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Revised Advice on Laboratory Containment Measures for work with Tissue Samples in Clinical Cytogenetics Laboratories

Supplement to: ACDP guidance on protection against blood-borne infections in the workplace: HIV and hepatitis

HSE
Health & Safety Executive

REVISED ADVICE ON LABORATORY CONTAINMENT MEASURES FOR WORK WITH TISSUE SAMPLES IN CLINICAL CYTOGENETICS LABORATORIES

Introduction

1. As a result of the re-assessment of risk associated with the culture of known or suspected HIV infected samples, revised measures for containment are now recommended. This revised guidance is issued following a risk assessment undertaken by a Working Group comprising representatives of the Association of Clinical Cytogeneticists and the Royal College of Pathologists. Their recommendations for revised containment measures in clinical cytogenetics laboratories have been accepted by the Advisory Committee on Dangerous Pathogens (ACDP), and are set out in the following paragraphs.

2. This revised guidance represents what is considered to be good practice by members of the ACDP. Following this guidance is not compulsory, and it is open to individuals to take other action, but if this guidance is followed it will normally be sufficient to comply with the law. Health and safety inspectors seek to secure compliance with the law and may refer to this guidance as illustrating good practice.

Work in cytogenetics laboratories

3. The advice in the following guidance applies to work in clinical cytogenetics laboratories.

4. Cytogenetics involves microscopic analysis of chromosome preparations derived from cells in the metaphase stage of mitotic division. *In vitro* cultivation of cells forms an integral part of the methodology. The processing of samples for analysis can be summarised as:

1. The sample is received and, where necessary, appropriate cell types selected;
2. The tissue is set up in culture. Culture time will vary depending on the sample type;
3. At the appropriate time, cultures are processed for cytogenetic preparations (i.e. harvesting);
4. Cultures for harvesting are blocked in mitosis using a spindle inhibitor, colcemid;
5. After a pre-determined time, cells are treated with a hypotonic solution, fixed (3:1, methanol:acetic acid), and the mitoses spread on clean glass slides;

6. After further processing with an enzyme, and staining to produce a banding pattern, the chromosomes are analysed by light microscopy.

5. Most laboratories use a Class 2 microbiological safety cabinet for sample and culture handling, in order to protect both the operator and the sample from air-borne contamination. The glass front panel also affords operator protection against contamination by droplets produced from splashing. At various stages of processing, and especially during harvesting, samples will need to be centrifuged.

6. Although all dividing cells can be processed for cytogenetic analysis, the cells and tissues that are routinely cultured for diagnostic purposes are discussed in the following paragraphs.

Current ACDP guidance

7. General guidance on safe working procedures applicable to all persons at risk of exposure to blood-borne viruses (BBVs) as a result of their occupation are set out in Part 3, paragraphs 83-88 of the ACDP publication "Protection against blood-borne infections in the workplace: HIV and hepatitis", 1995, HMSO ISBN 0 11 321953 9, (ACDP, BBV guidance), to which this revised guidance forms a supplement. **The general guidance remains extant.**

8. Guidance on laboratory work with blood-borne viruses is contained in Part 4, paragraphs 116 – 181 of the ACDP, BBV guidance. In particular paragraph 142 advises that "where materials that contain or may contain BBVs are being examined for purposes other than propagation or concentration of virus, Containment Level (CL) 2 is acceptable if additional precautions are taken." (i.e. CL2+). Paragraph 152 states that, for the purposes of propagation or concentration in the laboratory, work must be conducted at CL3.

9. Paragraph 156 goes on to advise that the cultivation of all cells from known or suspected cases of HIV or other primate (human or simian) retrovirus infection, must be conducted at Containment Level 3. However, **subject to the assessment of risk required under the COSHH Regulations**, permissive or mixed cells from those not known or suspected to be infected with retroviruses may be handled at CL2+. The guidance also points out that with prolonged incubation (e.g. beyond 3-4 days) there is increasing likelihood of significant HIV production, and a local risk assessment is necessary in each case, which should take account of the potential risks from HIV and other BBVs that may be present. **These recommendations have been reviewed following a re-assessment of the risks, as mentioned in paragraph 1 above, and annexed as an appendix to this supplement. The revised guidance is set out in paragraphs 11.2-11.7 below.**

10. Paragraphs 131 - 132 give advice on sample labelling requirements. There remains a need for known or suspected high risk of infection specimens to be appropriately labelled in order that laboratories can make a local risk assessment and apply local precautions, to ensure the health, safety and welfare of staff.

For purposes of emphasis the relevant requirements for labelling of high risk samples are set out in paragraphs 13-15 below, together with a reminder of the legal obligation to label risk of infection samples appropriately, under the Health and Safety at Work Act and COSHH Regulations.

Revised recommendations for containment in clinical cytogenetics tissue culture

11.1 In general, in clinical cytogenetics work, procedures should be undertaken at Containment Level 2+[*] because of the ever-present risk of contamination of samples with BBVs.

11.2 HIV risk samples

(i) The cultivation of lymphocytes (using PHA stimulation), and other permissive cells, with CD4 receptors, **from known or suspected cases of HIV infection** may be conducted at Containment Level 2+, provided the incubation of cultures does not exceed 100 hours.

(ii) The cultivation of bone marrow **from known or suspected cases of HIV infection** may be conducted at Containment Level 2+, provided incubation of cultures does not exceed 100 hours.

(iii) Where incubation of lymphocytes or other lymphoid tissue and other permissive cells **from known or suspected cases of HIV infection** exceeds 100 hours, work should be conducted at Containment Level 3.

(iv) The cultivation of chorionic villus cells, amniotic fluid cells, fibroblasts or other solid tissue cell types and non-lymphoid tumours in clinical cytogenetics, **from known or suspected cases of HIV infection**, may be conducted at Containment Level 2+, irrespective of the period of culture. This applies equally where the initial sample is contaminated with blood.

(v) For any procedure proposed using material **from known or suspected cases of HIV infection**, which does not fall within the above categories, a separate risk assessment will need to be undertaken.

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[*] NB: Throughout the document, Containment Level 2+ requires that a Class 1 or Class 2 microbiological safety cabinet should be used.

11.3 Hepatitis B risk samples

The cultivation of all cells (except liver cells) from known or suspected cases of HBV infection may be conducted at Containment Level 2+. If, very exceptionally, a liver cell culture is to be performed, a separate risk assessment should be undertaken.

11.4 Hepatitis C risk samples

The cultivation and processing of all cells from known or suspected cases of HCV infection may be conducted at Containment Level 2+.

11.5 All other routine samples not suspected to present a risk

All other samples may be handled and cultured at Containment Level 2+, provided they are not suspected of being infected with any other Hazard Group 3 or 4 pathogen.

11.6 Samples at risk of being infected with Hazard Group 3 or 4 pathogens other than HIV, HBV and HCV

A local risk assessment is necessary in all unusual cases. If significant risk of infection is shown, the appropriate Containment Level should be used.

11.7 Lymphoblastoid cell lines and other continuous lines of susceptible cells

In general, continuous lines of susceptible cells, including lymphoblastoid cell lines, may be handled at Containment Level 2+ unless, on the basis of a risk assessment, it is considered that they should be handled at a higher Containment Level.

Specifications for Containment Level 2+ in cytogenetics

12.1 In addition to the measures and facilities normally specified for work at Containment Level 2, the additional measures listed below should also apply when Containment Level 2+ is specified. These extra precautions should be in constant use to guard against percutaneous inoculation, contamination of the skin, mucous membranes and working surfaces.

- Work must be conducted in a designated area of the laboratory with sufficient space to work safely. Access of unauthorised persons to the work must be prevented, to ensure that the person conducting the work is free from the risk of disturbance or accidental contact with others. An appropriately sited Class 1 or 2 microbiological safety cabinet would meet this requirement, and is recommended for culturing and processing samples in clinical cytogenetics laboratories – and see paragraph 12.2 below.

- The use of "sharps" and glassware must be avoided wherever possible. Where such use is essential, particular care must be taken in their handling and disposal after contact with unfixed materials.

- Lesions on exposed skin should be covered with waterproof dressings.
- Protective clothing must include a laboratory coat, disposable gloves and other personal protective equipment appropriate to the task.
- The workstation must be cleared of unnecessary equipment.
- Sealed rotors or buckets should be used for the centrifugation of samples and unfixed cell suspensions. In the event of breakage, the rotor or bucket should be opened in a Class 1 or 2 cabinet.
- The bench surface and any equipment must be disinfected immediately after completion of work.
- A suitable disinfection policy must be in operation.
- Full consultation with staff in respect of containment must take place, and protocols for the safe conduct of the work must be agreed and strictly adhered to.
- The need for immunisation of laboratory staff with HBV vaccine, together with follow-up procedures, in line with the advice contained in paragraphs 87-88 of the BBV guidance, should be considered.

12.2 The use of microbiological safety cabinets

Containment Level 2 specifies that although work in general may be conducted on an open bench, for vigorous shaking or mixing a Class 1 microbiological safety cabinet, or other equipment designed to contain resultant aerosols and droplets, must be used. However, as BBVs are unlikely to be infectious by the airborne route, the use of a Class 2 cabinet for this purpose is acceptable when protection of the work from contamination is essential.

Labelling of samples

13. It is important that known or suspected high risk of infection specimens are appropriately labelled. This will allow laboratories to make a suitable local risk assessment, and apply relevant local precautions.

14. The ACDP made a clear recommendation in the BBV guidance, to which this forms a supplement, (see paragraphs 131-132) about the labelling of high risk specimens with regard to the culturing of BBV high risk samples. Furthermore, in order to comply with existing HSAC guidelines and legislative requirements, the use of "danger of infection" labels for specimens known or suspected to carry a BBV is strongly recommended.

15. For emphasis, the earlier recommendations are (in part) reproduced below.

> **"Anyone responsible for taking and/or despatching specimens or other potentially hazardous material for laboratory examination has duties under the Health and Safety at Work etc. Act (HSWA) and the COSHH Regulations to conduct the work safely. One specific requirement (covered by Section 3 of the HSWA) is to convey knowledge of known or suspected hazards to those who need to handle any material that is sent for examination, and this may be achieved by inclusion of clinical details on the request form which accompanies the specimen. Such information however is confidential and should not be readable by persons handling the specimen while it is still contained in its packaging.**
>
> **Use of some form of "<u>danger of infection</u>" label; (e.g. a yellow biohazard sticker) is appropriate for labelling specimens known or suspected to come from individuals with BBV infections. The specimen container should be labelled on the outside and be clearly visible. The accompanying paperwork should also be appropriately labelled. It is good practice for those requesting tests to provide as much information as is relevant, consistent with maintaining patient confidentiality, with any request for a laboratory investigation."**

ASSESSMENT OF RISK OF PROPAGATION OF HIV IN TISSUE CULTURE IN CYTOGENETIC LABORATORIES

RISK ASSESSMENTS FOR INDIVIDUAL SAMPLE-TYPE CULTURES

1. Lymphocyte cultures: Approximately 0.5–1ml. heparinised whole blood is added to 10ml. culture medium containing phytohaemagglutinin (PHA), a mitogen which stimulates T-cell lymphocytes to divide. Cultures are harvested at either 48 hours or 72 hours after initiation. Whilst the majority of blood samples processed in this way come from adults and children, fetal cord blood obtained *in utero* or at delivery can also be cultured in this way.

Risk assessment There have been concerns that this lymphocyte culture system might propagate HIV to dangerously high levels. However, experiences with routine culture in virology departments has indicated that this is not the case. Using culture conditions optimised for production of HIV (PHA stimulated blood supplemented with interleukin 2, with the addition of a feeder lymphocyte layer), it has been found that little or no virus production, measured by retrovirus specific reverse transcriptase activity and levels of p24 core antigen, occurs within 100 hours, and that significant viral production takes 10-17 days. The conclusion, therefore, is that there would be no HIV production in routine lymphocyte cultures in clinical cytogenetics, provided these do not exceed 100 hours.

Plasma from patients with a high viral load at the time of seroconversion when terminally ill with HIV infection would have a titre of between 10^3 and 10^7/ml. plasma. These samples are currently handled in biochemistry and haematology laboratories at CL2+. In clinical cytogenetics there would be approximately 1:10 dilution of the viral concentration when the sample is set up in culture. As there is no significant increase in the viral load during culture, the subsequent processing would not present a greater risk than activities in other laboratories handling HIV samples. Furthermore most harvesting of samples in UK cytogenetics laboratories occurs under exhaust protection and splash and droplet protection in Class 1 or 2 cabinets, by staff highly trained in aseptic techniques.

2. <u>B-cell lymphoblastoid cell cultures</u>: Immortalised cell lines, derived from B-cell lineages are established by transforming cells with Epstein Barr Virus. These can grow indefinitely, and are often held in long-term storage facilities using liquid nitrogen. Changes in technology in recent years have meant that these are initiated less frequently than in previous years, and they are used now more in research than in diagnostics.

Risk assessment There is evidence that some B-cells transformed with Epstein Barr Virus may support HIV-1 replication when experimentally exposed to T-cell-line adapted laboratory strains of HIV-1 and HIV-2. However, there is no evidence of lymphoblastoid cell lines derived from known HIV-positive donors being HIV positive. Although B-cells are less efficient at HIV propagation than T-cells, because of the length of time in culture the viral titre could eventually be high. Continuous lines of susceptible cells and lymphoblastoid cell lines can be handled at CL2+ unless a risk assessment shows significant risk of production of HIV. Such cell lines should not be distributed within the scientific community unless they have been tested and shown to be uninfected, or the receiving laboratory acknowledge their status, in writing, before receipt.

3. <u>Amniocyte cultures</u>: Amniocentesis is the standard procedure for cytogenetic analysis of the fetal karyotype. Usually performed mid-trimester, 15-20ml. amniotic fluid is removed under ultrasound guidance, and the various types of fetal and amnion cells contained therein are removed by centrifugation and set up in culture. Culturing usually takes 7-14 days, although this period may be longer if large numbers of cells are required for molecular or biochemical diagnosis.

Risk assessment There is no evidence that amniotic fluid cultures support the sustained production of HIV, and consequently there appears to be no risk of concentrating the virus by routine culture techniques. Blood stained amniotic fluid samples from HIV infected patients do not present any additional risk of propagation of HIV, because the lymphocytes within the blood require specific mitogens for transformation. The majority of blood cells are usually removed after a few days, when the tissue culture medium is replaced with fresh medium. However, as some residual infectivity may remain, care should be taken throughout to avoid percutaneous injury. Great care should be exercised in handling glass coverslips when *in situ* culture techniques are employed.

4. <u>Chorionic villus samples (CVS)</u>: Biopsies from the placenta are usually taken in the first trimester, from 10 weeks gestation onwards. Direct preparations of the trophoblast will yield spontaneously dividing

cells which, although of poor quality, can be used to diagnose many of the common cytogenetic abnormalities. Direct preparations do not require culturing, although sometimes the tissue may be placed in an incubator overnight prior to processing. CVS however are also usually cultured to improve diagnostic accuracy. Cells derived from the mesenchyme core of the villus are cultured in a way similar to amniocytes. A CVS sample needs to be manually processed on receipt, in order to remove any maternal decidua or clotted blood adhering to the villus. This is achieved most effectively by the use of sharp needles.

Risk assessment There is no evidence that CVS cultures support the production of HIV. While any associated macrophages may be infected with HIV, these cells do not grow well under routine culture conditions.

However, the initial sample, particularly if contaminated with blood from a patient infected with HIV, could present a risk during processing. There is epidemiological evidence to show that the risk from percutaneous injury with a hollow bore needle is greater than with a solid needle. For example, needlestick injuries in health care workers, involving hypodermic syringes, are associated with a greater risk of HIV transmission than injuries in surgeons using suture needles. In addition, there is experimental evidence to show that there is a significantly greater wiping effect (90%) through wearing disposable latex gloves in percutaneous injuries involving solid needles compared with hollow bore needles. This reinforces the need to wear disposable protective gloves when handling and processing samples, and to avoid the use of "sharps" wherever possible. When the use of "sharps" is essential, particular care must be taken, and solid needles should be used for dissection rather than syringe needles.

In HIV high risk cases, the use of plastic pipettes should also be considered for manipulating the specimen, where the condition of the sample allows it. Great care should also be exercised in handling glass coverslips when *in situ* culture techniques are employed.

5. Solid tissue samples: Tissue biopsies and *post mortem* samples are grown for cytogenetic, biochemical and molecular analysis from a variety of sources, and also stored as cell lines. Common samples used in diagnostics include tissues from early products of conception, fetal tissues (e.g. skin, fascia lata, pericardium, muscle, amnion, chorion etc.), and skin biopsies from neonates, children and adults. Cells are often in culture for 3-4 weeks.

Risk assessment There is no evidence that fibroblast cultures support the sustained production of HIV. Whilst Langerhans cells in skin samples may contain HIV, the cells do not grow well under routine culture conditions. The associated keratinocytes are unlikely to be susceptible. However, the initial sample, particularly if contaminated with blood from an HIV-infected patient, could present a risk during processing. As with CVS samples, when the use of "sharps" is essential, particular care must be exercised in their handling and disposal.

6. Marrow samples: Diagnosis and prognosis of leukaemia patients is assisted by cytogenetic analysis of marrow cells. Although some analysis is done on direct preparations, where culturing time is limited by the action time of the spindle inhibitor, colcemid, most laboratories use a range of culture times from overnight to 48 hours incubation, and occasionally as long as 72 hours. In some lymphoid disorders, a B-cell mitogen may be used. Leukaemia patients may be immunosuppressed, increasing chances of infection. Adult T-cell leukaemia may, rarely, be caused by HTLV1, but the prevalence of HTLV1 infection in the UK is thought to be very low.

Risk assessment It is very unlikely that there would be any significant production of HIV in the culture regimens normally employed with marrow samples.

7. Solid tumour samples: Samples from patients with other forms of cancer are increasingly being analysed using cytogenetic techniques. Although some malignant tissue, such as lymphomas, contain spontaneously dividing cells, long-term cell culture of 10-60 days is also necessary in most cases.

Risk assessment It is most unlikely that cultures established from solid tumours would be a productive source of HIV. Short-term cultures established from lymphomas present no significant risk. However, a separate risk assessment should be made in each case that may potentially involve permissive cells. Immortalised cell lines derived from lymphoid cells should probably be subject to the same containment conditions as lymphoblastoid cell lines, and handled at CL2+. In samples from patients presenting with lymphadenopathy, the possibility of HIV, or HTLV1 infection being the cause should be considered.