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## Determination of cholesterol in clinical samples

### Introduction

Cholesterol is the main sterol component in body tissues occurring mainly in the brain and spinal cord. Measurements in plasma help to classify hyperlipemias. Increased plasma levels are associated with atherosclerosis, nephrosis, diabetes mellitus, myxedema and obstruction jaundice. Decreased levels may occur in hyperthyroidism, anaemias, malabsorption and wasting syndromes. Normal levels are affected by stress, age, gender, hormonal balance and pregnancy. The normal range is 140-250 mg/100ml.

Use of a spectrophotometer makes cholesterol estimations very convenient, particularly for obtaining rapid results on relatively small numbers of samples, and also in non-laboratory situations. The analysis is performed directly in a cuvette at room temperature.

Provided the concentration range is 50 - 800mg/ml, foodstuff extracts can be analysed with the same method.

### Principle

Cholesterol is determined at 500nm after enzymatic hydrolysis and oxidation, via the formation of the indicator quinoneimine in the presence of phenol and peroxidase.

### Method

The reagent can be prepared ready for use or obtained as a kit (Randox Labs. UK)

Pipette 1 ml of reagent containing:

Cholesterol esterase 0.16U/ml  
Cholesterol oxidase 0.11U/ml  
4-amino antipyrine 0.25 mmol/l  
Phenol 25 mmol/l  
Peroxidase 5.5U/ml

into each of 2 disposable cells (80-2004-53).



## Libra S21/S22 operation

- Select Basic Modes (1)
- Select Concentration  
Select Wavelength to 500 press OK (F3)
- Enter Factor 0.533 press OK (F3)
  - The spectrophotometer can be set to read directly in concentration units (mg/100ml) using a calibration factor of 0.533 previously established experimentally.

Add 0.01ml of sample and 0.01ml water, for the blank analysis, respectively and mix.

After 10 minutes at 20-25°C:

- Insert blank cuvette. and press green run key.
  - A single blank as above suffices for subsequent analyses in the same series.
- Insert sample and press green run key.

The analysis is linear over the range 50-800 mg/100ml.

(For assays where there is no established concentration factor, calibration should be carried out using prepared standards.)

Print outs may be obtained by setting up the printer options in System Utilities and Preferences (3) prints results. This is automatic with autoprint.

Additionally press **.** to print result if auto-print is off, or to re-print result if auto-print is on

The above procedure can be easily used with other instruments in the Libra range by using the concentration mode and the 0.533 factor.

## Ordering Details

Libra S5	80-2115-00
Libra S11	80-2115-15
Libra S12	80-2115-10
Libra S21	80-2115-25
Libra S22	80-2115-20
Libra S32	80-2115-30

The reaction can be accelerated for increased sensitivity if warmed. For this purpose the Libra S21/S22 have the following accessories:

- 8 position water heated cell changer (80-2109-70) used with an external heating bath
- 6 position Peltier heated cell changer (80-2106-04) and Temperature Control Unit (80-2112-49)
- Single position water heated cell holder (80-2106-08) used with an external heating bath
- Single position electrical cell holder (80-2106-12), temperatures selectable from 25, 30 and 37°C
- Single position Peltier cell holder, temperatures selectable over the whole range from 20-49°C (80-2106-13).

The Sipper (80-2112-25) enables some automation of the analyses, and can be used together with a heated (not water heated) or non-heated single cell holder.