- Measuring semen pH (if required).
- Preparing a wet preparation for assessing microscopic appearance, sperm motility and the dilution required for assessing sperm number.
- Assessing sperm vitality (if the percentage of motile cells is low).
- Making semen smears for assessing sperm morphology.
- Making semen dilutions for assessing sperm concentration.
- Assessing sperm number.
- Performing the mixed antiglobulin reaction (MAR) test (if required).
- Assessing peroxidase-positive cells (if round cells are present).
- Preparing spermatozoa for the immunobead test (if required).
- Centrifuging semen (if biochemical markers are to be assayed).

Within 3 hours:

- Sending samples to the microbiology laboratory (if required).

After 4 hours:

- Fixing, staining and assessing smears for sperm morphology.

Later on the same day (or on a subsequent day if samples are frozen):

- Assaying accessory gland markers (if required).
- Performing the indirect immunobead test (if required).

2.2 Sample collection

2.2.1 Preparation

- The sample should be collected in a private room near the laboratory, in order to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis (see Sections 2.2.5 and 2.2.6 for exceptions).
- The sample should be collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence. If additional samples are required, the number of days of sexual abstinence should be as constant as possible at each visit.
- The man should be given clear written and spoken instructions concerning the collection of the semen sample. These should emphasize that the semen sample needs to be complete and that the man should report any loss of any fraction of the sample.
- The following information should be recorded on the report form (see Appendix 6, section A6.1): the man's name, birth date and personal code number, the period of abstinence, the date and time of collection, the completeness of the sample, any difficulties in producing the sample, and the interval between collection and the start of the semen analysis.

2.2.2 Collection of semen for diagnostic or research purposes

- The sample should be obtained by masturbation and ejaculated into a clean, wide-mouthed container made of glass or plastic, from a batch that has been confirmed to be non-toxic for spermatozoa (see Box 2.1).
- The specimen container should be kept at ambient temperature, between 20 °C and 37 °C, to avoid large changes in temperature that may affect the spermatozoa after they are ejaculated into it. It must be labelled with the man's name and identification number, and the date and time of collection.
- The specimen container is placed on the bench or in an incubator (37 °C) while the semen liquefies.
- Note in the report if the sample is incomplete, especially if the first, sperm-rich fraction may be missing. If the sample is incomplete, a second sample should be collected, again after an abstinence period of 2–7 days.

Box 2.1 Confirming the compatibility of semen collection vessels

Select several semen samples with high sperm concentration and good sperm motility. Place half of each specimen in a container known to be non-toxic (control) and the other half in the container being tested. Assess sperm motility (see Section 2.5) at hourly intervals in replicate at room temperature or at 37 °C for 4 hours. If there are no differences at each time point between control and test assessments ($P>0.05$ as judged by a paired *t*-test), the test containers can be considered to be non-toxic to spermatozoa and to meet semen collection requirements.

2.2.3 Sterile collection of semen for assisted reproduction

This is performed as for diagnostic collection (see Section 2.2.2) but the specimen containers, pipette tips and pipettes for mixing must be sterile.

2.2.4 Sterile collection of semen for microbiological analysis

In this situation, microbiological contamination from non-semen sources (e.g. commensal organisms from the skin) must be avoided. The specimen containers, pipette tips and pipettes for mixing must be sterile.

The man should:

- Pass urine.
- Wash hands and penis with soap, to reduce the risk of contamination of the specimen with commensal organisms from the skin.
- Rinse away the soap.
- Dry hands and penis with a fresh disposable towel.
- Ejaculate into a sterile container.

Note: The time between collection of the semen sample and the start of the investigation by the microbiological laboratory should not exceed 3 hours.

2.2.5 Collection of semen at home

- A sample may be collected at home in exceptional circumstances, such as a demonstrated inability to produce a sample by masturbation in the clinic or the lack of adequate facilities near the laboratory.
- The man should be given clear written and spoken instructions concerning the collection and transport of the semen sample. These should emphasize that the semen sample needs to be complete, i.e. all the ejaculate is collected, including the first, sperm-rich portion, and that the man should report any loss of any fraction of the sample. It should be noted in the report if the sample is incomplete.
- The man should be given a pre-weighed container, labelled with his name and identification number.
- The man should record the time of semen production and deliver the sample to the laboratory within 1 hour of collection.
- During transport to the laboratory, the sample should be kept between 20 °C and 37 °C.
- The report should note that the sample was collected at home or another location outside the laboratory.

2.2.6 Collection of semen by condom

- A sample may be collected in a condom during sexual intercourse only in exceptional circumstances, such as a demonstrated inability to produce a sample by masturbation.
- Only special non-toxic condoms designed for semen collection should be used; such condoms are available commercially.
- The man should be given information from the manufacturer on how to use the condom, close it, and send or transport it to the laboratory.
- The man should record the time of semen production and deliver the sample to the laboratory within 1 hour of collection.
- During transport to the laboratory, the sample should be kept between 20 °C and 37 °C.
- The report should note that the sample was collected by means of a special condom during sexual intercourse at home or another location outside the laboratory.

Note: Ordinary latex condoms must not be used for semen collection because they contain agents that interfere with the motility of spermatozoa (Jones et al., 1986).

Comment 1: Coitus interruptus is not a reliable means of semen collection, because the first portion of the ejaculate, which contains the highest number of spermatozoa, may be lost. Moreover, there may be cellular and bacteriological contamination of the sample, and the low pH of the vaginal fluid could adversely affect sperm motility.