

**VALEPORT LIMITED**

**400 Series Instruments**

**Additional Sensor Secondary  
Calibration Procedures**

Document Ref: 04008121C.doc

Date: June 2003

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Valeport Limited,  
St. Peter's Quay,  
Totnes, Devon,  
TQ9 5EW,  
UK

Tel: +44 (0)1803 869292  
Fax: +44 (0)1803 869293  
e-mail: sales@valeport.co.uk  
Web: www.valeport.co.uk

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# 1 PH SENSORS

## INTRODUCTION

pH sensors give an analogue output signal (in millivolts) – the accurate measurement of this signal is the function of the primary sensor calibration, which is given in the calibration section of this manual. This primary calibration has a high level of long-term stability, and should not need to be rechecked any more often than the conductivity, temperature or pressure sensors – typically every one or two years depending on the customer's own requirements.

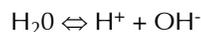
However, this primary data output may be subjected to a secondary, or "User" calibration within the instrument, so that the millivolt output is converted into standard pH units. Whilst the glass electrode pH sensor used on Valeport instruments is very stable in the short term, it is subject to long term drift errors like most similar sensors. For this reason, the user will find it necessary to perform a secondary calibration on the pH sensor at regular intervals. Frequency of calibration is very subjective, but Valeport recommends that re-calibration is carried out as often as is practical, and suggest the following guidelines:

For intermittent use (e.g. once per month)	Calibrate before each deployment
For regular use (e.g. every day)	Check against a standard buffer daily, recalibrate once per week, unless check shows significant drift
For long term deployments	Calibrate before deployment and after recovery. Assume linear drift between the two calibrations, and correct data for the drift over time.

## THEORY OF PH AND BUFFERS

pH is a measurement of Hydrogen ion concentration. It is a logarithmic scale, thus a change in pH of 1 unit signifies a change in  $H^+$  concentration (written as  $[H^+]$ ) of a factor of 10. pH is defined as  $-\log_{10}[H^+]$ .

In pure water, the water molecule is in equilibrium with its dissociated ionic form as follows:



At 25°C, the concentration of  $H^+$  ions is  $10^{-7}$  Molar ( $10^{-7}$  moles per litre, where 1 mole is approx  $6.022 \times 10^{23}$  molecules). Thus the pH of pure water is  $-\log_{10}[10^{-7}]$ , i.e. 7.

Acidic solutions have higher concentrations of  $H^+$  ions than water, and thus a lower pH. A solution of pH 4 will have  $[H^+]$  of  $10^{-4}$ M, which is 1,000 times greater than that of water. Conversely, alkaline solutions have lower  $[H^+]$  and thus a higher pH.

Certain combinations of compounds can be used to create solutions of very stable pH. Such solutions are called Buffers, and they can absorb the addition or removal of a certain level of acid or alkali without affecting pH. Buffers are mixed solutions of an acid and its so-called conjugate base (or base and conjugate acid). A good example is Ethanoic acid and its base Sodium Ethanoate. These substances exist in equilibrium:



If small amounts of acid are added, the extra  $H^+$  ions react with the ethanoate ( $CH_3OO^-$ ) ions and are therefore "absorbed" by the equilibrium. Similarly, small amounts of extra alkaline react with the  $Na^+$  ions. In both cases, the overall concentration of  $H^+$  ions is relatively unaffected, and therefore the pH remains stable.

By careful selection of appropriate compounds in appropriate concentrations, buffers can be created for specific  $[H^+]$ , i.e. specific pH values.

The Valeport pH sensor works by effectively creating a battery. The solution to be measured, with its concentration of  $H^+$  ions acts as one half of the cell, with a reference solution on the inside of the sensor acting as the other. The output of the sensor is therefore the potential difference between the two halves of the cell. The secondary calibration procedure defines how this relates to pH.

## PROCEDURE

The principle of the secondary calibration is very simple. The relationship between the voltage output of the sensor and pH is linear, so the calibration simply defines this straight line relationship.

To do this, the sensor must be placed in various buffers, and the output from the sensor at each pH noted. A best straight line fit can then be created for these measurements, and the equation then programmed into the instrument. An Excel spreadsheet for doing all the required calculations is provided on the software CD supplied with the instrument, numbered "04008118" (and a letter indicating version number; a, b, c, etc.).

To obtain accurate calibrations, the following protocol should be used. Remember that high quality calibrations can only be achieved with patience and attention to detail.

### Equipment:

#### Supplied with product

- Instrument with pH sensor
- Black acetal calibration pot
- pH reference solution, (0.5M KCl/AgCl)
- pH Buffer capsules, pH 4, 7 & 10
- plastic syringes
- Secondary calibration spreadsheet

#### Supplied by customer

- Distilled Water (at least 0.5litre)
- 4 plastic or glass beakers (200ml+)
- Plastic stirrers
- Paper towel
- Waste bucket
- PC running DataLog 400 or HyperTerminal, and with MS Excel.

Calibration may be carried out through the DataLog 400 software provided, or through entering commands in a terminal emulation program such as HyperTerminal. Instructions for both methods are given.

1. Begin by ensuring that the pH sensor itself is full of reference solution. Gently unscrew the protective black plastic guard from the pH sensor, exposing the glass electrode. Remove the Perspex protective cover from the end of the electrode as well, by loosening the screw in the end of the cover. This will allow the cover to then gently slide off the end of the electrode.
2. Tilt the instrument on its side, and check to see that no air bubbles are present within the sensor. If bubbles are present, this simply means that some of the internal reference solution has escaped, and must be replaced. Fit the white plastic nozzle to the top of the pH reference solution (marked 0.5M KCl/AgCl). Gently slide the rubber ring down the sensor, exposing the small refill hole in the side of the glass electrode. Orientate the instrument so the air bubbles in the electrode are beneath this hole, and then slowly use the nozzle on the reference bottle to fill the electrode. Once the electrode is full, replace the rubber ring to seal the solution inside.
3. Next, create three pH buffer solutions of 4, 7 and 10 pH. In a single 200ml beaker, dissolve the contents of one pH buffer capsule in 100ml water, stirring thoroughly until the powder has completely dissolved. Repeat for the other two buffer solutions

Note that once made, the pH buffer solutions may be kept for several days. If possible transfer them to sealed plastic bottles, which will help to keep them fresh. The indication that a solution is no longer fit for use is that the surface becomes cloudy with crystallised deposits or mould.

4. Use distilled water to clean the glass electrode of any crystallised deposits or other matter. Dry thoroughly using paper towel. Thoroughly rinse the black acetal calibration pot in distilled water to remove any deposits remaining from previous calibrations. Dry the pot using paper towel, and then screw it into place over the pH sensor.

Since we are using different concentrations of solution, it is standard practice to use the most dilute first. This is the pH 10 solution, which has a  $[H^+]$  of 1000 times less than pH 7, and 1,000,000 times less than the pH 4. It is easy to conceive that, despite the buffering capabilities of the solution, a single drop of pH 4 solution could easily contaminate the pH 10 solution. For this reason, using the most dilute solution first presents the least possible chance of contamination by a stray drop of buffer.

5. Using a syringe, inject 10ml of pH10 buffer into the calibration pot through the side hole. Although the sensor will react quickly to pH changes, the solution will take at least 2 minutes to stabilise, so now is a good time to begin communications with the instrument.
6. Communicating

Using DataLog 400, interrupt the unit. When the unit has been interrupted, DataLog 400 displays a summary screen detailing the serial number of the instrument, firmware version, amount of memory available, and various other parameters. At the bottom of this screen is a list of all the sensors fitted to the instrument, preceded by a two digit "module number". This is an address number for each sensor in the instrument; note down the module number of the pH sensor.

Set the instrument to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the pH sensor module is setup to output primary calibration data (mV). Refer to Section 4.2.3 of the software manual for details on how to do this.

Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Firstly, determine the module number (address) of the pH sensor by sending the command

```
#062<Enter>
```

This will list the sensors fitted and their module numbers. Make a note of the pH module number.

Next, ensure that the pH sensor is set to output data in mV by sending the command:

```
#020;<pH_module_number>;1;<space><Enter>
```

Set the unit to direct reading mode:

```
#041;DIR<Enter>
```

Set the instrument to run continuously at 1Hz:

```
#003;CONT;1;1;30;NONE;1<Enter>
```

Enter Run mode:

```
#028<Enter>
```

***Data should now be displayed and updated at 1Hz, whichever method is being used.***

7. Open the MS Excel spreadsheet supplied on the Valeport CD-ROM, numbered "04008118" (and a letter indicating version number). Cells where data must be entered are signified by red ## symbols.
8. At the top of the sheet "pH secondary calibration", fill in the instrument type, serial number and pH sensor serial number (printed on a small label on the base of the sensor). This is so that the documentation is complete in case you wish to print or save the secondary calibration record. Also enter the module number, determined from DataLog 400 or HyperTerminal – this is essential for generating the data string to send to the instrument after the calibration.
9. Whilst the buffers are relatively stable, they do show a slight variation in pH with temperature. This is shown on the sheet titled "Temperature correction" in the 04008118 spreadsheet. Whilst this variation is small, it should be accounted for if the calibration is to be as accurate as possible. Read the temperature value from the instrument output, and enter the value in the Air Temperature cell in the spreadsheet – a stability of 0.1°C is more than acceptable for this correction. The nominal pH values in the table will automatically be corrected accordingly.
10. By now, the pH 10 readings should have stabilised; 10 consecutive identical readings is an acceptable indication of stability. Enter this mV value into the spreadsheet, against pH value 10.
11. Unscrew the calibration pot from the electrode, and empty. It is acceptable to re-use this solution.
12. Thoroughly rinse the calibration pot with distilled water, and dry with a paper towel. Likewise, clean the glass electrode with distilled water and dry.

13. Refit the calibration pot over the sensor, and use a clean syringe to put 10ml of pH7 buffer inside. Leave to stabilise for at least 2 minutes.
14. Again, view the data output in either HyperTerminal or DataLog 400. When the readings are stable, enter the mV output into the appropriate cell in the spreadsheet.
15. Repeat steps 12 - 14 for the remaining pH 4 buffer, leaving the calibration pot with pH 4 buffer in place after taking the final reading.
16. Having entered all three mV calibration readings in the spreadsheet, view the "Calibration curve" page. This should show a straight black line (the calibration fit) running over a blue curve (actual data measured). For a perfect calibration, the blue curve will be entirely hidden by the black line.
17. The equation of the black calibration line should be displayed on the graph. Click this equation with the mouse, and then drag the mouse to highlight the text. Make sure every character is highlighted. Copy this text and paste it into the "pH secondary calibration" sheet, in the green cell as indicated.
18. Look at the calculated pH error – this should be within the specification of the sensor as indicated. The sheet will automatically indicate if the calibration has passed or failed for each measured point. If the calibration has failed at any point, the entire procedure should be repeated – note that the point at which failure is indicated is not necessarily the incorrect reading, it is simply the point at which the straight line fit is most inaccurate. This could be because any of the measurement points has skewed the straight line.
19. If the sensor repeatedly fails the calibration procedure, it is likely that the small ceramic frit in the side of the sensor has become contaminated with crystalline deposits. Soak the sensor in pH7 solution for several hours to remove these deposits, and then retry the calibration procedure.
20. If the calibration is acceptable, the next step is to enter the calibration into the unit, which is done in the form of a text string, either through DataLog 400 or HyperTerminal, as follows.

In DataLog 400, interrupt the unit's operation once again, and enter the Module Setup page. Highlight the pH sensor. Now enter "pH" in the User Units box. Copy the calibration string from the DataLog 400 box in the spreadsheet, and paste it into the calibration string box in DataLog 400. Press the "Setup Sensor" button, and the string is sent to the instrument.

In HyperTerminal, once again interrupt by repeatedly sending #<Enter> until the message ERROR appears.

Copy the calibration string from the HyperTerminal box in the spreadsheet and paste it into HyperTerminal. The unit should repeat the string back to confirm acceptance.

Next, set the correct units by entering the following string:

```
#020;<pH_module_number>;1;pH<Enter>
```

21. Now, the unit should output calibrated pH data. Set it into Run mode again (either by selecting Run in DataLog 400 or by sending the command #028<Enter> in HyperTerminal).
22. The calibration pot with pH4 solution should still be in place. Check the output of the sensor, and enter the calibrated value into the Measured pH cell in the spreadsheet, under the Post Calibration Check area. The error shown should be within specification – again a Pass/Fail status will be indicated.
23. If required, further post-calibration checks can be performed at pH10 and pH 7. If this is done, it is advised to check pH10 first, followed by pH7. Be sure to thoroughly and repeatedly wash and dry the sensor and the calibration pot before using the pH10 solution – a single drop of the pH4 buffer in the pH10 buffer could drastically alter the pH.
24. When calibration is complete, put a few drops of the sensor reference fluid into the Perspex sensor cap, and re-fit, tightening the screw when the cover is in place. Finally, screw the protective black acetal guard back over the glass electrode.

## 2 REDOX (E<sub>H</sub>)

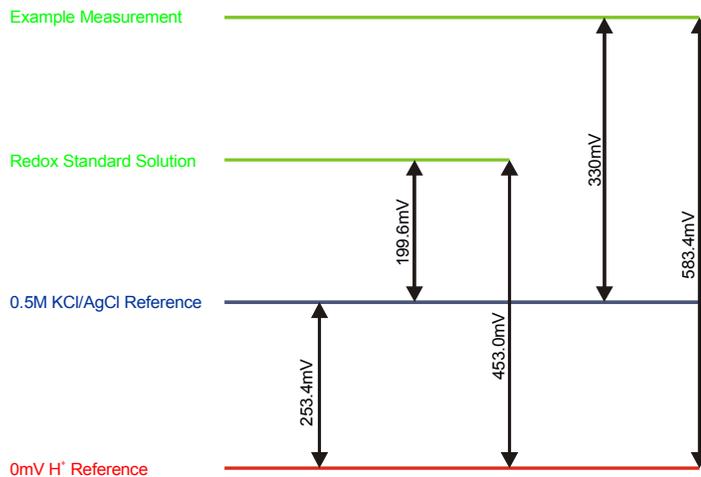
### INTRODUCTION

Redox, or E<sub>H</sub> sensors give an analogue output signal (in millivolts) – the accurate measurement of this signal is the function of the primary sensor calibration, which is given in the calibration section of this manual. This primary calibration has a high level of long-term stability, and should not need to be rechecked any more often than the conductivity, temperature or pressure sensors – typically every one or two years depending on the customer's own requirements.

### PRINCIPLE OF REDOX MEASUREMENT

Redox measurements are an actual millivolt measurement of the electric potential (e.m.f.) generated between a reference fluid (inside the sensor), and a sample fluid (outside the sensor). The magnitude of this e.m.f. is a measurement of the capacity of the fluid to cause a chemical reaction (reduction & oxidation of the species involved). However, Redox measurements are usually theoretically referenced to a Hydrogen electrode, which is defined as having zero electric potential. Hydrogen electrodes are expensive, and not suitable for field measurements. For this reason, field Redox sensors such as that fitted to Valeport instruments use a stable reference fluid as the internal reference, and compare the Redox potential of the sample fluid to this value.

The reference fluid in the Valeport Redox sensor is 0.5M KCl/AgCl. Compared to a zero potential Hydrogen electrode, this fluid itself has a Redox potential of approximately +253.4mV at 25°C. Any Redox potentials measured by this sensor are therefore offset from the true Redox potential by this value. For example, if the sensor is reading a Redox potential of +330mV, the actual Redox potential, with respect to a zero potential Hydrogen electrode would be +583.4mV. This is denoted by the symbol E<sub>H</sub>



The output from the sensor is the actual measured millivolt output (+330mV in the above example). The user has the option within the DataLog 400 software to correct this value to the true Hydrogen electrode referenced Redox potential, if required.

The offset figure of +253.4mV is only valid at 25°C, since the Redox potential of the reference solution is temperature dependent, shifting by 0.7mV per °C. In correcting the measured Redox potential to E<sub>H</sub>, DataLog 400 uses the output from the Temperature sensor to apply the correct correction factor; this can be summarised in the form of an equation:

$$\text{Correction factor} = 271.1 - 0.71 \times T$$

DataLog 400 uses this equation as default, but the equation can be manually altered if required, within the Sensor Setup page. The user may wish to do this if a different reference solution is used. To make it as easy as possible, the user is asked to simply enter the characteristics of their reference. Any standard reference buffer used in a Redox sensor will have defined characteristics. Just enter the reference potentials of the fluid at two different temperatures as requested, and the software will calculate the appropriate gain and offset figures.

The nature of Redox potential measurements means that regular calibration is not required, as with pH sensors. However, it is possible that with time, a certain amount of reference fluid may leak out of the sensor, and be replaced with water. This will naturally alter the reference potential. To check the performance of the sensor, it is therefore supplied with a quantity of standard solution 0.1M K<sub>4</sub>Fe(CN)<sub>6</sub> / 0.05M K<sub>3</sub>Fe(CN)<sub>6</sub> (Potassium Ferro-Cyanate). This solution is temperature dependent, but at 25°C will give a Redox potential of +199.6mV (+453mV E<sub>H</sub>).

## PERFORMANCE CHECK

A check on the performance of the Redox sensor may be made using either the DataLog 400 software or a terminal emulation program such as HyperTerminal. The procedures for both methods are shown below.

Using DataLog 400, interrupt the unit. When the unit has been interrupted, DataLog 400 displays a summary screen detailing the serial number of the instrument, firmware version, amount of memory available, and various other parameters. Click OK to proceed to the setup page.

Set the instrument to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the Redox sensor module is setup to output primary, uncorrected calibration data (mV). Refer to Section 4.2.3 of the software manual for details on how to do this.

Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Ensure that the Redox sensor is set to output data in mV by sending the command:

```
#020;<Redox_module_number>;1;<space><Enter>
```

Set the unit to direct reading mode:

```
#041;DIR<Enter>
```

Set the instrument to run continuously at 1Hz:

```
#003;CONT;1;1;30;NONE;1<Enter>
```

Enter Run mode:

```
#028<Enter>
```

1. Unscrew the protective guard from the Redox sensor, and remove the Perspex protective cover from the end of the electrode by loosening the screw in the end of the cover. This will allow the cover to then gently slide off the end of the electrode, exposing the 5mm metal tip.
2. Wash the calibration pot in distilled water and dry thoroughly.
3. Using a syringe (supplied), slowly inject 10ml of the Redox standard solution into the pot through one of the side holes.
4. View sensor output using the chosen method.
5. Alternatively, if just a quick check is required, the exposed sensor can simply be placed into the full pot of Redox standard solution, removing the need for the calibration pot.
6. When the check is complete, put a few drops of the sensor reference fluid into the Perspex sensor cap, and re-fit, tightening the screw when the cover is in place. Finally, screw the protective black acetal guard back over the glass electrode.

**Thoroughly rinse the sensor in water after immersion in the standard solution**

The sensor should output the following values in the standard solution:

Temperature (°C)	mV	E <sub>H</sub>
10	212.5	476.6
15	207.3	468.8
20	202.2	461.1
25	199.6	453.0
30	192.0	445.7

If the sensor gives a different reading in this standard solution, this indicates that the potential of the reference solution has changed and the sensor should be checked using the procedures described overleaf.

### **REFRESHING THE SENSOR**

1. Gently unscrew the calibration pot from the Redox sensor, exposing the glass electrode.
2. Tilt the instrument on its side, and check to see that no air bubbles are present within the sensor. If bubbles are present, this simply means that some of the internal reference solution has escaped, and must be replaced. Fit the white plastic nozzle to the top of the Redox reference solution (marked 0.5M KCl/AgCl). Gently slide the rubber ring down the sensor, exposing the small refill hole in the side of the glass electrode. Orientate the instrument so the air bubbles in the electrode are beneath this hole, and then slowly use the nozzle on the reference bottle to fill the electrode. Once the electrode is full, replace the rubber ring to seal the solution inside.

If there are no air bubbles present, then the solution may need to be replaced.

1. Tilt the instrument on its side, and gently slide the rubber ring down the sensor, exposing the small refill hole in the side of the glass electrode. Using a syringe (supplied) gently extract the old reference solution from the sensor.
2. Fit the white nozzle to the bottle marked 0.5M KCl/AgCl. Slowly squeeze the bottle, injecting the solution into the sensor, ensuring that no air bubbles are present. Slide the black rubber ring back in place to seal the solution inside.

Once the solution has been replaced, repeat the test against the standard solution.

### 3 DISSOLVED OXYGEN

#### INTRODUCTION

The measurement of dissolved oxygen levels used to be carried out chemically – this was time consuming and not really suitable for field measurements. The development of an electrical technique by Hosiiah Clark allowed quick and easy measurements to be taken in the field. The principle of the Clark cell is that oxygen diffuses across a semi-permeable membrane, acting as the link between two half-cells. The first half cell is a (usually) lead electrode in KCl reference solution; the other being a platinum electrode in the solution to be measured. The rate of diffusion of oxygen across the membrane (and thus the e.m.f. generated by the “battery”) is related to the partial pressure of oxygen in the measured solution.

Valeport offer two types of DO sensor. For shallow water measurements (up to 2000m), an Oxyguard DO sensor is used. For deep water measurements (up to 7000m), an Idronaut pressure balanced DO sensor is used. Both are variations on the standard Clark cell.

#### OXYGUARD (2000M)

Advantages of this cell type include:

- You do not have to apply a potential to it to make it work - it generates its own electricity;
- The Oxyguard type of oxygen sensor is always ready for use - it does not need to "warm up";
- This type of sensor reacts quickly to changes in oxygen level;
- The Oxyguard type cell does not decay or lose sensitivity with age - it maintains its efficiency - so you do not have to perform maintenance on a regular basis.

You do, of course, need to keep the membrane of any membrane covered oxygen sensor reasonably clean since deposits (bacteria, fat etc.) on the membrane will act as a barrier to the oxygen the cell is trying to measure. The membrane is reasonably robust, and will tolerate being washed and dried with paper towel.

If the membrane becomes severely wrinkled, it should be renovated, as described below.

There are two main procedures that the user will need to carry out to obtain good results:

- Calibration
- Renovation

#### CALIBRATION

As with other sensor fitted to the Valeport instruments, the Oxyguard DO sensor outputs a voltage signal. The primary calibration of the sensor is simply to allow the accurate measurement of this voltage. This is very stable calibration, which should not need to be rechecked any more often than the other sensors fitted to the instrument, typically every one or two years depending on the user's own requirements.

However, the secondary calibration of this voltage output into meaningful dissolved oxygen values requires more regular attention. We recommend the following intervals:

For intermittent use (e.g. once per month)	Calibrate before each deployment
For regular use (e.g. several time per day)	Calibrate every other day
For long term deployments	Calibrate before deployment and after recovery. Assume linear drift between the two calibrations, and correct data for the drift over time.

Dissolved Oxygen is usually given either as an absolute concentration (mg/l) or as % saturation. In an equilibrated system at a fixed temperature, the water will be 100% saturated with Oxygen – the actual mg/l concentration of Oxygen will be dependent on the temperature (and salinity) of the water. Chemical and Biological process in the water will decrease this Oxygen level. The Valeport Oxyguard sensor is designed

to give % saturation values of DO, and automatically compensates for changes in temperature. The secondary calibration procedure is thus a simple one.

The performance of the sensor is linear, so a unit change in DO will give rise to a fixed change in voltage output. To perform a secondary calibration, it is therefore only necessary to define two points to characterise the straight line fit. Fortunately, the nature of the sensor means that if there is no Oxygen in the water, no e.m.f. will be generated; we therefore know that the first point on the line will pass through the origin, i.e. 0%sat = 0v.

To define the second point, an equilibrated system must be created, with 100% saturated water. In such an equilibrium, the Oxygen concentration in the water just below the surface is the same as that in the air just above the surface. The sensor can therefore be calibrated in air-saturated water, or in water-saturated air.

The important point to note about performing the calibration is that this equilibrium takes time to establish, and is temperature related. It is therefore recommended that a small amount of water be left to stand at room temperature for period of at least 30 minutes before the calibration takes place.

Communications and the calibration procedure may be performed either in DataLog 400 or through HyperTerminal:

Using DataLog 400, interrupt the unit. When the unit has been interrupted, DataLog 400 displays a summary screen detailing the serial number of the instrument, firmware version, amount of memory available, and various other parameters. At the bottom of this screen is a list of all the sensors fitted to the instrument, preceded by a two digit "module number". This is an address number for each sensor in the instrument; note down the module number of the DO sensor.

Set the instrument to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the DO sensor module is setup to output primary calibration data (mV). Refer to Section 4.2.3 of the software manual for details on how to do this.

Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Firstly, determine the module number (address) of the DO sensor by sending the command

```
#062<Enter>
```

This will list the sensors fitted and their module numbers. Make a note of the DO module number.

Next, ensure that the DO sensor is set to output data in mV by sending the command:

```
#020;<DO_module_number>;1;<space><Enter>
```

Set the unit to direct reading mode:

```
#041;DIR<Enter>
```

Set the instrument to run continuously at 1Hz:

```
#003;CONT;1;1;30;NONE;1<Enter>
```

Enter Run mode:

```
#028<Enter>
```

***Data should now be displayed and updated at 1Hz, whichever method is being used.***

1. Open the MS Excel spreadsheet supplied on the Valeport CD-ROM, numbered "04008120" (and a letter indicating version number; a, b, c, etc.). Cells where data should be entered are signified by red ## symbols.
2. At the top of the sheet "DO secondary calibration", fill in the instrument type, serial number and DO sensor serial number (printed on a small label on the base of the sensor). Also enter the module number, determined from DataLog 400 or HyperTerminal.

3. Remove the cap from the DO sensor, and dry the membrane thoroughly with a paper towel.
4. Remove the small sponge from the DO sensor cap, and dry the cap thoroughly. Put just a few drops of “equilibrated” water into the bottom of the cap, and screw the cap lightly onto the sensor.
5. At this point, view the data output on the PC. It is an unfortunate consequence of the measurement method of DO, that the actual measurement depletes the oxygen levels. Over a period of time, it is possible to see a steady decrease in DO levels whilst performing the calibration. The user must therefore make a judgement on when the sensor has stabilised after the cap fitted, but before the oxygen begins to deplete. The readings given approximately 10 seconds after application of the cap should be taken as being representative of the 100% saturation figure.
6. Note this millivolt reading and enter it into the spreadsheet.
7. Remove the cap from the sensor, and dry it and the membrane thoroughly
8. View the “Calibration curve” page. This should show a straight black line (the calibration fit) running over a blue curve (actual data measured). For a perfect calibration, the blue curve will be entirely hidden by the black line.
9. The spreadsheet will also calculate this straight line fit, and will automatically apply it to the measured values.
10. Look at the calculated DO error – this should be zero, since both points should be on the straight line.
11. If the calibration is acceptable, the next step is to enter the calibration into the unit, which is done in the form of a text string, either through DataLog 400 or HyperTerminal, as follows.

In DataLog 400, interrupt the unit’s operation once again, and enter the Module Setup page. Highlight the DO sensor. Now enter “sat%” in the User Units box – note that the first character in the User Units must be a letter so “%sat” is not acceptable. Copy the calibration string from the DataLog 400 box in the spreadsheet, and paste it into the calibration string box in DataLog 400. Press the “Setup Sensor” button, and the string is sent to the instrument.

In HyperTerminal, once again interrupt by repeatedly sending #<Enter> until the message ERROR appears.

Copy the calibration string from the HyperTerminal box in the spreadsheet and paste it into HyperTerminal. The unit should repeat the string back to confirm acceptance.

Next, set the correct units by entering the following string:

```
#020;<DO_module_number>;1;sat%<Enter>
```

1. Now, the unit should output calibrated DO data as % saturation. Set it into Run mode again (either by selecting Run in DataLog 400 or by sending the command #028<Enter> in HyperTerminal).
2. Add a few drops of water to the cap, and refit it to the sensor. Again, after a few seconds the readings will be representative, but slowly decreasing. Enter these values in the post calibration check section of the spreadsheet, and confirm that they are within specification.

## RENOVATION

Renovation will be necessary if the membrane is damaged, wrinkled, fouled, or if the readings are so unstable as to prevent calibration.

1. Clean the outside of the probe. Unscrew the membrane cap, discard the used membrane and O-ring. Clean and dry the cap.
2. Soak the probe end in electrolyte - how long depends on how long ago it was last soaked.
3. Inspect the cathode face. If the wooden insert has lifted above the cathode or edge wet the grade 300 wet-or-dry emery paper delivered with the probe with electrolyte and use it to smooth it down until no edges can be felt, using the electrolyte as lubricant.
4. Put a new O-ring in place in the bottom of the cap and place a new membrane above it. It is very important that the membrane is placed concentrically in the bottom of the cap above the O-ring. Take care not to touch the face of the membrane.
5. Dip the cathode face in electrolyte.
6. Hold the probe with the cathode face up and gently screw the cap with O-ring and membrane onto it.
7. Tighten the cap firmly. The membrane should not wrinkle, if it does discard it and start again with a new membrane.
8. Renovation is complete. Calibrate the probe. Remember to store with the protector fitted. The sponge in the protector must be kept moist at all times.

## IDRONAUT 7000M

The pressure balanced Idronaut sensor will operate down to 7000m depth, and is offered as an option instead of the standard Oxyguard type. Although the sensor is quite robust, you do need to keep the membrane reasonably clean since deposits (bacteria, fat etc.) on the membrane will act as a barrier to the oxygen the cell is trying to measure. The membrane is reasonably robust, and will tolerate being washed and dried with paper towel.

As well as calibration of the sensor, to ensure continuity of performance of the oxygen sensor, periodic renewal of electrolyte and membrane is to be performed. It is suggested that electrolyte is renewed every month, and that the membrane is renewed every 3 months.

## CALIBRATION

As with other sensor fitted to the Valeport instruments, the DO sensor outputs an analogue signal, in this case nano-Amps (nA). The primary calibration of the sensor is simply to allow the accurate measurement of this current. This is a very stable calibration, which should not need to be rechecked any more often than the other sensors fitted to the instrument, typically every one or two years depending on the user's own requirements.

However, the secondary calibration of this current output into meaningful dissolved oxygen values requires more regular attention. We recommend the following intervals:

For intermittent use (e.g. once per month)	Calibrate before each deployment
For regular use (e.g. several time per day)	Calibrate every other day
For long term deployments	Calibrate before deployment and after recovery. Assume linear drift between the two calibrations, and correct data for the drift over time.

Dissolved Oxygen is usually given either as an absolute concentration (mg/l) or as % saturation. In an equilibrated system at a fixed temperature, the water will be 100% saturated with Oxygen – the actual mg/l concentration of Oxygen will be dependent on the temperature (and salinity) of the water. Chemical and Biological process in the water will decrease this Oxygen level.

The performance of the sensor is linear, so a unit change in DO will give rise to a fixed change in current output. To perform a secondary calibration, it is therefore only necessary to define two points to characterise the straight line fit. Fortunately, the nature of the sensor means that if there is no Oxygen in the water, no current will be generated; we therefore know that the first point on the line will pass through the origin, i.e. 0%sat = 0nA.

To define the second point, we must simply measure the output of the sensor in water that we know to be saturated with oxygen, or in air that is saturated with water. This occurs at the boundary between water and air, so the calibration is best performed with a cup of water that has been allowed to stabilise and equilibrate with the atmosphere.

Communications and the calibration procedure may be performed either in DataLog 400 or through HyperTerminal:

Using DataLog 400, interrupt the unit. When the unit has been interrupted, DataLog 400 displays a summary screen detailing the serial number of the instrument, firmware version, amount of memory available, and various other parameters. At the bottom of this screen is a list of all the sensors fitted to the instrument, preceded by a two digit "module number". This is an address number for each sensor in the instrument; note down the module number of the DO sensor.

Set the instrument to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the DO sensor module is setup to output primary calibration data (nA). Refer to Section 4.2.3 of the software manual for details on how to do this.

Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Firstly, determine the module number (address) of the DO sensor by sending the command

```
#062<Enter>
```

This will list the sensors fitted and their module numbers. Make a note of the DO module number.

Next, ensure that the DO sensor is set to output data in nA by sending the command:

```
#020;<DO_module_number>;1;<space><Enter>
```

Set the unit to direct reading mode:

```
#041;DIR<Enter>
```

Set the instrument to run continuously at 1Hz:

```
#003;CONT;1;1;30;NONE;1<Enter>
```

Enter Run mode:

```
#028<Enter>
```

*Data should now be displayed and updated at 1Hz, whichever method is being used.*

1. Open the MS Excel spreadsheet supplied on the Valeport CD-ROM, numbered "04008120" (and a letter indicating version number; a, b, c, etc.). Cells where you will need to enter data are signified by red ## symbols.
2. At the top of the sheet "DO secondary calibration", fill in the instrument type, serial number and DO sensor serial number (printed on a small label on the base of the sensor). Also enter the module number, determined from DataLog 400 or HyperTerminal.
3. Dry the membrane thoroughly with a paper towel.
4. At this point, view the data output on the PC. It is an unfortunate consequence of the measurement method of DO, that the actual measurement depletes the oxygen levels. Over a period of time, it is possible to see a steady decrease in DO levels whilst performing the calibration. The user must therefore make a judgement on when the sensor has stabilised after the cap fitted, but before the oxygen begins to deplete. The readings given approximately 10 seconds after application of the cap should be taken as being representative of the 100% saturation figure.
5. Note this reading and enter it into the spreadsheet.
6. Remove the cap from the sensor, and dry it and the membrane thoroughly
7. View the "Calibration curve" page. This should show a straight black line (the calibration fit) running over a blue curve (actual data measured). For a perfect calibration, the blue curve will be entirely hidden by the black line.

8. The spreadsheet will also calculate this straight line fit, and will automatically apply it to the measured values.
9. Look at the calculated DO error – this should be zero, since both points should be on the straight line.
10. If the calibration is acceptable, the next step is to enter the calibration into the unit, which is done in the form of a text string, either through DataLog 400 or HyperTerminal, as follows.

In DataLog 400, interrupt the unit's operation once again, and enter the Module Setup page. Highlight the DO sensor. Now enter "sat%" in the User Units box – note that the first character in the User Units must be a letter so "%sat" is not acceptable. Copy the calibration string from the DataLog 400 box in the spreadsheet, and paste it into the calibration string box in DataLog 400. Press the "Setup Sensor" button, and the string is sent to the instrument.

In HyperTerminal, once again interrupt by repeatedly sending #<Enter> until the message ERROR appears.

Copy the calibration string from the HyperTerminal box in the spreadsheet and paste it into HyperTerminal. The unit should repeat the string back to confirm acceptance.

Next, set the correct units by entering the following string:

```
#020;<DO_module_number>;1;sat%<Enter>
```

3. Now, the unit should output calibrated DO data as % saturation. Set it into Run mode again (either by selecting Run in DataLog 400 or by sending the command #028<Enter> in HyperTerminal).
4. Observe the readings again. Again, after a few seconds the readings will be representative, but slowly decreasing. Enter these values in the post calibration check section of the spreadsheet, and confirm that they are within specification.

### **RENEWING ELECTROLYTE**

- 1) Using the procedure described above, perform a calibration on the Oxygen sensor.
- 2) Unscrew the end cap of the oxygen sensor, keeping the membranes intact and in place. Pay attention not to damage the glass tip of the electrode.
- 3) Wash the silver and glass assembly with distilled water and dry it with a lint free paper towel. Do not touch the internal parts of the sensor.
- 4) In this condition, with the sensor tip cleaned and dried, the sensor should output less than 2nA raw current (less than 2ppm, or less than 3% saturation, approximately). If the readout is more than this, there is probably still a thin film of moisture on the sensor. Dry it thoroughly and repeat.
- 5) Carefully fill the membrane cap with the electrolyte supplied, ensuring that no air bubbles are caught on the membrane or in the cap.
- 6) Gently screw the membrane cap back onto the sensor body, and tighten securely.
- 7) Dry the sensor and membrane with a lint free paper towel.
- 8) Using the procedure described in the calibration manual, recalibrate the Oxygen sensor.

#### **Note**

Maximum stability of readout is achieved approximately 30 minutes after electrolyte replacement, once the sensor has repolarised.

## **RENEWING MEMBRANE**

### **AVAILABLE MEMBRANES**

The performance of the oxygen sensor depends on the type of membrane being used. Three membranes are available: “green” and “blue” as internal measurement membranes; “red” as an additional external protective membrane. The choice between the green and blue membrane affects the time constant, the blue membrane being faster but more subject to stirring effects. For normal use (Single Point or standard Profiling acquisition at >0.2m/s), the green membrane should be used. The blue membrane should only be used in fast profiling applications (>1m/s).

The outer protective red membrane can be added in order to:

- Increase the life of the measuring membrane
- Limit the effect of fouling
- Avoid the need of sample stirring

The use of the external red membrane slows the response of the sensor. For samples with a very low oxygen content, or whenever fast reading is required (profiling), it is recommended to not use the red membrane, and to use the green or blue membrane only.

The following table lists the recommended configurations to obtain maximum performance for different applications:

Application	Membrane		Time Constant	Stirring Effect
	Internal (measurement)	External (protection)		
Single Point	Green	Red	15s	3%
Profiling	Green	None	3s	25%
Fast Profiling	Blue	None	0.9s	40%

Notes:

- The time constant is the time taken for the sensor to respond to a step change from Nitrogen to air.
- The stirring effect is the difference in measurement between a stirred sample and a stagnant sample.
- All above values are at 25°C.
- Sensor is delivered with green membrane installed.

### **MEMBRANE REPLACEMENT**

Conditions which could require the membrane to be replaced are:

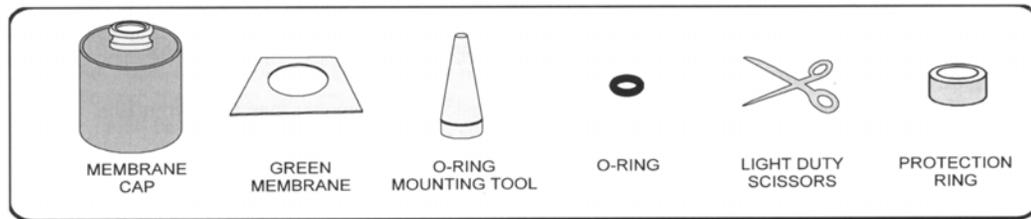
- The sensor responds more slowly than usual or drifts
- The membrane shows leakage, holes or scratches
- A reading of over 2nA/0.2ppm or 3% is given when the sensor is used in the absence of oxygen
- The sensor has been stored for a long time outside the recommended temperature range (-10°C to +40°C)

The Model 606+ is supplied with a DO sensor maintenance kit. Locate the following parts in the kit:

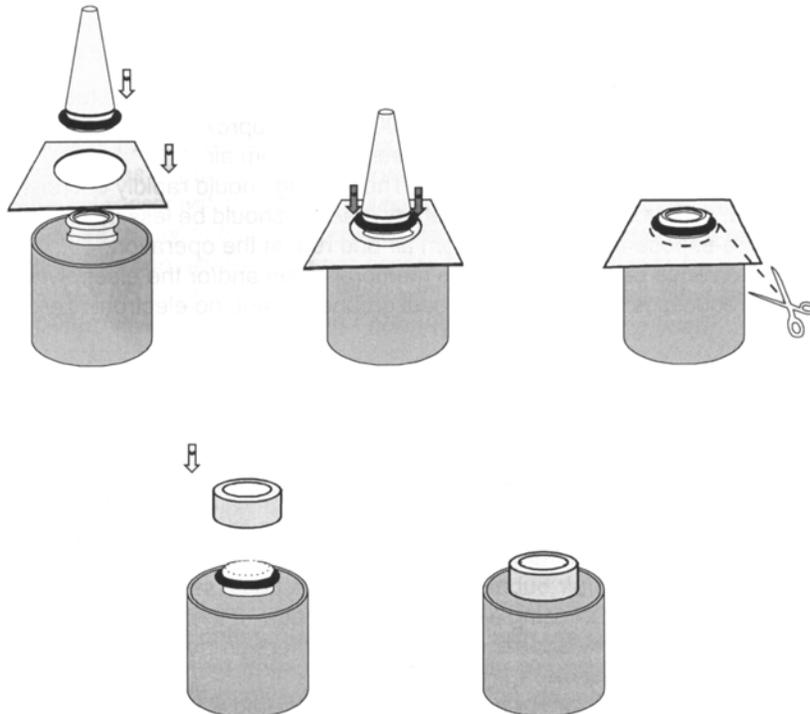
- 1 x Red membrane (if required)
- 1 x Green or Blue membrane (as necessary)
- 1 x o-ring
- 1 x o-ring mounting tool

In addition, a pair of scissors will be required.

It is advisable to carry out this procedure with the membrane cap removed from the sensor.



- 1 Remove the white protection ring from the cap. Remove and discard the old o-ring and membrane(s).
- 2 Fit the new o-ring over the mounting tool and roll it down to the widest part.
- 3 Place the cap on a flat surface, with the membrane end up.
- 4 Position the measuring membrane (green or blue) over the top of the cap.
- 5 If the red (protective) membrane is required, remove the white plastic backing. Place this membrane on top of the first membrane.



- 6 Place the o-ring tool over the membrane(s), and carefully roll the o-ring down, securing the membrane(s) in place.
- 7 Cut away the excess membrane, taking care not to damage the o-ring.
- 8 Place the white protection ring over the membrane and o-ring.
- 9 Refill the membrane cap with new electrolyte, and replace it as described above.

## SENSOR CLEANING

Rarely (every 2 or 3 years), replenishing the electrolyte and membrane will fail to restore the output current to normal levels. For reference, acceptable current output levels for the sensors are:

<b>Measurement Membrane</b>	<b>Current Output nA (approx)</b>
Green	40 – 70
Blue	80 - 120

- 1 If the output current is lower than these levels, it is necessary to clean the sensor tip.
- 2 Remove the membrane cap as described in the electrolyte changing procedure.
- 3 Very gently, rub the tip with the abrasive paper included in the maintenance kit, and wash with distilled water or electrolyte.
- 4 If the silver anode is excessively black or covered in debris, use the abrasive paper to clean this also, until it is restored to a normal silver colour. Again, rinse with distilled water or electrolyte.
- 5 It will be noticed that after these procedures, the output may be higher than expected for a few hours, but will slowly drop to normal levels.

## 4 TURBIDITY

### INTRODUCTION

Turbidity sensors are optical sensors rather than Electro-chemical sensors, but they still output a voltage signal related to the turbidity measured. As with other Valeport sensors, the primary calibration of the sensor is simply to allow the accurate measurement of that voltage output. The relationship between that voltage output and the turbidity level will require secondary calibration by the user.

The problem with turbidity sensors is that the output of the sensor is not only related to the amount of suspended particulate matter, but also the nature of that matter. The principle of measurement is to emit a beam of infrared light, and then measure how much of that light is scattered or reflected by the particles in the water. In absolutely pure water, with no particles, no light will be reflected back to the sensor. In highly turbid water, all (or nearly all) of the light will be reflected off the particles back to the sensor.

It is easy to see how the nature of the particles can affect the performance of the sensor; again, looking at extreme situations, the water sample could be full of small glass beads. This is particulate matter in high concentration, say up to 500g/l. However, the glass beads will hardly reflect any light at all, resulting in a low voltage output from the sensor, despite the obviously high amount of suspended matter. Conversely, the water sample may contain just a single particle with a highly mirrored surface. This will obviously reflect a good deal of light, resulting in a high voltage output from the sensor, despite the fact that only a single particle is present.

Within these extreme circumstances, there are variations in performance between different sediment types. A sandy sediment will cause a different response from the sensor than a clay-like sediment. To attempt to overcome these variations, a standard measurement system has evolved, based on the response of the turbidity sensor to a standard suspended particle. The chosen substance is Formazin, resulting in a standard unit of FTU (Formazin Turbidity Unit). This is sometimes interchanged with the unit NTU (Normalised Turbidity Unit). Despite the existence of this “standard” unit, care should be taken not to compare data from different environments with different types of suspended matter.

Seapoint Turbidity sensors as fitted to Valeport 400 series instruments have a normalised output, and as such, all perform similarly to different Formazin concentrations. This standard Formazin calibration is held within DataLog 400, and may be set as a secondary calibration from a drop down menu; refer to Section 4.2 of the Software Operation Manual for details of how to do this. A feature of the Seapoint Turbidity sensor is that it has software selectable gain control, so that the sensor output can be optimised for the expected turbidity range. The appropriate secondary calibration fit for the selected gain is automatically used, and is sent to the sensor when the “Sensor Setup” button is pressed.

To accurately measure the actual suspended sediment concentration at a specific site, rather than just relying on the FTU standard (which may be misleading), it will be necessary to perform a calibration on the sensor.

To do this, the sensor must be placed in various solutions containing different sediment concentrations, and the output from the sensor at each concentration noted. A polynomial fit can then be created for these measurements, and the equation then programmed into the instrument. A spreadsheet for doing all the required calculations is provided on the software CD supplied with the instrument, numbered “04008119”.

Note that whilst polynomial calibrations provide the most accurate fit over the range of data used, they may give rise to spurious readings outside this range. For example, if calibration is carried out up to a sediment concentration of 5g/l, readings above this value should not be trusted. If it is expected that readings outside the calibration range will occur, then a straight line fit should be used instead.

**Equipment:****Supplied with product**

- Instrument with turbidity sensor
- Black acetal calibration pot
- Secondary calibration spreadsheet

**Supplied by customer**

- Distilled Water (several litres)
- Dried sediment sample
- Accurate weighing balance
- Plastic or glass beakers (500ml+)
- Plastic stirrers
- Paper towel
- Waste bucket
- PC running DataLog 400 or HyperTerminal, and with MS Excel.

To obtain accurate calibrations, the following protocol should be used. Remember that high quality calibrations can only be achieved with patience and attention to detail. This protocol assumes that the user has the facilities and general know-how to create solutions of known sediment concentration, but general guidance is offered.

**PREPARATION**

1. Take water samples of the proposed site, taking care to ensure that the water that is taken is representative of what the instrument will measure. There is no point taking surface water samples if the instrument will be deployed on the bed, or vice versa. It is not always a good idea to use actual bed sediment for samples, since this may not be representative of the suspended sediment. It is suggested that several litres of water sample are collected.
2. In the laboratory, remove the instrument from its deployment frame (if fitted), and position it so that it is stable, with easy access to the sensors. Thoroughly clean and dry the black plastic calibration pot, taking care to remove any deposits from previous work. Place the turbidity calibration pot over the turbidity sensor, ensuring that the sealing o-ring is in place, and the drain hole blocked. Also take care to ensure that the window of the turbidity sensor is clean and dry, and faces the widest area of the pot.

It is recommended that the calibration procedure is carried out under fluorescent lighting, since this has minimal output at the frequencies measured by the sensor.

3. The next step is to determine the appropriate gain setting for the calibration and subsequent deployment. Fill the calibration pot with a sample of water from the proposed site. The sampling volume of the sensor is of an area over the whole sensor window, extending approximately 5cm from the face.

Using DataLog 400, interrupt the unit. When the unit has been interrupted, DataLog 400 displays a summary screen detailing the serial number of the instrument, firmware version, amount of memory available, and various other parameters. At the bottom of this screen is a list of all the sensors fitted to the instrument, preceded by a two digit "module number". This is an address number for each sensor in the instrument; note down the module number of the turbidity sensor.

Set the instrument to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the turbidity sensor module is setup to output primary calibration data (mV). Choose a gain setting for the sensor. Refer to Section 4.2 of the software manual for details on how to do this.

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Firstly, determine the module number (address) of the turbidity sensor by sending the command

```
#062<Enter>
```

This will list the sensors fitted and their module numbers. Make a note of the turbidity module number.

Next, ensure that the turbidity sensor is set to output data in mV by sending the command:

```
#020;<turbidity_module_number>;1;<space><Enter>
```

Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

Set the unit to direct reading mode:

#041;DIR<Enter>

Set the instrument to run continuously at 1Hz:

#003;CONT;1;1;30;NONE;1<Enter>

Enter Run mode:

#028<Enter>

*Data should now be displayed and updated at 1Hz, whichever method is being used.*

4. Repeat the above procedures at a different gain setting, until a suitable output level is reached. It is suggested that the user aim for an output in the region of 1 – 2.5 volts for this sample. A higher output than this suggests that the gain is too high, and the maximum output of 5v will be reached at turbidity levels only slightly greater than that of the sample. Conversely, a very low reading will mean that resolution is lost for turbidity levels lower than that of the sample. Common sense is the best guide here.
5. Once the appropriate gain setting has been established, open the MS Excel spreadsheet supplied on the Valeport CD-ROM, numbered “04008119” (and a letter indicating version number; a, b, c, etc.). Cells where you will need to enter data are signified by red ## symbols.
6. At the top of the sheet “Turbidity secondary calibration”, fill in the instrument type, serial number and turbidity sensor serial number (marked on the base of the sensor). Also enter the module number, determined from DataLog 400 or HyperTerminal.
7. The next stage is to decide on the various dilutions and concentrations required for the calibration. Generally, a reasonable starting point would be the following range, relative to the original sample concentration:

Pure water  
 1/8 dilution  
 1/4 dilution  
 1/2 dilution  
 original sample  
 2x concentration

Variation on these ranges to the user's choice may certainly be used, but at all times remember that the maximum output will be 5v. The user should ensure that the higher concentrations used do not exceed this value.

8. Now, determine the actual sediment concentration in the original sample. Filter and dry the sediment from a litre of the sample, and weigh accurately. Based on the choice of calibration concentrations above, it should now be possible to enter the proposed sediment load values in the 04008119a.xls spreadsheet.
9. Manufacture the diluted samples using a simple dilution technique. Take 1litre of the original sample, and add 1 litre of distilled water to make up to 2 litres. This is the 1/2 dilution sample. Take 1 litre of this and add to 1 litre of distilled water to create the 1/4 dilution sample, and again for the 1/8 dilution.
10. To make the concentrated sample, simply add the dried and weighed sediment from step 8 to distilled water to make up a volume of 0.5litre. This is then twice as concentrated as the original sample.

Calibration may be carried out through the DataLog 400 software provided, or through entering commands in a terminal emulation program such as HyperTerminal. Instructions for both methods are given.

11. By now, it is likely that the sample solution in the calibration pot used for gain setting will have settled slightly. We therefore recommend that the pot be emptied using the drain hole before the remainder of the procedure.
12. Use distilled water to clean the pot and sensor of any deposited matter. Dry thoroughly using paper towel. Fit the pot over the sensor, as before.

Since we are using different concentrations of solution, it is standard practice to use the most dilute first. It is easy to conceive that a single drop of high sediment solution could easily contaminate the low sediment solution, whereas a drop of the more dilute solution would have little effect on the more concentrated one. For this reason, using the most dilute solution first presents the least possible chance of contamination.

### Communicating

Using DataLog 400, interrupt the unit. When the unit has been interrupted, set it to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the turbidity sensor module is still set to output primary calibration data (mV). Refer to Section 4.2 of the software manual for details on how to do this.

Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Ensure that the turbidity sensor is set to output data in mV by sending the command:

```
#020;turbidity_module_number>;1;<space><Enter>
```

Set the unit to direct reading mode:

```
#041;DIR<Enter>
```

Set the instrument to run continuously at 1Hz:

```
#003;CONT;1;1;30;NONE;1<Enter>
```

Enter Run mode:

```
#028<Enter>
```

*Data should now be displayed and updated at 1Hz, whichever method is being used.*

13. Fill the pot with distilled water.
14. The mV output from the turbidity sensor should be reasonably stable. Enter the mV value into the appropriate cell in the 04008119 spreadsheet.
15. Empty the fluid from the calibration pot using the drain hole, and dry.
16. Repeat steps 13 to 15 using increasing concentrations of sediment, taking care to clean and dry the pot between solutions.
17. Having entered all the mV calibration readings in the spreadsheet, view the “Calibration curve” page. This should show a black line (the calibration fit) running over a blue curve (actual data measured). For a perfect calibration, the blue curve will be entirely hidden by the black line.
18. The equation of the black calibration line should be displayed on the graph. Click this equation with the mouse, and then drag the mouse to highlight the text. Make sure every character is highlighted. Copy this text and paste it into the “turbidity secondary calibration” sheet, in the green cell as indicated.
19. Look at the calculated turbidity error – this should be within the specification of the sensor (currently  $\pm 2.0\%$ ).

20. If the calibration is acceptable, the next step is to enter the calibration into the unit, which is done in the form of a text string, either through DataLog 400 or HyperTerminal, as follows.

In DataLog 400, interrupt the unit's operation once again, and enter the Module Setup page. Highlight the turbidity sensor. Now enter "mg/l" in the User Units box. Copy the calibration string from the DataLog 400 box in the spreadsheet, and paste it into the calibration string box in DataLog 400. Press the "Setup Sensor" button, and the string is sent to the instrument.

In HyperTerminal, once again interrupt by repeatedly sending #<Enter> until the message ERROR appears.

Copy the calibration string from the HyperTerminal box in the spreadsheet and paste it into HyperTerminal. The unit should repeat the string back to confirm acceptance.

Next, set the correct units by entering the following string:

```
#020;<turbidity_module_number>;1;mg/l<Enter>
```

21. Now, the unit should output calibrated turbidity data. Set it into Run mode again (either by selecting Run in DataLog 400 or by sending the command #028<Enter> in HyperTerminal).
22. The calibration pot with the highest sediment concentration solution should still be in place. Check the output of the sensor, and enter the calibrated value into the "Measured mg/l" column in the spreadsheet, under the Post Calibration Check area. The error shown should be within specification.
23. If required, further post-calibration checks can be performed at other solution concentrations. If this is done, it is advised to check with distilled water first, followed by increasing sediment concentrations. Be sure to thoroughly and repeatedly wash and dry the sensor and the calibration pot before using the distilled water – a single drop of sediment in the water could drastically alter the readings.

## 5 FLUOROMETER (CHLOROPHYLL-A)

### INTRODUCTION

Chlorophyll is an organic compound contained in all plant cells, and is a key substrate in the photosynthesis pathway that creates Oxygen from Carbon Dioxide. The world's oceans are the prime sites of photosynthesis, through the presence of phytoplankton. These tiny plant cells have a total mass that far exceeds that of all land-based vegetation, and each phytoplankton cell contains chlorophyll.

The amount of chlorophyll contained in each cell is a function of climate, season, time of day and species, but the measurement of chlorophyll concentration still provides an excellent indicator of phytoplankton numbers. The only truly accurate method of determining chlorophyll concentration is to extract it from a sample of water containing phytoplankton. This is a laboratory procedure and the methodology is not covered in detail here. It involves the destruction of the phytoplankton cells, and the removal of the chlorophyll molecules with a solvent (acetone).

However, it has long been known that chlorophyll fluoresces under UV light – the greater the chlorophyll concentration, the greater the fluorescent intensity. This feature is used by many companies to provide approximate chlorophyll levels with the use of a fluorometer, which measures the fluorescence created in a water sample under specific light wavelengths.

In laboratory conditions, this a predictable and repeatable response, varying in intensity with chlorophyll concentration. Using pure chlorophyll in acetone it is therefore possible to calibrate a fluorometer to “pure chlorophyll” conditions. However, care must be taken when applying such a calibration to field conditions, since the behaviour of chlorophyll in the field will be significantly different to the laboratory. The amount of fluorescence from the chlorophyll depends on many factors, most particularly the species of phytoplankton in which it exists. A fluorometer calibrated against a pure chlorophyll standard cannot therefore be relied upon to accurately represent the chlorophyll concentration in the ocean.

A more useful calibration method is to calibrate against the fluorescence of a known species of phytoplankton. Valeport use the Seapoint SCF Fluorometer, which is calibrated against a known culture of the algae *Isochrysis Galbana*. The actual amount of chlorophyll in a population of this species is determined by extraction with acetone as described above, and the sensor is set to provide its maximum output at a concentration of 150µg/l. Note however that this concentration of chlorophyll will result in a different amount of fluorescence with a different species, or even with a pure extract in acetone. As such, it is important to recognise that use of a fluorometer of *any* type to determine chlorophyll concentration should be treated as a comparative exercise rather than an absolute value, since any data is only approximate. For true chlorophyll readings, the use of a fluorometer should be in conjunction with water samples, serving to improve spatial and temporal data resolution in small areas only. Such data should then be recalibrated against measured chlorophyll concentrations extracted from the water samples.

As mentioned, it is assumed that the user has experience and understanding of the detailed methodology of extracting chlorophyll from water samples; information on this procedure is not available from Valeport. One important point to note however is the following:

**Do not expose the Seapoint SCF Chlorophyll Fluorometer to the solvent acetone. Acetone will damage the sensor housing and windows irreparably. Chlorophyll samples dissolved in acetone should be measured through glass vials.**

The Seapoint SCF fluorometer has 4 gain settings, selected through the software, allowing the user to choose the most appropriate range and resolution for the environment to be measured. Each Seapoint chlorophyll fluorometer is tuned to give a maximum output at 150µg/l chlorophyll in *Isochrysis Galbana*, at Gain setting x1. The linear equations for this relationship and for the other gain settings are embedded in the DataLog 400 software, so that the user may get a calibrated µg/l output from the sensor at any gain setting. This calibrated output is accurate for the algae *Isochrysis Galbana*, but readings may vary with different species, or against pure chlorophyll. The user may perform a further calibration on the data, either to convert the standardised readings from the Seapoint SCF into accurate chlorophyll readings from a water sample, or to cross-calibrate against another fluorometer type. Calibration of data against water samples should be done in post-processing using MS Excel, and it is assumed that the user will have a standard procedure for doing this if required. The next section describes how to cross calibrate against an alternative fluorometer.

## CALIBRATION

Cross –calibration against another fluorometer type will enable comparable data to be measured and logged. The Valeport CD-ROM supplied with the instrument contains a spreadsheet numbered “04008154”. This spreadsheet is designed to allow a linear cross-calibration against the output from another device, at a specific gain setting only. The user may calculate a polynomial equation of their own if they so desire, up to 5<sup>th</sup> order.

Note that whilst polynomial calibrations provide the most accurate fit over the range of data used, they may give rise to spurious readings outside this range. For example, if calibration is carried out up to a chlorophyll concentration of 50µg/l, readings above this value should not be trusted. If it is expected that readings outside the calibration range will occur, then a straight line fit should be used instead.

### Equipment:

#### Supplied with product

- Instrument with Fluorometer
- Secondary calibration spreadsheet

#### Supplied by customer

- Alternative fluorometer or other chlorophyll measuring device.
- Container of typical seawater containing chlorophyll.
- Container of Distilled water.
- PC running DataLog 400 or HyperTerminal, and with MS Excel.

To obtain accurate calibrations, the following protocol should be used. Remember that high quality calibrations can only be achieved with patience and attention to detail.

### PREPARATION

1. Take a water sample of the proposed site, taking care to ensure that the water that is taken is representative of what the instrument will measure. It is suggested that several litres of water sample are collected.
2. In the laboratory, remove the fluorometer from its deployment frame (if fitted), keeping it connected to the main instrument. Thoroughly clean and dry the sensor taking care to remove any deposits from previous work.

It is recommended that the calibration procedure is carried out under natural lighting, since this will be typical of the conditions found in the field. Fluorescent lighting should be avoided.

3. The next step is to determine the appropriate gain setting for the calibration and subsequent deployment. Place the sensor in a sample of water from the proposed site.

Using DataLog 400, interrupt the unit. When the unit has been interrupted, DataLog 400 displays a summary screen detailing the serial number of the instrument, firmware version, amount of memory available, and various other parameters. At the bottom of this screen is a list of all the sensors fitted to the instrument, preceded by a two digit “module number”. This is an address number for each sensor in the instrument; note down the module number of the fluorometer.

Set the instrument to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the fluorometer module is setup to output primary calibration data (mV). Choose a gain setting for the sensor. Refer to Section 4.2

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Firstly, determine the module number (address) of the fluorometer by sending the command

#062<Enter>

This will list the sensors fitted and their module numbers. Make a note of the fluorometer module number.

Next, ensure that the fluorometer is set to output data in mV by sending the command:

of the software manual for details on how to do this.  
Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

```
#020;<fluorometer_module_number>;1;<space><Enter>

Set the unit to direct reading mode:

#041;DIR<Enter>

Set the instrument to run continuously at 1Hz:

#003;CONT;1;1;30;NONE;1<Enter>

Enter Run mode:

#028<Enter>
```

*Data should now be displayed and updated at 1Hz, whichever method is being used.*

4. Repeat the above procedures at a different gain setting, until a suitable output level is reached. It is suggested that the user aim for an output in the region of 1 – 2.5 volts for this sample. A higher output than this suggests that the gain is too high, and the maximum output of 5v will be reached at chlorophyll levels only slightly greater than that of the sample. Conversely, a very low reading will mean that resolution is lost for chlorophyll levels lower than that of the sample. Common sense is the best guide here.
5. Once the appropriate gain setting has been established, open the MS Excel spreadsheet supplied on the Valeport CD-ROM, numbered 04008154. Cells where you will need to enter data are signified by red ## symbols.
6. At the top of the sheet “Fluorometer secondary calibration”, fill in the instrument type, serial number and fluorometer serial number (marked on the base of the sensor). Also enter the module number, determined from DataLog 400 or HyperTerminal.

Calibration may be carried out through the DataLog 400 software provided, or through entering commands in a terminal emulation program such as HyperTerminal. Instructions for both methods are given.

### Communicating

Using DataLog 400, interrupt the unit. When the unit has been interrupted, set it to operate in Continuous mode at 1Hz, with direct reading data output. Ensure that the fluorometer module is still set to output primary calibration data (mV). Refer to Section 4.2 of the software manual for details on how to do this.

Now Run the instrument. Once running, view the data output in your preferred mode – the scrolled data screen provides the easiest method of seeing that the output has stabilised.

Using HyperTerminal, ensure that the correct comm port and baud rate have been set. Interrupt unit operations by sending the command #<Enter> until the instrument responds with ERROR.

Ensure that the fluorometer is set to output data in mV by sending the command:

```
#020;<fluorometer_module_number>;1;<space><Enter>

Set the unit to direct reading mode:

#041;DIR<Enter>

Set the instrument to run continuously at 1Hz:

#003;CONT;1;1;30;NONE;1<Enter>

Enter Run mode:

#028<Enter>
```

*Data should now be displayed and updated at 1Hz, whichever method is being used.*

7. Place the alternative sensor in the container of distilled water, and measure the calibrated chlorophyll output from that device. It should be zero, since there is no chlorophyll or other fluorescence source in this sample. Enter this value in the “known chlorophyll” section of the spreadsheet.
8. Now place the Seapoint fluorometer in the distilled water. The mV output from the sensor should be reasonably stable, and near zero. Enter this mV value into the appropriate cell in the 04008154 spreadsheet.
9. Now place the alternative sensor in the container of sampled sea water, and measure the calibrated chlorophyll output from that device. Enter this value in the “known chlorophyll” section of the spreadsheet.
10. Place the Seapoint fluorometer in the sea water sample, and enter this mV value into the appropriate cell in the 04008154 spreadsheet.
11. Having entered the mV calibration readings in the spreadsheet, view the “Calibration curve” page. This should show a black line (the calibration fit) running over a blue curve (actual data measured). For a perfect calibration, the blue curve will be entirely hidden by the black line.
12. The spreadsheet will also calculate this straight line fit, and will automatically apply it to the measured values.
13. Look at the calculated error – since we are using a straight line fit, this should be zero.
14. If the calibration is acceptable, the next step is to enter the calibration into the unit, which is done in the form of a text string, either through DataLog 400 or HyperTerminal, as follows.

In DataLog 400, interrupt the unit's operation once again, and enter the Module Setup page. Highlight the fluorometer. Now enter “ $\mu\text{g/l}$ ” in the User Units box. Copy the calibration string from the DataLog 400 box in the spreadsheet, and paste it into the calibration string box in DataLog 400. Press the “Setup Sensor” button, and the string is sent to the instrument.

In HyperTerminal, once again interrupt by repeatedly sending #<Enter> until the message ERROR appears.

Copy the calibration string from the HyperTerminal box in the spreadsheet and paste it into HyperTerminal. The unit should repeat the string back to confirm acceptance.

Next, set the correct units by entering the following string:

```
#020;<fluorometer_module_number>;1;ug/l<Enter>
```

15. Now, the unit should output calibrated chlorophyll data. Set it into Run mode again (either by selecting Run in DataLog 400 or by sending the command #028<Enter> in HyperTerminal).
16. The sensor should still be in the seawater sample. Check the output of the sensor, and enter the calibrated value into the “Measured  $\mu\text{g/l}$ ” column in the spreadsheet, under the Post Calibration Check area. The error shown should be within specification.