Special tools/requirements/spares

A general tool kit and lens tissues are required.

Spares: source lamps
fuses
cuvettes
photocell

A calibrating filter is required to check the wavelength accuracy of spectrometers.

Maintenance

When the instrument is cool, and with the electricity turned off:

1. Clean the filters and optical windows with lens tissue.
2. Keep the cuvettes clean (see page 44).

Service

The window and/or front surface of the photodetector should be inspected periodically, and cleaned with lens tissues.

Check lamp alignment (see page 42).

Wavelength calibration (spectrometers only)

By inserting a calibrating filter in the cuvette compartment in place of a normal cuvette, the wavelength calibration may be checked as follows:

1. Turn the wavelength control slowly and identify the peaks described in the data sheet accompanying the filter.
2. If the instrument is more than 5 nm off calibration, apply the manufacturer's instructions for recalibration.

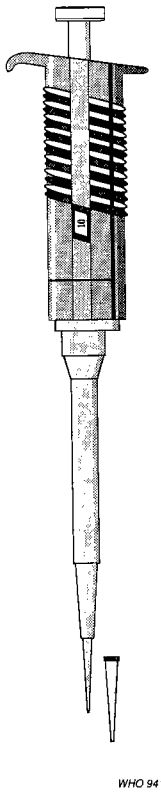
Repair

1. If there is no display response, but light is passing through the system, then change the photocell.
2. If there is no light passing through the system change the lamp. This may also be necessary if the light signal does not remain constant during measurement (as indicated by unreproducible results of extinction obtained from repeated measurements using the same cuvette).

Pipettes, autopipettes, and dispensers

Pipettes are instruments that are used for transferring a predetermined volume of liquid from one vessel to another. They are not connected to a reservoir. There are so many types of pipette that it is difficult to discuss the subject systematically. It should be noted that the replacement of broken conventional calibrated pipettes is often very costly, and that it may be cheaper in the long run to use mechanical pipettes.

Fig. 2.22. A mechanical micropipette.



Mechanical pipettes

Mechanical micropipettes (Fig. 2.22) can only be recommended where a reliable supply of new disposable tips is readily available. They are used for the delivery and/or dilution of biological samples in the volume range 5–1000 µl. They are usually of air displacement (indirect) or direct displacement design. To avoid contamination between consecutive samples, most pipettes have a disposable tip that is discarded after each delivery. This greatly increases the cost per test. The practice of washing and reusing disposable tips is not recommended, as any cleaning procedures will change the "wettability" of the plastic. In addition, drying at only slightly elevated temperatures may distort the tip, and prevent a good pneumatic seal with the pipette body.

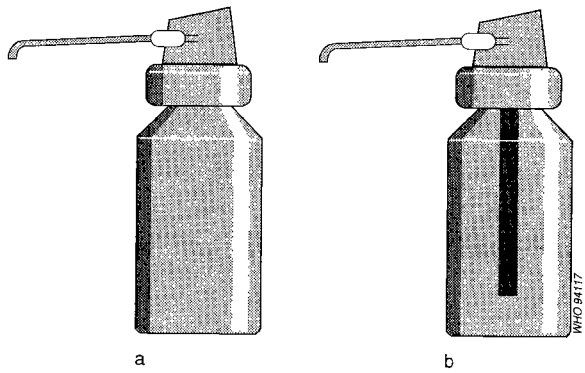
Alternative sample pipettes

Any system that requires mouth-pipetting of biological samples is unacceptable because of the high risk of infection from accidental aspiration of contaminated material. Thus, the traditional shell-backed pipettes used with a haemocytometer-type tube and mouthpiece should never be used.

Sanz pipettes

A pipette that meets the requirements of safe handling and precision is the Sanz pipette. It is available in two forms; one is for the accurate measurement of samples (Fig. 2.23a), the other is for the repeated delivery of reagents in the range 5–100 µl (Fig. 2.23b).

Fig. 2.23. Sanz pipettes.

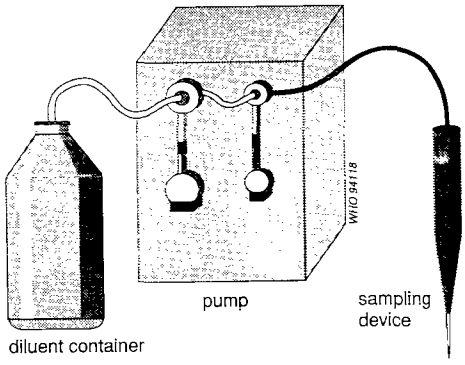


Sanz pipettes have a high precision (coefficient of variation: 0.5% in the range 5–100 µl), are very robust, and can be made locally.

Dispensers and dilutors

Dispensers are instruments for delivering predetermined volumes of liquid from a reservoir. The reservoir may be an integral part of the instrument, or connected externally. Dilutors are instruments for taking up different liquids (e.g., sample and diluent) and delivering them together in a predetermined ratio and/or predetermined volume. The reservoirs of the diluent may be an integral part of the instrument, or connected externally (Fig. 2.24).

Fig. 2.24. Diluter.



Maintenance and repair

It is virtually impossible to give helpful general advice on the maintenance and repair of dispensers and autopipettes because there are so many different types. The manufacturer's instructions and recommendations should be followed.

Pipetting

When the specimen is being mixed with a reagent and buffer solution, the appropriate pipette (or pipette tip) must be used for each individual step of the procedure. Pipetting by mouth should be forbidden because of the biological and chemical hazards. A small rubber bulb (Peleus ball) with two valves (Fig. 2.25) should be fixed to the top of the pipette. The pipette is held vertically while being filled by suction. The position of the bottom of the meniscus on the pipette scale indicates the exact volume (Fig. 2.26). When the solution is expelled, the pipette must also be held vertically. It should be kept in this position for 5 seconds after the outflow of the last drop. After use, semi-automated pipettes must be kept in an *upright* position and thoroughly cleaned periodically.

Fig. 2.25. Peleus ball.

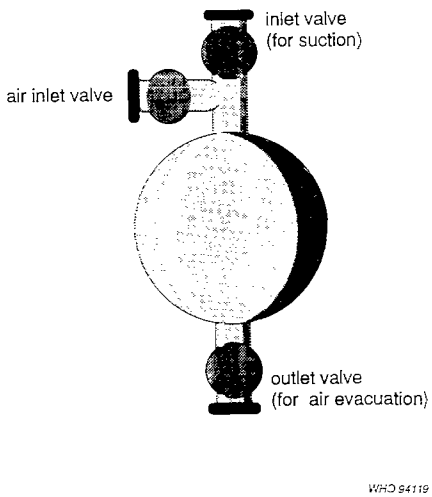
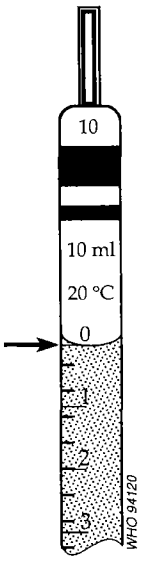


Fig. 2.26. Use of a glass pipette.



Semi-automated pipettes should be calibrated every 3 months, using appropriate dye solutions.

Plastic pipette tips must be tested for air-tightness. If there is not a good seal, the solution will leak out. Cheap pipette tips are often of poor quality, and therefore useless. A single pipette tip can be used for serial pipetting of a solution. Although the same tip may be used repeatedly for the same solution, it must be replaced before different solutions or samples are pipetted.

Testing and calibration

Requirements: distilled water
analytical balance
thermometer (readable to 0.1 °C)
barometer (± 25 mbar)
weighing vessel (10–50 times the test volume, with cover or cap).

When verifying the performance of an instrument, pipetting must be repeated at least 10 times to estimate accuracy and at least 30 times to estimate within-run precision. For subsequent control evaluations, the estimate for within-run precision should be made after pipetting at least 10 times, and the estimate of accuracy after pipetting at least 4 times. The general procedure is based on gravimetric analysis of water samples delivered by the instrument. The values are corrected for evaporation. True mass and volume are then calculated simultaneously, based on the density of water at specific temperatures, and corrections for air buoyancy.

Note: For safety reasons the use of mercury for gravimetric calibration should be discouraged.

Procedure

1. Deliver a total of n samples into a covered weighing vessel and weigh each sample after delivery. Replicate as precisely as possible all motions and time intervals in each sampling cycle. Use a randomly selected pipette tip either only once for each sample weighing or repeatedly for the n weighings.
2. Measure a control blank by dispensing a pre-tared volume of water into the covered weighing vessel, to estimate the degree of evaporation under the experimental conditions. Then duplicate all motions and time intervals as in normal pipetting, with the exception that no more liquid is added to the weighing vessel. Use the resultant mean loss of weight as the correction value for evaporation.
3. Measure and record the temperature of the water to 0.1 °C, before and after the weighing procedure. The temperature (t) is the average of the two measurements of water temperature, rounded to the nearest 0.5 °C.

Calculations

Calculate the mean volume (V) delivered at the test temperature (t) from the mean weighing result (w) by adding the mean evaporation (e) and correcting the sum by an appropriate factor that allows for density and buoyancy corrections when water is weighed in air, at the test temperature and pressure, and standard humidity.

1. Calculate the individual weighing results (w_i) by subtracting the tare reading from the sample reading for each sample.
2. Calculate the mean weight ($\bar{w}$) from the individual weighings (w_i):

$$\bar{w} = \frac{\sum w_i}{n}$$

where n = number of samples.

3. Calculate the evaporation e from the number of determinations as follows:

$$e = \frac{\sum e_i}{n_e}$$

where e_i = individual determination and n_e = number of control blanks.

4. Calculate the mean volume of the liquid samples ($\bar{V}_t$) from the mean weight ($\bar{w}$):

$$\bar{V}_t = (\bar{w} + e) \times z$$

where z = conversion factor (µl/mg) at the test temperature and pressure. (Values of z for distilled water, at various test temperatures, are listed in Table 2.10.)

5. Calculate the percentage inaccuracy ($\bar{E}_t$) of the instrument at the test temperature (t) as the difference between the nominal volume of the instrument (V_o) and the calculated mean volume $\bar{V}_t$:

$$\bar{E}_t = \frac{\bar{V}_t - V_o}{V_o} \times 100$$

Table 2.10. Values for z (µl/mg), as a function of temperature and pressure, for distilled water

Temperature (°C)	Air pressure					
	600	640	680	720	760	800 (mmHg)
	800	853	907	960	1013	1067 (mbar)
	80.0	85.3	90.7	96.0	101.3	106.7 (kPa)
15	1.0018	1.0018	1.0019	1.0019	1.0020	1.0020
15.5	1.0018	1.0019	1.0019	1.0020	1.0020	1.0021
16	1.0019	1.0020	1.0020	1.0021	1.0021	1.0022
16.5	1.0020	1.0020	1.0021	1.0022	1.0022	1.0023
17	1.0021	1.0021	1.0022	1.0022	1.0023	1.0023
17.5	1.0022	1.0022	1.0023	1.0023	1.0024	1.0024
18	1.0022	1.0023	1.0024	1.0024	1.0025	1.0025
18.5	1.0023	1.0024	1.0025	1.0025	1.0026	1.0026
19	1.0024	1.0025	1.0025	1.0026	1.0027	1.0027
19.5	1.0025	1.0026	1.0026	1.0027	1.0028	1.0028
20	1.0026	1.0027	1.0027	1.0028	1.0029	1.0029
20.5	1.0027	1.0028	1.0028	1.0029	1.0030	1.0030
21	1.0028	1.0029	1.0030	1.0030	1.0031	1.0031
21.5	1.0030	1.0030	1.0031	1.0031	1.0032	1.0032
22	1.0031	1.0031	1.0032	1.0032	1.0033	1.0033
22.5	1.0032	1.0032	1.0033	1.0033	1.0034	1.0035
23	1.0033	1.0033	1.0034	1.0035	1.0035	1.0036
23.5	1.0034	1.0035	1.0035	1.0036	1.0036	1.0037
24	1.0035	1.0036	1.0036	1.0037	1.0038	1.0038
24.5	1.0037	1.0037	1.0038	1.0038	1.0039	1.0039
25	1.0038	1.0038	1.0039	1.0039	1.0040	1.0041
25.5	1.0039	1.0040	1.0040	1.0041	1.0041	1.0042
26	1.0040	1.0041	1.0042	1.0042	1.0043	1.0043
26.5	1.0042	1.0042	1.0043	1.0043	1.0044	1.0045
27	1.0043	1.0044	1.0044	1.0045	1.0045	1.0046
27.5	1.0044	1.0045	1.0046	1.0046	1.0047	1.0047
28	1.0046	1.0046	1.0047	1.0048	1.0048	1.0049
28.5	1.0047	1.0048	1.0048	1.0049	1.0050	1.0050
29	1.0049	1.0049	1.0050	1.0050	1.0051	1.0052
29.5	1.0050	1.0051	1.0051	1.0052	1.0052	1.0053
30	1.0052	1.0052	1.0053	1.0053	1.0054	1.0055

6. Calculate the within-run imprecision (coefficient of variation, CV) from the distribution of the individual weighings (w_i) about their mean ($\bar{w}$), corrected for error due to evaporation:

$$CV = \frac{100 \times s}{(\bar{w} + e)}$$

$$\text{where } s = \frac{(w_i - \bar{w})^2}{n - 1}$$

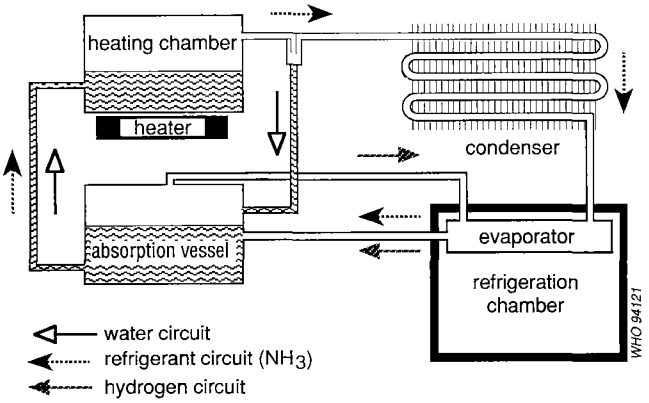
Refrigerators

Refrigeration is the result of the absorption of energy (heat) during the evaporation of a liquid. A refrigerant liquid is circulated through a closed system of pipes, in which on one side (refrigeration chamber) it is vaporized and on the other side (outside the refrigeration chamber) it is condensed. Common refrigerant liquids are ammonia (boiling point −33 °C), and low relative molecular mass chlorofluorocarbons (boiling point near −30 °C). The vaporization of the refrigerant liquid is achieved by either absorption or compression.

Absorption

The absorption system is used mainly in small refrigerators, because it requires more energy input than the compressor system. The closed system of an absorption refrigerator consists of an evaporator, an absorption vessel, a heating chamber and a condenser (Fig. 2.27). The liquid contains ammonia as refrigerant and water as absorbant. The third component in the system is hydrogen, which accelerates the evaporation of ammonia and maintains a constant pressure in the circuit.

Fig. 2.27. Working principle of an absorption refrigerator.



The circuit works at constant pressure and has no moving parts. The operation of the circuit is based on the following principles:

- Water can absorb large quantities of ammonia at ordinary temperatures. The absorption of ammonia in water occurs so fast that a "compression" effect results.
- At modestly elevated temperatures, ammonia separates from water into the gaseous phase.
- Hydrogen does not dissolve in water.