation ratio
0.01g/L	HDL	0.002	0.543	5.00g/L	HDL	0.002	0.656
	LDL	0.003			LDL	0.005	
0.05g/L	HDL	0.003	0.909	10.00g/L	HDL	0.003	0.649
	LDL	0.005			LDL	0.007	
0.10g/L	HDL	0.004	1.210	20.00g/L	HDL	0.002	0.433
	LDL	0.005			LDL	0.007	
0.50g/L	HDL	0.004	1.212	40.00g/L	HDL	0.002	0.337
	LDL	0.005			LDL	0.009	
1.00g/L	HDL	0.004	1.515	80.00g/L	HDL	0.003	0.413
	LDL	0.004			LDL	0.011	

By TABLE 6 data, it can get conclusion as following:

When A-90 concentration is smaller, dissociation effects on both HDL and LDL are not good, when A-90 concentration is 1g/L, dissociation ratio arrives at maximum value, now inhibiting LDL dissociation selectivity is the best, with respect to NUST-6 and NUST-9, its inhibiting LDL dissociation selectivity is relatively good.

With A-90 concentration increases, dissociation difference almost remains unchanged, dissociation degrees changes on HDL are smaller, inhibition dissociation efficiency on HDL is very good, but dissociation degrees on LDL become larger, inhibition dissociation selectivity on LDL reduces, and is just opposite to the experiment purpose of selecting inhibiting LDL dissociation.

CONCLUSION

The paper on the basis of summarizing high, low density lipoprotein cholesterols diagnostic reagent developing learns current most widely applied detected method is homogenous method. And then, for homogenous method, it makes theoretical analysis, it analyzes PEG modification enzymatic method、immune-separation method、selective suppression method and clearance four regular detection methods and their principles, which builds theoretical basis for different reference materials seeking and their impacts on HDL-C and LDL-C dissociation degrees. Finally, take A(HDL)/A(LDL)as specific evaluation indicator, it explores NUST-9、NUST-6、B-66 and A-90 four kinds of reference materials to HDL and LDL dissociation degree influence rules in automatic analyzer. Result indicates :

Non-ionic surfactant selectivity is good, especially for HLB non-ionic surfactant selectivity is better in the range 13~14, as B-66, its HLB value is 13.2.

For surfactant dissociating lipoprotein mechanism, possible explanation is that surfactant is combined into lipoprotein hydrophobic region, its affinity with lipoprotein is strong, it replaces original lipid so that breaks through lipoprotein particle to release particle cholesterol and enzyme reagent response.

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