SECTION 3

OPERATION

3.1 GENERAL PRINCIPLES

The Genova is a grating spectrophotometer with a selectable wavelength range of 198 – 1000nm. For specific nucleotide and protein assays software is on-board to facilitate rapid analysis of these compounds.

Using spectroscopy, the quantitative analysis of these assays has now become a routine method in many laboratories. It includes absorption measurement, primarily in the ultraviolet range. Proteins are measured directly at 280nm, nucleic acids at 260nm and colorimetric protein determination is carried out within the range of 550 to 600nm.

Nucleic Acid Determination

DNA, RNA and oligonucleotides can be measured directly in aqueous solutions in a diluted or undiluted form. Aqueous buffers with low ion concentrations (e.g. TE buffer) are ideal for this method. The concentration is determined by measuring at 260nm against a blank and then evaluating against factor.

The absorption of 1 OD (A) is equivalent to, approximately:
50μg/ml dsDNA, 37μg/ml ssDNA, 40μg/ml RNA or 30μg/ml for oligonucleotides.

Purity determination of DNA interference by contaminants can be recognised by the calculation of ratio. The ratio A260/A280 is used to estimate the purity of nucleic acid, since proteins absorb at 280nm.

Pure DNA should have a ratio of approximately 1.8; pure RNA 2.0. Absorption at 230nm reflects contamination of the sample by substances such as peptides, phenols, aromatic compounds or carbohydrates. In pure samples the ratio should be approximately 2.2.

Referring to a blank value where no absorption should occur is commonly required. On the Genova this default reference is 320nm. Should you then wish to change or modify these wavelengths, this flexibility is in-built.

Protein Determination

Several analytical procedures can be used to determine the protein content of a preparation. Evaluation can be carried out either via a calibration curve or a factor using up to 6 standards.
The Genova uses the following methods of analysis:

B.C.A. (second order curve fit; quadratic)	562nm
Bradford (second order curve fit; quadratic)	590 or 595nm
Lowry (second order curve fit; quadratic)	550/750nm or 500/750nm
Biuret (first order curve fit; quadratic)	540 or 550nm
Direct UV (multi-wavelength)	

B.C.A. – (Bicinchoninine acid assay)

This test is a highly regarded alternative to the Lowry assay, being much easier to carry out and sensitivity can be varied using different temperatures. The dye complex is very stable. This test, however, can be susceptible

Bradford assay

This method is twice as sensitive as the B.C.A. or Lowry test and is the most sensitive quantitative dye assay. It is the easiest to handle and the most rapid method. It also has the additional advantage that a series of reducing substances (e.g. DTT and mercaptoethanol) have no adverse effect on results. It is, however, sensitive to detergents. The main disadvantage with this method is that identical amounts of different standard proteins can cause considerable differences in the resulting absorption coefficients.

Biuret assay

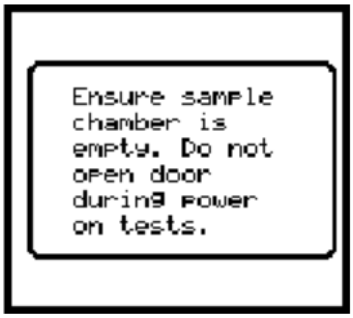
The principle of the Biuret is similar to that of the Lowry. However, it involves a single incubation of 20 minutes. There are very few interfering agents (ammonium salts being one such agent). The Biuret consumes more material. This assay is a good general protein assay for batches of material for which yeild is not a problem.

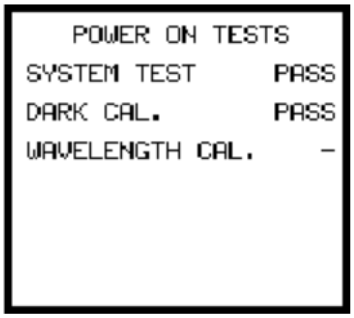
Lowry assay

The principal target is to reduce the high susceptibility to interference. In comparison to the Biuret assay, the sensitivity of this assay has greatly increased. The Lowry method, however, is adversely affected by a wide range of non-proteins. Additives such as EDTA, ammonia sulphate or Triton X-100 in particular are incompatible with the test.

3.2 POWER ON SELF TEST

Prior to switching the unit on check that the voltage select switch is set to the voltage supply being used. When the unit is switched on a self test routine will automatically be performed.





SYSTEM TEST
This test checks the validity of the operating parameters. The following messages may be displayed during this test.

"CRITICAL ERROR – CALIBRATION DATA FAILURE."
This error is non-recoverable and means the calibration data for the detector is not working and therefore the unit cannot operate. If this message is displayed during the test the manufacturer or local distributor should be contacted immediately for advice.

"SYSTEM ERROR – OPERATING PARAMETERS FAILURE"
This error is recoverable and means that the setup and operating parameters have been reset to their default values, possibly due to memory corruption. To continue press any key.

DARK CALIBRATION TEST
The message **"SYSTEM ERROR – DARK LEVEL CALIBRATION FAILURE"** may be displayed if the unit receives too much light when trying to perform a dark level calibration. Pressing any key clears this message and the unit will retry calibration. It will repeat this process until it passes the test satisfactorily.

WAVELENGTH CALIBRATION
This part of the self test checks the optical alignment of the unit.
If it fails this test an error message **"SYSTEM ERROR – WAVELENGTH CALIBRATION FAILURE"** will be displayed. This message is displayed permanently on screen and can only be removed by powering the unit off. If this message is displayed during the test the manufacturer or local distributor should be contacted immediately for advice.

Once the power on self test is successfully completed the main menu will be displayed:

3.3 KEYPAD OPERATION

The following information applies to all setup and operating modes:

UP/DOWN KEYS used to move the highlight around menu/screen options unless editing a parameter. In this instance these keys are used to adjust the highlighted parameter.

LEFT/RIGHT KEYS used to move the highlight around menu/screen options. If editing a numeric value the highlighted digit can be altered. If the highlight is moved off the left most digit the data editing will be aborted and the previous value will be re-instated.

ENTER KEY used to select the highlighted menu option or to store the current parameter being entered.

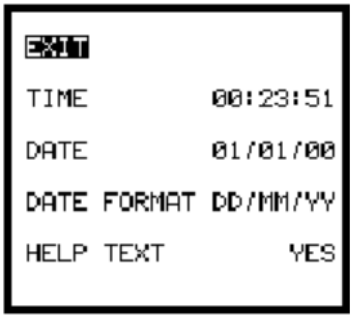
CAL KEY initiates a calibration routine.

PRINT KEY provides a printout of the current reading with an incremental sample number. When pressed for the first time after a calibration, the print out will give calibration information. The incremental sample number will be reset after a calibration.

3.4 GLOBAL SETUP PARAMETERS

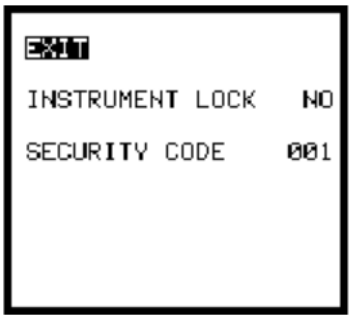
INSTRUMENT SETUP MENU

This option will allow all instrument related setup (i.e. non-mode specific) parameters to be set up. The options available through the Instrument Setup menu are as follows:



EXIT Returns to the main menu screen
TIME Allows the user to set the current time into the instrument in the form of HH:MM:SS
DATE Allows the user to set the current date into the instrument in the form of either DD:MM:YY or MM:DD:YY depending on the setting of the date format field.
DATE FORMAT Allows the user to choose between either the DD:MM:YY formatting of dates or MM:DD:YY formatting. The selected format is applicable to all displayed dates.
HELP TEXT Additional help messages will be displayed if the YES option is selected. These messages provide assistance with general operation. If the message does not appear a

SECURITY



EXIT Returns to the main menu screen

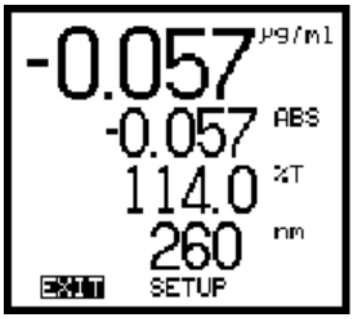
INSTRUMENT LOCK Yes or no option.
When set to Yes the up and down arrow keys are disabled within set up screens thus preventing operating parameters of the unit from being changed. Standard curve and absorbance zero calibrations are possible when the instrument lock is activated. The Photometrics mode is not affected by the instrument lock feature. When the instrument lock is active, entry of a locking code (0 to 999, set when the instrument lock is activated), is required to re-enter the security option.

SECURITY CODE Needed to get back into security menu to turn it off. 0-999. If no security number is available turn the unit off and then on holding down the enter key. This then resets to no security code and also any other previously set parameters. Will prompt first message refering to operating parameters indicated by a failure in System Test.

3.5 PHOTOMETRICS MODE

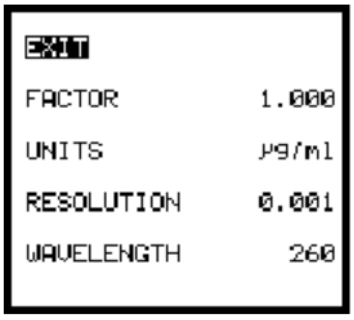
NOTE: Absorbance, %T and Concentration values are displayed simultaneously, i.e. they are not individually selectable.

Having selected **PHOTOMETRICS** from the main menu the following display will be shown:



It is recommended that setup parameters be reviewed prior to calibration or measurement to ensure the selected values are correct.

Select **SETUP** and the following options will be displayed:



EXIT	Allows the user to exit this menu
FACTOR	Used for the concentration calculation where concentration = Factor x Abs
UNITS	Allows selection of the units used to display against the concentration reading. The choice available: µg/ml, µg/l, %, M, g/l, mg/l, ppm, mM, none, ng/ml, mg/ml.
RESOLUTION	Allows the user to specify the resolution of the displayed reading (1, 0.1, 0.01, 0.001). The maximum resolution that concentration readings are displayed to can be set up to 3 decimal places. The instrument will automatically display concentration readings to the maximum possible resolution using this parameter.
WAVELENGTH	Allows user setting of preferred wavelength for the test being performed.

Performing a measurement

To perform a measurement it is necessary to set the unit to the required wavelength and perform a calibration.
Place a blank solution into the sample chamber and close the lid. Press the **CAL** key.
The instrument will momentarily show **CAL** indicating that the calibration is being performed.
Once calibrated, the display values will update to show ABS, %T and Concentration readings.