Comment 2: If a man cannot provide a semen sample, the postcoital test (see Section 3.3.1) may provide some information about his spermatozoa.

2.2.7 Safe handling of specimens

Semen samples may contain dangerous infectious agents (e.g. human immunodeficiency virus (HIV), hepatitis viruses or herpes simplex virus) and should therefore be handled as a biohazard. If the sample is to be processed for bioassay, intra-uterine insemination (IUI), in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (see Section 5.1), or if semen culture is to be performed (see Section 2.2.4), sterile materials and techniques must be used. Safety guidelines as outlined in Appendix 2 should be strictly followed; good laboratory practice is fundamental to laboratory safety (WHO, 2004).

2.3 Initial macroscopic examination

Semen analysis should begin with a simple inspection soon after liquefaction, preferably at 30 minutes, but no longer than 1 hour after ejaculation, to prevent dehydration or changes in temperature from affecting semen quality.

2.3.1 Liquefaction

Immediately after ejaculation into the collection vessel, semen is typically a semi-solid coagulated mass. Within a few minutes at room temperature, the semen usually begins to liquefy (become thinner), at which time a heterogeneous mixture of lumps will be seen in the fluid. As liquefaction continues, the semen becomes more homogeneous and quite watery, and in the final stages only small areas of coagulation remain. The complete sample usually liquefies within 15 minutes at room temperature, although rarely it may take up to 60 minutes or more. If complete liquefaction does not occur within 60 minutes, this should be recorded. Semen samples collected at home or by condom will normally have liquefied by the time they arrive in the laboratory.

Normal liquefied semen samples may contain jelly-like granules (gelatinous bodies) which do not liquefy; these do not appear to have any clinical significance. The presence of mucus strands, however, may interfere with semen analysis.

Note 1: Liquefaction can be recognized both macroscopically, as described above, and microscopically. Immobilized spermatozoa gain the ability to move as the semen liquefies. If immobilized spermatozoa are observed on microscopic examination, more time must be allowed for the liquefaction process to be completed.

Note 2: During liquefaction, continuous gentle mixing or rotation of the sample container on a two-dimensional shaker, either at room temperature or in an incubator set at 37 °C, can help to produce a homogeneous sample.

Note 3: If the semen does not liquefy within 30 minutes, do not proceed with semen analysis but wait for another 30 minutes. If liquefaction has not occurred within 60 minutes, proceed as in Section 2.3.1.1.

2.3.1.1 Delayed liquefaction

Occasionally samples may not liquefy, making semen evaluation difficult. In these cases, additional treatment, mechanical mixing or enzymatic digestion may be necessary.

1. Some samples can be induced to liquefy by the addition of an equal volume of physiological medium (e.g. Dulbecco's phosphate-buffered saline; see Appendix 4, section A4.2), followed by repeated pipetting.
2. Inhomogeneity can be reduced by repeated (6–10 times) gentle passage through a blunt gauge 18 (internal diameter 0.84 mm) or gauge 19 (internal diameter 0.69 mm) needle attached to a syringe.
3. Digestion by bromelain, a broad-specificity proteolytic enzyme (EC 3.4.22.32), may help to promote liquefaction (see Box 2.2).

Box 2.2 Preparation of bromelain

Prepare 10 IU/ml bromelain in Dulbecco's phosphate-buffered saline (see Appendix 4, section A4.2); it is difficult to dissolve but, with mixing, most should dissolve within 15–20 minutes. Dilute semen 1 + 1 (1:2) with the 10 IU/ml bromelain, stir with a pipette tip, and incubate at 37 °C for 10 minutes. Mix the sample well before further analysis.

Comment: These treatments may affect seminal plasma biochemistry, sperm motility and sperm morphology, and their use must be recorded. The 1 + 1 (1:2) dilution of semen with bromelain must be accounted for when calculating sperm concentration.

2.3.2 Semen viscosity

After liquefaction, the viscosity of the sample can be estimated by gently aspirating it into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long.

Alternatively, the viscosity can be evaluated by introducing a glass rod into the sample and observing the length of the thread that forms upon withdrawal of the rod. The viscosity should be recorded as abnormal when the thread exceeds 2 cm.

In contrast to a partially unliquefied sample, a viscous semen specimen exhibits homogeneous stickiness and its consistency will not change with time. High viscosity can be recognized by the elastic properties of the sample, which adheres strongly to itself when attempts are made to pipette it. The methods to reduce viscosity are the same as those for delayed liquefaction (see Section 2.3.1.1).

Comment: High viscosity can interfere with determination of sperm motility, sperm concentration, detection of antibody-coated spermatozoa and measurement of biochemical markers.

2.3.3 Appearance of the ejaculate

A normal liquefied semen sample has a homogeneous, grey-opalescent appearance. It may appear less opaque if the sperm concentration is very low; the colour may also be different, i.e. red-brown when red blood cells are present (haemospermia), or yellow in a man with jaundice or taking certain vitamins or drugs.

2.3.4 Semen volume

The volume of the ejaculate is contributed mainly by the seminal vesicles and prostate gland, with a small amount from the bulbourethral glands and epididymides. Precise measurement of volume is essential in any evaluation of semen, because it allows the total number of spermatozoa and non-sperm cells in the ejaculate to be calculated.

The volume is best measured by weighing the sample in the vessel in which it is collected.

- Collect the sample in a pre-weighed, clean, disposable container.
- Weigh the vessel with semen in it.
- Subtract the weight of the container.
- Calculate the volume from the sample weight, assuming the density of semen to be 1 g/ml (Auger et al., 1995). (Semen density varies between 1.043 and 1.102 g/ml (Huggins et al., 1942; Brazil et al., 2004a; Cooper et al., 2007).)

Note: Empty specimen containers may have different weights, so each container should be individually pre-weighed. The weight may be recorded on the container before it is given to the client. Use a permanent marker pen on the vessel itself or on a label. If a label is used for recording the weight, it should be attached before the empty container is weighed.

Alternatively, the volume can be measured directly.

- Collect the sample directly into a modifed graduated glass measuring cylinder with a wide mouth. These can be obtained commercially.
- Read the volume directly from the graduations (0.1 ml accuracy).

Note: Measuring volume by aspirating the sample from the specimen container into a pipette or syringe, or decanting it into a measuring cylinder, is not recommended, because not all the sample will be retrieved and the volume will therefore be underestimated. The volume lost can be between 0.3 and 0.9 ml (Brazil et al., 2004a; Iwamoto et al., 2006; Cooper et al., 2007).