

QUADRAPED™

HDL-D CHOLESTEROL KIT

(Direct Enzymatic Method)
(For veterinary invitro diagnostic use only)

INTENDED USE

QUADRAPED™ HDL-D Cholesterol kit is used for the determination of HDL Cholesterol in serum/plasma.

SUMMARY

HDL particles serve to transport lipoproteins in the blood-stream. HDL is known as "good cholesterol" because high levels are thought to lower the risk of heart disease and coronary artery disease. Low HDL cholesterol levels, are considered a greater heart disease risk. Clinical diagnosis should not be made on a single test result but should integrate clinical and other laboratory data.

PRINCIPLE

Direct determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample. The method depends on the properties of a detergent which solubilizes only the HDL so that the HDLc is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoproteins LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to absorption of the detergents on their surfaces. The intensity of the color formed is proportional to the HDLc concentration in the sample.

EXPECTED VALUES

Species	HDL Cholesterol (mg/dl)
Dog	72-189
Cat	60-120
Cow	56-112
Buffalo	40-110
Horse	56-112
Pig	40-120
Sheep	24- 35
Goat	24- 35
Rabbit	40-120

It is recommended that each laboratory establish its own range as reference ranges may vary between laboratories.

PRESENTATION

REF	1126120040
Pack Size	40 ml
L1 HDL-D Reagent 1	30 ml
L2 HDL-D Reagent 2	10 ml
C Calibrator (for 1 ml)	1 No.

COMPOSITION

Goods Buffer 30 mM ; Dextran Sulphate ; Magnesium Sulphate Heptahydrate; 4-Aminoantipyrine 3 mM; Cholesterol Esterase 6000 U; Cholesterol Oxidase 10000 U; Peroxidase 15000 U; Surfactant and Preservatives.

STORAGE / STABILITY

Contents are stable at 2-8°C till the expiry mentioned on the labels.

REAGENT PREPARATION

Reagents L1 and L2 are ready to use. Cap the bottles immediately after use and avoid contamination.

Calibrator : Reconstitute with 1 ml of D.W. Mix gently to dissolve the contents. Once re-constituted the calibrator is stable for 2 weeks at 2-8°C or 3 months at -20°C. Do not repeatedly thaw and refreeze.

SAMPLE MATERIAL

Serum or Heparinized plasma. Serum should be separated from the clot, as soon as possible. HDL is reported to be stable in the sample for 1 week when stored at 2-8°C.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

PROCEDURE

Wavelength / filter : 578 nm / 630 nm (Bichromatic)
Temperature : 37°C
Light path : 1 cm

Pipette into clean dry test tubes labelled as Blank (B), Calibrator (C) and Test (T)

Addition Sequence	B (ml)	C (ml)	T (ml)
HDL-D Reagent 1 (L1)	0.450	0.450	0.450
Calibrator	-	0.006	-
Sample	-	-	0.006
Mix and incubate for 5 mins. at 37°C and			
HDL-D Reagent 2 (L2)	0.150	0.150	0.150

Mix well and incubate for 5 mins. at 37°C and read the absorbance of the Calibrator and the Test against Blank at 578 nm / 630 nm.

CALCULATIONS

$$\text{HDLc in mg/dl} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Calibrator}} \times \text{Conc. of Calibrator}$$

QUALITY CONTROL

The following process is recommended for QC during the assay of HDL-D Cholesterol. Define and establish acceptable range for your laboratory.

- Two levels of control (Normal and Abnormal) are to be run on a daily basis.
- If QC results fall outside acceptance criteria, recalibration may be necessary.
- Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

LOD: 1.0 mg/dl

LOQ: 2.0 mg/dl

Lower Limit: 1.0 mg/dl

Higher Limit: 150 mg/dl

Interferences:

Sample when spiked with interferent such as upto 40 mg/dl Bilirubin, 500 mg/dl haemoglobin, 1000 mg/dl intralipid does not affect the ability of the kit to determine the HDL - D Cholesterol concentration.

Precision:

Within run

Within run	n	Mean	SD	% CV
Sample 1	10	47.9	0.44	0.91
Sample 2	10	95.8	0.47	0.49
Sample 3	10	55.4	0.14	0.22

Between run

Between run	n	Mean	SD	% CV
Sample 1	10	48.1	0.34	0.71
Sample 2	10	98.5	0.40	0.41
Sample 3	10	55.4	0.16	0.28

Method of comparison:

Comparative studies were done to compare our reagent with another commercial HDL-D Cholesterol Assay. No significant differences were observed. Details of the comparative study are available on request.

NOTE

In vitro diagnostic reagent for laboratory and professional use only Not for medicinal use. The reagent contain sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantity of water. Only clean and dry glassware must be used. Components from human origin in the calibrator have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

Do not use the reagents if there are particles or turbidity.

Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances. Do not use turbid, deteriorated or leaking reagents.

REFERENCES

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- Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, Kenneth S. Latimer, ISBN Jane Wardrop, 6th Edition – 2010.
- Clinical Biochemistry of Domestic Animals, Sixth Edition, 2008 by Kaneko J.J., Harvey J.W. & Bruss M.L.
- Data on file: Coral Clinical Systems.

System Parameters

Reaction	: End Point	Interval	: ---
Wavelength	: 578 / 630 nm (Bichromatic)	Sample Vol.	: 0.006 ml
Zero Setting	: Reagent Blank	Reagent Vol.	: 0.600 ml
Incub. Temp.	: 37°C	Standard	: See Calibrator
Incub. Time	: 5 min. + 5 min.	Factor	: ---
Delay Time	: ---	React. Slope	: Increasing
Read Time	: ---	Linearity	: 150 mg/dl
No. of read.	: ---	Units	: mg/dl

SYMBOL KEYS

Store at 2-8°C	Manufacturer	IVD In vitro Diagnostic Medical Device	L1 HDL-D Reagent 1	Direct Enzymatic
Use by (Last day of stated month)	Consult Instructions for use	LOT Batch Number	L2 HDL-D Reagent 2	Direct Enzymatic Method
Date of Manufacture	REF Catalogue Number		C Calibrator (for 1 ml)	This way up



Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

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