HIV

and the Practice of

Pathology

Report of the Working Party

of the

Royal College of Pathologists

July 1995

PUBLISHED BY THE MARKS AND SPENCER PUBLICATIONS UNIT
OF THE ROYAL COLLEGE OF PATHOLOGISTS

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Registered Charity No. 261035

ISBN 0 9518574 4 4

Produced by the Marks and Spencer Publications Unit of the Royal College of Pathologists

Further copies of this publication can be obtained from the Deputy College Secretary, Royal College of Pathologists, 2 Carlton House Terrace, London SW1Y 5AF, price £10.00; cheques to be made payable to the Royal College of Pathologists.

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INTRODUCTION

The first report of the Royal College of Pathologists' Working Party on HIV Infection was directed towards defining the risks of acquisition of HIV-1 by healthcare workers in the United Kingdom and the risk of transmission of HIV-1 from infected healthcare workers to their patients during exposure prone procedures. The report provided background information for other Royal Colleges, Faculties and professional groups which could then establish their own codes of practice in the light of the report.

This second report, whilst updating some of the information provided in the first report, is directed mainly towards providing those who work in laboratories with data which may be useful for their own practice. In addition, it provides information which is frequently requested by clinicians and those working in different branches of laboratory medicine. Although the main focus of the document is HIV, much of the information will also, of course, be applicable to other blood-borne infections.

This report also provides a number of different standards relevant to many aspects of HIV infection including those applicable to laboratory safety, HIV testing, and management of occupational risks and includes suggestions for suitable audits.

HIV is now widely distributed throughout the world. The College is aware that a significant number of its Members and Fellows reside abroad, many in resource-poor countries. It is hoped that some of the guidance provided in this report will be of value to them.

In the preparation of this report the College's HIV Working Party was assisted by a number of experts who are listed on page *i*.

Human Immunodeficiency Viruses (HIV)

HIVs are enveloped particles, 100-120nm in diameter. Virions comprise a conical protein core (p24), containing the RNA complex and reverse transcriptase, surrounded by an envelope with glycoprotein peplomers (gp120 and gp41) plus an inner matrix protein (p18) ***(Fig 1)***. HIVs are classified within the lentivirus subfamily of the Retroviridae. Two distinct HIVs are recognised: HIV-1, found world-wide, and HIV-2, confined mostly to western Africa and Portugal (although infection has been reported from all continents). Recent studies have demonstrated two HIV-1 types: type M (consisting of subtypes A-H) and type O, and five subtypes of HIV-2 (A-E).

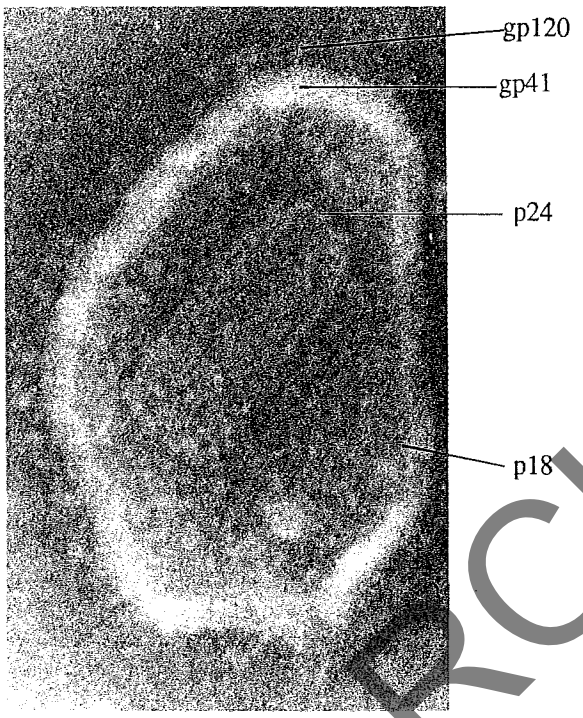


Figure 1: Electronmicrograph of negatively stained HIV

HIV-1 is transmitted primarily through sexual contact, by vaginal or anal intercourse with an HIV-1 positive person, or from an infected woman to her child *in utero*, at delivery, or through breast feeding. Other routes of transmission are by exposure to infected blood, blood products or donor tissues, and through the use of contaminated needles and syringes. Routes of transmission for HIV-2 are as for HIV-1 but transmission efficiency may be lower. For example, perinatal HIV-2 transmission is $<1\%$ compared to 12-40% for HIV-1.

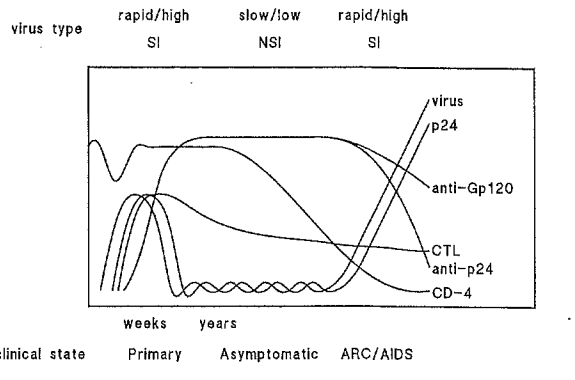
After HIV-1 infection has occurred, antibody develops. The median time between exposure to virus and development of an antibody response is less than one month if 3rd generation tests are employed *(see p 20)*. The standard method for establishing a diagnosis is the detection of HIV-1 antibody in a serum sample. HIV-1 may be present in the blood, semen, or vaginal secretions, and has also been isolated from saliva, tears, breast milk, urine, and cerebrospinal, synovial, and amniotic fluids from infected individuals. Concentrations of virus in blood and body fluids may be particularly high around the time of seroconversion and when AIDS develops. Although a diagnosis of infection may be made by isolating the virus or by detecting its components in blood or other body fluids or host tissue, these techniques, in most cases, offer no advantage over the HIV-1 antibody test.

The median interval between HIV-1 infection and the development of AIDS is 8-10 years, during which time infection is likely to be asymptomatic. The incidence of AIDS in HIV-2 positive individuals is lower, the rate of progression much slower and therefore the incubation period is much longer. Figure 2 shows the clinical and laboratory features of HIV infection.

Commercial antibody tests are designed to detect both HIV-1 and HIV-2.

Epidemiology

The epidemiology of HIV 1 and HIV 2 has been reviewed recently *(Mertens et al, 1994; Kanki and De Cock, 1994)*. HIV-1 infection has a world-wide distribution; only a few countries in Asia and some small islands in Oceania claim to be AIDS free. By the end of 1994, it was estimated that about 18 million adults had been infected with HIV, mostly HIV-1, about 50% of infections occurring in those aged less than 25 years. By the year 2000 it is estimated that some 30 to 40 million people will have been infected and that over 1 million will be dying of AIDS each year. Although the current female:male ratio is 2:3, this is expected to approach 1:1 by the year 2000. The rising rate of infection among women is accompanied by a similar increase in HIV infected children (estimated total 1.5 million). Globally, the majority of infections are heterosexually acquired. Details of regional estimates of infection by HIV-1 and AIDS are provided in Tables 1 and 2 respectively.



CTL – Cytotoxic T-lymphocytes
SI – Syncytium inducing

Figure 2: The clinical laboratory features of HIV infection

Table 1
Adult HIV-1 infections by continent or region (estimated)

Region	Cumulative total	Adults still alive
Western Europe	>500 000	450 000
Eastern Europe/ Central Asia	>50 000	>50 000
North America	>1 million	>750 000
Latin America/ Caribbean	2 million	>1.5 million
North Africa/ Middle East	>100 000	>100 000
Sub-Saharan Africa	11 million	>8 million
Eastern Asia/Pacific	50 000	50 000
Australasia	25 000	20 000
South/South East Asia	>3 million	>2.5 million
Global Total	**>18 million**	**>13-15 million**

(based on WHO Report, 1995)

Table 2
Total number of AIDS cases (adults and children) from late 1970s to end of 1994

Region	Reported (%)	Estimated (%)
Africa	350 000 (34)	3 155 000 (>70)
Americas (less USA)	123 000 (12)	450 000 (>9)
USA	400 000 (39)	405 000 (9)
Asia	20 000 (2)	270 000 (>6)
Europe	127 000 (12.5)	180 000 (4)
Oceania	5 000 (0.5)	40 000 (>1)
Total	**1 025 000**	**4 500 000**

(based on WHO Report, 1995)

In North America, Europe and Australasia, the majority of HIV-1 infections occur among homosexual/bisexual men. However, transmission through injecting drug use (IDU) and via heterosexual exposure is increasing. Latin America and the Caribbean are experiencing a marked increase in HIV-1 infection resulting from a combination of homosexual, heterosexual and IDU exposure. Over 70% of infections world-wide have occurred in Sub-Saharan Africa, the major route of transmission being heterosexual. The prevalence is highest in Central and Eastern Africa with rates of up to 40% being reported in some urban populations; among prostitutes and those attending departments of genitourinary medicine even higher rates are reported. Infection is now spreading increasingly into Western, Southern and North Africa. South East Asia represents another part of the world which is experiencing a markedly expanding epidemic. Infection was initially reported among IDUs and prostitutes but is now being detected increasingly in the general population.

As in the rest of Europe, HIV infection in the UK has been reported most frequently in homosexual/bisexual men and IDUs. There has recently been an increase in infection acquired heterosexually but the majority of these infections were acquired either in HIV endemic countries or as a result of exposure to those who were infected in those countries. Although infection has been reported in all parts of the UK, the highest prevalence rates for HIV and AIDS are in London, particularly in inner city areas.

HIV-2 infection is confined mainly to West Africa (being the dominant virus in Guinea-Bissau, Senegal, and The Gambia) and to Portugal (which has close links with Guinea-Bissau) although infection, with, to date, little spread, has been reported in all continents. However, in many parts of West Africa the incidence of HIV-1 infection has increased dramatically in recent years and currently exceeds that of HIV-2. It should be expected, therefore, that HIV-1 prevalence will increase also and that no area of West Africa will remain HIV-1 free.

Dual serological reactivity to HIV-1 and HIV-2 has been reported frequently, especially in Côte d'Ivoire (33% among prostitutes), Burkina Faso and Mali, as well as in Bombay, India. Such dual reactivity can result from both cross-reactivity and dual infection and care needs to be taken when interpreting data.

Occupational acquisition of HIV

The first case of occupationally acquired HIV infection was reported in 1984 *(Anon, 1984)*. By early 1995, a further 213 cases had been reported, mainly from resource rich countries ***(Tables 3 and 4)*** *(Heptonstall et al, 1995)*. Of these, 73, over half of which were reported between 1991 and 1994, were health care workers in whom seroconversion was documented after a specific exposure at work. The remaining 141 cases were infections in health care workers or other carers, who denied major behavioural risks for HIV infection, which, for various reasons, were presumed to have been occupationally acquired, or were cases which were reported in insufficient detail to allow them to be categorised as documented seroconversions. The number of recognised cases of occupationally-acquired HIV-1 infection is certain to increase as the HIV-1 epidemic progresses.

Table 3
Summary of Cases of Occupational Acquisition of HIV-1 reported to May 1995

	USA	Europe	UK	Rest of World	Total
Documented seroconversion after specific occupational exposure	43	21	4	5	**73**
Possible occupationally acquired infection	91	34	7	9	**141**
TOTAL	**134**	**55**	**11**	**14**	**214**

Sixty five of the 73 documented seroconversions occurred after percutaneous exposure. Of these, 90% involved exposure to HIV infected blood; usually via hollow needles, although three followed injury with a blood-contaminated, sharp, solid object *(Perez et al, 1993; Henderson et al, 1986; Tait et al, 1991)*. The remainder include two infections which followed percutaneous exposure to visibly bloody body fluid (pleural fluid after thoracocentesis *(Oksenhendler et al, 1986)*, and an unspecified fluid) and a seroconversion after percutaneous exposure to concentrated infectious HIV in the laboratory. Six of the documented seroconversions followed prolonged or heavy mucocutaneous exposure to HIV infected blood (5 cases) or to concentrated live virus (one case). One further seroconversion followed exposure to concentrated live HIV, but the nature of the exposure was not specified.

Thirty four infections, almost 50% of reported seroconversions, many of which followed injuries sustained during or after venepuncture, occurred in workers categorised as nurses. Nearly a quarter of seroconversions (17) occurred among clinical laboratory workers, at least nine of whom were employed as phlebotomists ***(Table 4)***. Although no surgeon has been reported to have seroconverted after a specific occupational exposure, ten surgeons, four of whom had worked in Africa, have been classified as having possible occupationally acquired infections. One of these cases has been described in detail and is of particular interest. A cardiothoracic surgeon, who had not worked outside the USA was found to be HIV infected during a routine insurance medical in 1990. The source of his infection is believed to have been an unreported percutaneous exposure sustained in 1985 while rewiring the sternum of a patient who had received HIV infected blood 15 days earlier and who was probably in the acute phase of HIV infection *(Rotheram, 1994)*. In addition, two cases of occupationally-acquired SIV have been reported amongst laboratory workers caring for infected macaques *(CDC 1992)*; both persons remain asymptomatic.

Table 4
Occupationally Acquired HIV Infection (all reports) to May 1995

Occupation	Documented	Possible	**Total**
Nurse/midwife	34	38	72
Doctor	7	15	22
Surgeon	0	10	10
Dentist/dental worker	0	8	8
Clinical laboratory worker	17	17	17
Ambulanceman/ paramedic	0	10	10
Non clinical lab worker	3	3	3
Embalmer/ morgue technician	0	2	2
Surgical technician/oda	2	2	2
Dialysis technician	1	2	3
Respiratory therapist	1	2	3
Health aide/attendant/ nurse aide	1	12	13
Housekeeper/porter/maintenance	1	8	9
Other/unspecified HCW	6	12	18
TOTAL	**73**	**141**	**214**

Prospective studies to which health care workers are recruited immediately after reporting a significant occupational exposure to a known HIV infected source and subsequently serially tested for HIV antibody, provide the best measures of the transmission rates for HIV after different types of occupational exposure. At least twenty studies of this type have been conducted. Current data (20 seroconversions after 6325 exposures) suggest that the risk of transmission after a single percutaneous exposure to HIV is around 1 in 320 or 0.32% (upper limit of 95% confidence interval = 0.18 to 0.45%). The chance that a single mucocutaneous exposure will result in seroconversion is considerably lower, and probably less than 1 in 1000 or 0.1%.

Table 5

European Health Care Workers Percutaneous Exposures and HIV Seroconversions Data To May 1995

PROCESS	PROCEDURE					
	Vene-puncture	IVC insertion	Arterial puncture	Other	Not reported	Total
Failed procedure	1	-	-	-	-	1
Transfer to container	3	-	-	-	-	3
"Accidental"	2*	-	-	-	-	2
Prior to disposal	2	-	-	-	-	2
During disposal	2	2	-	-	-	4
Recapping	1	-	1	1	-	3
After disposal	1	-	-	-	-	1
Not reported	3	-	-	-	5	8
TOTAL	15	2	1	1	5	24

* 1 dropped syringe on foot
1 while picking up syringe which had been dropped

The majority of seroconversions reported occurred within three months of exposure and a state of prolonged "silent" HIV infection after a specific exposure has not been demonstrated *(Gerberding, 1994)*. This suggests that a final test for anti-HIV at six months after exposure is sufficient to exclude transmission.

There is ample evidence to suggest that the main risks to health care workers stem from percutaneous exposure to HIV infected blood, but measures designed to prevent hollow needle injuries have not yet effected a sustained reduction in the rate at which such injuries occur. There remains a need to ensure that health care workers are effectively educated about sharps' disposal, trained in techniques which may minimise the risk of injury, and encouraged to use gloves appropriately. Research designed to discover more about the circumstances in which percutaneous exposures occur and the practices and procedures with which they are associated should continue.

Needlestick injuries and their management

Occupational health and laboratory workers

Laboratory practices involving HIV and other blood borne viruses are covered by the Control of Substances Hazardous to Health *(COSHH)Regulations (1994)*, which should include safe methods of handling the virus. Nonetheless transmission of HIV and SIV *(CDC, 1992)* within the laboratory to scientists working with the viruses, has occurred *(see pp 2 & 3)*. It must be remembered that hepatitis B and C virus infections are common in HIV-positive patients *(Li et al, 1995)*. Precautions against inoculation will minimise infection, and those at risk should be vaccinated against HBV.

Preventing needlesticks and accidental exposure to HIV-infected blood

Under the COSHH Regulations employers are obliged to inform employees about risks in their work and to provide them with training to enable them to undertake their work safely *(see p 6)*. All hospitals should have clear policies and guidelines as to the procedures for taking blood and disposing of sharps. Failure to adhere to basic safe practice may increase the risk of inoculation injuries. For example, of four needlestick injuries reported to Communicable Diseases Surveillance Centre (CDSC) which resulted in seroconversion to HIV, two occurred when resheathing a needle, a practice which is known to increase risk of accidental inoculation injuries. In one case, the recipient was not wearing gloves. Evidence now indicates that wearing gloves reduces percutaneous exposure to blood during an incident by 46-86%. Details of percutaneous exposures and seroconversions in European health care workers are in ***Table 5***.

Individuals who regularly perform exposure prone procedures, or who work with high titre virus, may wish to have a blood specimen stored lest they become HIV positive

Table 6
Reported Failures of Zidovudine Prophylaxis after Occupational Exposure

Case	Country	Year	Exposure	Hours to first dose	Seroconversion documented
1	Australia	1990	hollow needle	6	6 weeks
2	South Africa	1990	lancet	12	day 24
3	USA	1990	16G IV cannula	3-7	day 94
4	France	1990	phlebotomy needle	½	day 16
5	USA	1991	21G phlebotomy needle	¾	3 months
6	South Africa	1992	IV cannula	½	6 weeks
7	USA	1992	21G syringe needle	2	day 121
8	UK	1992	18-20G IV cannula	1	day 56

in the absence of a documented seroconversion. Retrospective testing might help to establish that the infection had occurred recently and might therefore have been occupationally acquired. Ensuring the confidentiality and security of stored specimens is important. Adequate counselling and explicit consent must precede testing.

Staff and students, for example, medical students on elective projects, who plan to work in areas of the world in which the prevalence of HIV is high, and the facilities for protecting staff about blood borne infections are poor, should be advised about the risks of working in disciplines in which exposure to large quantities of blood is unavoidable. In particular, inexperienced individuals, such as medical students, would be advised not to undertake surgical and obstetric attachments in these countries unless it can be established that they would be adequately supervised. Emergency packs can be purchased for travel to countries with high rates of HIV and poor facilities for safe administration of intravenous medication or fluids. Such packs are available from, for example, The London School of Hygiene and Tropical Medicine, British Airways, and Trailfinders Ltd.

Training

Good practice when taking blood and performing exposure prone procedures should continue to be reinforced. Whilst the evidence that general campaigns to reduce needle capping are effective in reducing injuries is conflicting, one study showed that specific training in universal precautions resulted in a reduction in incidents of non-parenteral blood exposure. Audit of the circumstances in which sharps injuries occur amongst surgeons is of importance, with the object of improving practice.

Environment and equipment

Poor environmental conditions such as lighting and space, and fatigue may contribute to blood-exposure incidents *(McKinnon et al, 1992)*. Post-disposal injuries may be reduced by the introduction and correct placing of sharps disposal boxes. The design of the equipment used may also influence the incidence of sharps injuries. For example, one study demonstrated a fourfold reduction in the number of needlestick incidents when a device with a retractable blade for finger pulp sampling was used *(A Cockcroft, personal communication)*.

Management

The risk of acquiring HIV occupationally is very low *(see p 3)*. However, the risks of acquiring infection by such other blood-borne viruses as hepatitis B and C are higher and, consequently, healthcare workers sustaining percutaneous exposure to blood or blood-stained body fluids should be given advice regarding infection by these viruses and followed up appropriately. In addition all healthcare workers should be vaccinated against hepatitis B virus. Despite the rarity of occupationally acquired HIV infection, many of those exposed experience considerable anxiety, much of which may be relieved by reassurance. Obtaining a serum sample as soon as the incident is reported, followed by further samples at 3 and 6 months is recommended to determine whether seroconversion has occurred. Liaison between Occupational Health and Microbiology/Virology departments is of importance.

Current UK guidance states that prophylaxis with zidovudine (AZT) cannot be considered a necessary component of post-exposure management *(UK Health Departments, 1990)* but requires health districts to formulate local policy on its use. Given its acute side effects, the lack of information about long-term teratogenicity or oncogenicity, and the uncertainty about its efficacy, the use of zidivudine remains

controversial. It must be pointed out that 8 of the 73 documented seroconversions occurred despite the administration of zidovudine *(Table 6)*.

HIV Infected Staff in Pathology Departments

HIV infected pathologists face two specific problems in their workplace: the risk of transmission of HIV to a patient, and the risk of acquiring infection occupationally.

Pathologists are not involved in performing exposure-prone procedures on patients. Such procedures as bone marrow aspirations and fine needle cytology aspirations are not considered to be exposure-prone and there are not, therefore, any practice restrictions.

HIV-infected pathologists, MLSOs, and mortuary technical officers face similar problems relating to the risk of acquiring opportunistic infections from their workplace. HIV-induced immunosuppression renders individuals more susceptible to many infections, particularly tuberculosis. Although it may not be wise for such individuals to expose themselves to this risk, current recommendations emphasise individual responsibility and choice, and awareness of risk.

Health care workers who know or who suspect that they are HIV infected must seek appropriate expert medical and occupational health advice *(UK Health Dept, 1994)*. Those who may be exposed to potentially infectious pathogens should also seek confidential advice from the local occupational health physician.

Safety in Pathology Laboratories

Legislation

The following information is based on legislation and guidelines currently in operation in the United Kingdom.

Health and Safety at Work, etc. Act 1974

Employers, employees and the self-employed have specific duties to protect, as far as reasonably practicable, those at work and those who may be affected by any work activity in which they are engaged.

The Control of Substances Hazardous to Health Regulations 1994 (COSHH)

Full reference must be made to COSHH and a detailed risk assessment of laboratory procedures should be made as required by Regulation 6. The definition of a "substance hazardous to health" in COSHH has been amended to implement an EC Directive on the protection of workers from exposure to biological agents. A biological agent is defined as : "any micro-organism, cell culture or human endoparasite, including any which have been genetically modified, which may cause any infection, allergy, toxicity or otherwise create a hazard to human health".

Blood-borne viruses are included under the definition of "micro-organism" and the requirements of health and safety legislation apply if there is a risk of infection for employees no matter how infrequently this may occur. Similarly, there is a duty to protect others who may be exposed to contaminated waste, servicing contaminated equipment, or receiving specimens at a distant site.

The Management of Health and Safety at Work Regulations 1992

The duties of the Management of Health and Safety at Work Regulations (MHSWR) are wide-ranging and overlap with other health and safety legislation. Compliance with more specific regulations will normally meet the corresponding requirement of MHSWR. For example, the COSHH assessment will not need to be repeated for the purposes of MHSWR. Where duties laid down in MHSWR go beyond those in the more specific regulations, additional measures will be needed. Both MHSWR and COSHH specifically require that employees receive appropriate information, instruction and training on any identifiable risks to their health arising from their work.

Local safety policies and codes of practice

Where any hazard is known to exist, employers must provide a local safety policy and should develop codes of practice for the safe conduct of work. All staff, including newcomers, must be made aware such codes and instruction on their day-to-day application should be provided. Local codes of practice should reflect the principles expressed in law, but should be tailored specifically to local circumstances. Accident reporting procedures should be clearly defined and performance of the application of the policies should be monitored regularly.

The Reporting of Incidents, Diseases and Dangerous Occurrences Regulations 1985 (RIDDOR)

There is a requirement in RIDDOR for employers to report to the local Health and Safety Executive "acute illness requiring medical treatment where there is a reason to believe that this resulted from an exposure to a pathogen or infected material". In addition, any dangerous occurrence such as the uncontrolled or accidental release of a pathogen must also be reported.

Control measures

General

The measures listed in *Table 7* are applicable to all laboratory and clinical workers and form a reasonable basis for the institution of a universal approach to safe handling of any clinical or other pathogenic material.

The use of single-use disposables is generally safer and more practical than attempting to recycle contaminated items. Disposable gloves are prone to perforation during use and should not be washed and re-used. Needles should not be resheathed unless a safe means is available for doing so. Approved sharps containers (BS 7320:1990) should be available for disposal of needles, broken glass, *etc.*

Table 7
General precautions to prevent infection with HIV and other blood-borne viruses

Protect all breaks in exposed skin by means of waterproof dressings and/or gloves
Prevent puncture wounds, cuts and abrasions
Avoid use of, or exposure to, sharps (including needles, glass and metal) when possible. If this is unavoidable, take particular care in handling and disposal
Protect the eyes and mouth of the person or clothing by use of waterproof/water resistant protective clothing
Wear rubber boots or plastic disposable overshoes when the floor or ground is likely to be contaminated
Use good basic hygiene practices including handwashing and avoidance of hand-to-mouth contact
Control surface contamination by blood and body fluids by containment and appropriate decontamination procedures
Dispose of all contaminated waste safely

The use of universal precautions to prevent accidental exposure to blood and body fluids is officially advised in both the UK *(UK Health Department, 1990; ACDP, 1995a)* and USA. In practice very few hospitals have adopted full universal precautions. This may be due to a perceived low cost-benefit, especially in areas with low prevalence of reported blood-borne viruses or perceived problems by the staff in implementing such policies *(Cockcroft and Elford, 1994)*. Introduction of universal precautions should be considered when a significant proportion of the work of an institution involves patients at high risk for carriage of blood borne infections, *eg* patients with AIDS and patients attending for liver, renal, and haematological disorders. Evidence of a high prevalence of blood-borne viruses in unscreened admissions *(Poznansky et al, 1994)* or among patients involved in needlesticks *(Zuckerman et al, 1994)* may also justify the cost of introducing universal precautions.

Decontamination

Virus survival

For details of virus survival *see page 11*

Methods of decontamination

When selecting methods of decontamination it is essential to ensure that all pathogens will be destroyed by the process. Chemical disinfectants, for example, have their limitations and reliable inactivation of infectivity is difficult to achieve under some conditions. The presence of blood and other proteinaceous material may markedly reduce their action. Heat is the method of choice for destroying all infectious agents and this includes HIV and the other blood-borne viruses.

(a) **Autoclaving and Boiling**

Autoclaving is the optimal method of decontamination for items which are heat stable. Large and small steam sterilizers are available to suit particular requirements. When autoclaving is impracticable immersion in boiling water for a minimum of 5 minutes is acceptable.

(b) **Dry Heat**

A temperature of at least 160°C maintained for one hour is necessary to ensure sterilisation and therefore only metals and glass are likely to withstand this process.

(c) **Laundering**

The Department of Health has produced the following guidance:

"The effects of high temperature and the considerable dilution achieved by the washing process in a properly monitored and commissioned washing machine will render contaminated items safe to handle. Suitable time-temperature relationships are 80°C for 1 minute and 70-71°C for 3 minutes. These temperatures inactivate HIV but there is some uncertainty as to the minimal temperatures required to inactivate hepatitis B virus. However, the use of detergents and the dilution effect of the washing process are sufficient to eliminate the possibility of transmission and this assurance is further increased by the use of elevated temperatures."

(d) **Chemical Disinfectants**

Hypochlorite and other chlorine-releasing compounds, formaldehyde (4%) and activated glutaraldehyde (2%) have been shown to be effective in inactivating HIV and hepatitis B virus under most conditions of use. Prior to

cleaning of articles is important in order to reduce the load of organic matter and facilitate decontamination. All disinfectants are, by their nature, potentially hazardous and must be used with caution. For example, exposure to formaldehyde vapour >2ppm or glutaraldehyde vapour >0.7 ppm should be avoided by, for example, the use of fume hoods. In addition, reactions with other chemicals may present hazards.

Spillage
Details of management of spillage and other forms of contamination should be specified in local codes of practice. Gloves should be worn. Small spills can be wiped up with a paper towel soaked in hypochlorite solution (10,000 ppm available chlorine). Larger spills can either by covered with paper towels and flooded with hypochlorite solution (10,000 ppm available chlorine) or covered with dichloroisocyanurate granules. The contact time should be at least 2 minutes before clearing up. If glass is present, bunches of paper towels, pieces of card, or a plastic dustpan should be used to remove the fragments which should be placed in a sharps container.

Waste Disposal
The safe disposal of all hazardous waste is part of the statutory duty of employers under the Health and Safety at Work Act, COSHH, and regulations made under the Environmental Protection Act. Full details of waste disposal should be included in local codes of practice.

Full guidance on the definition of clinical waste and current requirements for its disposal is available in "Waste Management Paper No. 25 on Clinical Waste" produced by the Department of the Environment.

Detailed guidance on handling clinical waste on site can be found in "Safe disposal of clinical waste", Health and Safety Commission, Health Services Advisory Committee, 1992 HMSO ISBN 0-11-886355-X.

Clinical pathology laboratories

Any laboratory that processes samples from humans for any reason may, unknowingly, receive material from individuals infected with HIV or other blood-borne viruses. The prevalence of infection varies in the United Kingdom but it must be remembered that samples imported from other countries may present a significantly higher risk. Health and safety law in the UK demands that those who need to know are alerted to identified risks of infection by the labelling of samples and accompanying forms with a hazard warning label. However, as not all infected individuals will be identified, it is important to adopt consistently high standards of work practices.

Centrifugation
When centrifugation of clinical samples is necessary, sealed buckets or rotors should always be used. The use of transparent lids has the advantage of allowing the operator to detect breakage or leakage of specimen containers. In the case of breakage, and depending on the degree of potential contamination, the centrifuge, rotor, or bucket should be opened in a microbiological safety cabinet. Buckets and rotors are preferably decontaminated by heat treatment but if autoclaving is used it should only be done in accordance with the manufacturer's instructions. As an alternative, formaldehyde or glutaraldehyde may be used but operator exposure to vapour from these agents should be carefully controlled *(see above)*.

Ultracentrifugation poses specific problems in that virus may be being concentrated and a rotor failure may contaminate the centrifuge chamber. It is therefore recommended that ultracentrifuges used with HIV are located within containment level 3. In the event of rotor failure the use of respiratory protective equipment when opening the centrifuge may be necessary. In such a case both centrifuge and laboratory should be fumigated.

Laboratory precautions
Containment level 2 *(with extra precautions listed in Table 8)* is acceptable for handling materials known to contain HIV and other blood-borne viruses providing propagation or concentration of the viruses is not undertaken. This level of containment is appropriate for many types of laboratory including : clinical chemistry, haematology, histopathology, cytology, transfusion, serology, immunology, forensic work and drug assays.

Microbiological Safety Cabinets
In general, Class I or Class III microbiological safety cabinets *(British Standard 5726 : 1992)* should be used when handling Hazard Group 3 agents. However, as blood-borne viruses are unlikely to be infectious by the airborne route, Class II cabinets may be used when protection of the work from contamination is essential.

Analytical equipment
Local codes of practice should specify procedures for the safe use of all analytical equipment. All equipment should be decontaminated in accordance with the manufacturer's instructions. Analytical equipment requiring inspection, servicing or repair should, if possible, be decontaminated before the work begins.

Guidance on decontamination of equipment is available from the Department of Health (HC(91)33, under revision) and service engineers should request formal permission to work before beginning their work.

Specimen transport
Details on transportation of samples and the necessary construction and decontamination of containers are available in the Health Services Advisory Committee document "Safe working and the prevention of infection in clinical laboratories" *(HSAC, 1991a)*. More recently, within the UK, new Health and Safety Executive Regulations entitled "Classification, packaging and labelling of goods for carriage by road and rail" came into force in 1994.

Table 8
Precautions to be adopted by laboratories handling samples known or suspected to contain HIV and other blood-borne viruses

Protocols for safe conduct of work should be agreed and strictly adhered to
Each procedure whould be conducted in a designated area of the laboratory with sufficient space for safe working
If infected material may be dispersed (eg homogenisation, vigorous mixing) a microbiological safety cabinet or another form of primary containment should be used
Unnecessary equipment should be removed from the area
The worker should be free from disturbances; unauthorised persons should not have access to the area
Gloves and other appropriate protective clothing should be worn throughout the work
The use of sharps and glassware should be avoided if possible
Surfaces and any equipment should be decontaminated immediately on completion of a work session
A satisfactory disinfection policy must be in operation

Clinical material containing HIV and other blood-borne viruses may be sent by post providing the conditions stipulated by the Post Office are met. To minimise the possibility of leakage or breakage it is recommended that screw capped containers are used.

Carriage of infectious material by international post is subject to the regulations of the International Air Transport Association (IATA) and the Universal Postal Union (UPA). Details of packaging requirements and necessary declaration certificates may be obtained from Royal Mail International RM 12.1, Room 300, 52 Grosvenor Gardens, London SW1W 6AA. If goods are to be transported by air cargo, IATA, the airline, or a recognised air cargo agent should be consulted.

Propagation or concentration of HIV and other blood-borne viruses

In the UK, the COSHH Regulations 1994 (which are now consolidated and amended to implement European Community Directives) require employers to notify the Health and Safety Executive of *first use* of biological agents in Hazards Groups 2, 3 and 4. There is, however, no requirement to specify which agents are to handled. Nevertheless, those intending to cultivate or concentrate HIV for diagnostic, research or production purposes, or planning to inoculate experimental animals with HIV, are invited to inform the Technology and Health Sciences Division of the Health and Safety Executive. If there is any intention to conduct work involving genetic modification of HIV or other infectious agents, the Health and Safety Executive must, by virtue of the Genetically Modified Organisms (contained use) Regulations 1992, be notified in advance. In some cases, the formal consent of HSE is necessary before projects begin.

Propagation or concentration of HIV in the laboratory must be conducted at Containment Level 3. Some of the containment measures for laboratory work with pathogens and with infected animals are now a statutory requirement in COSHH 1994. Part II of schedule 9 of COSHH gives details of these. Other features of containment that remain as guidance are available in the document "Categorisation of biological agents according to hazard and categories of containment, HSE Books, London 1995" *(ACDP 1995b)*.

Inadvertent cultivation of retroviruses

There is a possibility that cell culture systems maintained for other purposes, *eg* cytogenetics or immunology studies, may support the replication of HIV and other retroviruses *(see p 14)*. Peripheral leucocyte cell cultures are the most important example although the viruses are unlikely to reach significant levels within 3-4 days incubation. Similarly, some susceptible cell lines of human or simian origin may be chronically infected with primate retroviruses, or virus may be introduced accidentally during passage in the laboratory. To reduce the risk of unsuspected exposure to infection from HIV and other retroviruses it is recommended that cultivation of all cells from known or suspected cases of HIV or other human retrovirus infection should be conducted at Containment Level 3. However, permissive cells (particularly if CD4 antigen positive) or mixed cells from those not known or suspected to be infected with retroviruses may be handled at Containment Level 2 with appropriate awareness of potential risks and satisfactory working practices. With more prolonged incubation (*eg* beyond 3-4 days) the need to conduct the work in Containment Level 3 should be considered on the basis of a detailed risk assessment. Although the risk of retrovirus replication in non-permissive cell lines is low, risk assessment should consider the prevalence of HIV and other retroviruses in the

population from which the cells are obtained.

HIV testing of donors of cells or samples being used in laboratory studies should be done only after appropriate counselling and informed consent have been given. This requirement is not necessary for samples which cannot be traced back to named donors.

Histopathology Laboratories and Autopsies

Histopathology laboratories

Risks of HIV-infected material

Specimens from HIV positive patients are being sent to histopathology laboratories in increasing numbers. The potential risk to technical and pathology staff arises if fresh unfixed HIV-infected material arrives in the laboratory. Inoculation of HIV by sharp injury, and inhalation of pathogens such as *Mycobacterium tuberculosis* are then possible. However, material from patients known to be HIV-positive should arrive at the laboratory already in fixative and, as the routine fixatives for histopathology, based on formaldehyde, rapidly inactivate HIV, no hazard arises from trimming adequately fixed material or from cutting paraffin-embedded tissue blocks. Similarly, the fixation and embedding procedures for electronmicroscopy also completely inactivate HIV.

An additional risk is the use of cryostat machines for frozen sections where there is the potential of cutting the hand in the presence of unfixed material. There are, as yet, no reports of such cuts leading to HIV seroconversion. While the likelihood of HIV infection by this route is probably less than that following hollow-bore needle-stick injury, it cannot be considered to be non-existent *(Reichert, 1992)*.

Staff should remain aware that, although the use of 'Danger of infection' stickers on material from known HIV-infected patients is widespread, inevitably a proportion of HIV-infected patients will not have been identified prior to tissue sampling or the high risk label may have been omitted.

Risk reduction

Guidelines on safe working practices in the laboratory are published by the Health Services Advisory Committee (HSAC)*(HSAC, 1991a and 1991b)*. These include the universal precaution of handling all tissues with gloved hands *(see also p 12 et seq)*.

As stated above, material from patients known to be HIV-positive should arrive at the laboratory already in fixative, and surgical staff should be encouraged to comply with this procedure. If it is necessary to store unfixed material from HIV-positive patients, such material should be adequately contained and not stored with HIV negative samples. Departments undertaking HIV-related research that involves fresh tissues should have instituted the appropriate containment facilities and training of staff in protection against accidental infection.

The preparation of frozen sections for rapid diagnosis on known HIV-positive material is strongly discouraged, unless the laboratory can utilise a dedicated cryostat and experienced operator. When receiving fresh material for cryostat cutting from patients of unknown HIV status, the laboratory should routinely enquire about the HIV risk status of the patient (this is similar to enquiring about the likelihood of active tuberculosis, a disease for which cryostat preparations are also not encouraged). The use of frozen section diagnosis in many hospitals is declining. Systems of rapid tissue fixing and processing may be used on high-risk and known HIV-positive material where an urgent (same day) diagnosis is required.

Mortuaries and autopsy work

Since the start of the HIV pandemic, autopsies have been fundamental in delineating the clinical pathology of AIDS *(Lucas, 1993)*. The rationale for performing autopsies on known HIV-positive cadavers includes: *(i)* clinico-pathological follow-up, *(ii)* validating end-points in HIV clinical trials, *(iii)* documenting adverse drug reactions, *(iv)* documenting specific organ pathologies (*eg* brain, spinal cord), and *(v)* medico-legal examination of unexplained death in HIV-positive people.

Among pathologists, there is a wide range of opinion as to whether autopsies should be carried out on HIV-positive cadavers. Some are of the opinion that there is no justification for a pathologist to refuse to perform an autopsy because the patient is HIV-infected *(Geller, 1990a)* while others suggest that a risk-benefit analysis be conducted by the pathologist and clinician on the likely value of information that could be obtained from an autopsy *(Reichert, 1992)*. Some pathologists refuse to conduct autopsies on HIV positive patients, an attitude often reinforced by the reluctance of mortuary technical staff to assist with such cases. The low overall prevalence of HIV in the UK has yet to render such an attitude a contractual problem with an employing authority. Nonetheless, where good reason exists for requesting an autopsy (outside of Hazard group 4 infection cases), the autopsy should be performed.

Many thousands of autopsies have been performed on HIV-positive cadavers of adults, children and on perinatal deaths. There are no reports of a pathologist acquiring HIV infection from an autopsy. However, in the USA, three morticians have acquired possible occupation-related HIV-1 infection; no details are given *(CDC, 1993)*.

Risks of HIV infection

The major hazards to pathology and technical staff in performing HIV-positive autopsies come from cuts and pricks to the skin from sharp instruments and bone spicules, and from inhaling virulent pathogens such as *M. tuberculosis (see p 13)*. Oral and conjunctival infection is possible but readily prevented by simple barriers. During 10% of autopsies pathologists sustain a glove puncture and technicians sustain rather more *(Weston and Locker, 1992; Harris, 1993)*. The incidence of cuts depends on experience. One study found

that pathology trainees sustained a cut injury once per 11 autopsies, whilst for the more experienced consultant pathologists the rate was one per 53 autopsies *(O'Briain, 1991)*. Although a study among surgeons in San Francisco, a city where HIV prevalence is high and where there is heightened awareness of risk, demonstrated that the rate of percutaneous exposures was not affected by prior knowledge of the patient's HIV status *(Gerberding et al, 1990)*, this may not be applicable elsewhere. Nevertheless there is evidence that, over time, the overall rate of such exposures has declined *(Lowenfels et al, 1995)*. Whether a scalpel injury incurred whilst dissecting a cadaver carries a greater or lesser risk of HIV transmission than a needle-stick injury is unknown *(Reichert, 1992)*.

Depending on relative humidity and other factors viruses may remain infectious for long periods in the environment. The infectivity of HIV decays with time, but slowly and variably *(Cao et al, 1993)*. However, HIV, in high concentration, may remain infectious for up to 3 weeks and infectious virus has been recovered from liquid blood held at room temperature for 2 months. HIV-1 has been recovered from cadaveric blood and tissue samples. The largest study to date found virus in 21/41 (51%) of plasma and/or blood mononuclear cell fractions from HIV infected cadavers *(Bankowski et al, 1992)*. The longest post-mortem time tested was 37.5 hours, but virus was found only up to 21.5 hours. In other, smaller, investigations, HIV-1 virus has been recovered 18 hours to 11 days after death *(Henry et al, 1989; Nyberg et al, 1990; Ball et al, 1991)*. HIV was detected in skull bone at 6 days post mortem but not in samples of bone sawdust. HIV could be recovered from spleen specimens stored for up to 14 days *(Nyberg et al, 1990)*. Refrigeration of cadavers did not appear to diminish the recovery of virus. HIV-2 has also been cultured from cadaveric blood 16.5 days after death *(Doucheron et al, 1993)*.

Since autopsies are usually performed within a few days of death, HIV-positive cadavers must be assumed to contain viable infectious HIV.

Epidemiology of HIV infection in autopsy work

The prevalence of HIV infection in adult cadavers varies according to location and case selection. About 800 deaths per year from AIDS in the UK are currently reported to CDSC *(CDR Report, 1995)*. Seventy percent of patients with AIDS reside in London. Although the annual trends show a slow increase in reports of HIV infection and cases of AIDS in the UK as the epidemic continues, the likelihood that HIV prevalence among unselected adult cadavers will approach the 50% that is observed in some cities in Africa is remote *(De Cock et al, 1990; Lucas et al, 1993)*.

There have been no studies of HIV prevalence among unselected cadavers arriving in mortuaries in the UK. However, in 1990 in Tayside, of 511 consecutive cadavers where forensic autopsy had been requested, 5 (1%) were HIV-1-positive. All were IDU and suspected or already known to be HIV-positive; 4/12 known IDUs were HIV-1-positive *(Sadler et al, 1992)*. In north west London, 14% of 56 cadavers of IDUs were HIV-1-positive *(West I, personal communication)*. A similar HIV seroprevalence was found among dead IDUs in Copenhagen, Denmark, in 1991 *(Kringsholm and Theilade, 1993)*. In Cardiff, between 1985-92, two out of 300 high risk patients' cadavers were HIV positive *(Claydon, 1993)*.

There are no published data on the proportion of HIV-positive adult cadavers, on whom an autopsy is requested, that is known to be HIV-positive before autopsy. Although data from UK death certificates have suggested that only 40-65% of HIV-positive people dying are known to be HIV-infected, this study did not consider inter-doctor communications about HIV status *(McCormick, 1991)*. Furthermore, there is anecdotal evidence that suggests the proportion is now >90%, as clinicians are becoming more familiar with HIV disease and HIV sero-testing.

HIV seroprevalence is high among homosexual and bisexual men, IDUs, and those from areas of high HIV prevalence. In one London hospital Accident and Emergency unit, 2.4% of attending patients aged 16-45 years were HIV-1-seropositive *(Poznansky et al, 1994)*. Suicide in young men in UK is increasing, and HIV infection is suspected as one of the reasons *(Hawton, 1992)*.

Unless other investigations are performed, it must be assumed that all fetuses and children aged <18 months from HIV-positive mothers are infected, since routine serology cannot establish whether infection has been transmitted from mother to baby *(see p 16)*.

Pre-autopsy HIV testing

Pathologists are concerned about the likelihood of unknown HIV-positive cadavers being presented for autopsy, either through a hospital request or via the Coroner (or Procurator Fiscal). For medico-legal autopsies, the Coroner can grant permission for HIV serology to be done as a test intended to provide information which may assist a pathologist in making a diagnosis. For hospital autopsies, where permission for autopsy is given by the possessor of the body, a pathologist can test likewise, since a major role of the autopsy is to establish a diagnosis. Legal opinion obtained by the Royal College of Pathologists and the statements of the General Medical Council are unanimous on this point. Prior consent of relatives is not required when HIV infection is suspected in a cadaver. Discussions are in progress between the GMC and DoH whether pathologists are justified in testing all cadavers for HIV prior to autopsy. In practice, outside certain risk groups (*eg* known IDUs) there appears to be little epidemiological or economic justification to do this at present.

If the previously unknown HIV-seropositive state of a patient is discovered through testing at autopsy, the duty of care and confidentiality does not cease with the patient's death. If there is a surviving spouse or sexual partner, then a breach

of confidentiality is justified by the need to advise him or her of exposure to infection. In practice, such a responsibility usually devolves through the pathologist to the patient's clinicians *(BMA, 1993)*.

Practical considerations: general
Whilst it may be preferable to have a separate suite for high risk cases within a mortuary, this is not obligatory. However, the structure of, and working conditions within, a mortuary should meet the standard guidelines *(HSAC, 1991b)*.

In principle, a policy of universal precautions may be advocated for all autopsy work but, in the UK, where the prevalence of HIV infection is low, this may be currently unrealistic. The Health Services Advisory Committee (HSAC) guidelines adopt a two-tier approach on safety measures with regard to HIV and other high risk infections *(HSAC, 1991b)*. A similar approach is advocated by the Clinical Pathology Accreditation scheme. However, standards of hygiene in medical practice are constantly increasing, and it is likely that the reasonable protective measures outlined below will become standard for all autopsy work.

The epidemiology of HIV is changing and familiarity with HIV disease is increasing. As AIDS becomes normalised as a disease, the circumstances of mortuary practice will probably change, with less anxiety being manifested over performing HIV autopsies. Currently, in those units where HIV-positive autopsies are not performed, cases for autopsy may be referred to another unit. Requests from the Coroner for autopsy of suspected high risk cases can be evaluated by HIV serology and proceeding if the HIV status is negative. If the test is positive, the Coroner can arrange another venue for autopsy.

Practical considerations: specifics
Detailed guidelines on the performance of high risk autopsies, including HIV-positive cases, and on the mortuary working environment are published by the HSAC *(HSAC, 1991b)*. The following recommendations are a brief guide on the performance of autopsies on known HIV-positive cadavers. For anatomical pathology technicians, guidelines appropriate for HIV-positive cadavers are also available *(Royal Institute of Public Health and Hygiene, 1991)*.

All pathology, scientific and technical staff in the post-mortem room during examination of HIV-positive cadavers must be trained in safe working practices, and it is recommended that the number of persons involved in the autopsy be limited to three:a pathologist, technician assistant, and a circulator (who remains uncontaminated). Although the pathologist need not be of consultant or equivalent status, he/she must have substantial experience of general autopsy work and appropriate training for higher risk cases.

Clothing
The pathologist and assistant should change into 'surgical' trousers and shirt and wear: a waterproof gown or jump-suit that covers the legs, all of the arms, and tucks into the gloves; an apron covering the trunk and legs; water resistant boots; a visor that fully protects the face or a face mask and glasses, or goggles, to protect the eyes; a hat that covers the scalp if it is not already covered by a jump-suit. Disposable gowns, suits, aprons, masks and hats are recommended. The necessity for down-draft ventilated visors has not been established *(but see p 13)*.

There is no consensus on the best means of protecting the hands against cuts and sharp injuries whilst maintaining appropriate sensitivity for dissection and tissue analysis (Geller, 1990b). Most pathologists experienced in HIV work prefer wearing more than just a single pair of latex gloves. The options include: double gloving, the outer pair being thicker household rubber gloves; chain mail worn between two latex gloves; synthetic polyolefin cut-resistant gloves worn under an outer glove or between two latex gloves; heavy duty neoprene or other types of reinforced glove. While pathologists and assistants will determine their preferred option, it is strongly recommended that more protection than single latex gloves be worn during HIV autopsy work. Double gloving significantly decreases the rate of inner glove perforation and probably reduces the amount of blood transferred if percutaneous exposure occurs.

Instruments to be used in autopsies
The number of sharp instruments should be reduced to a minimum and should always be kept in sight. Wherever possible, blunt-end instruments should be used. These include organ-slicing knives, scissors, and body-opening PM40 knives modified to remove the sharp point (now available commercially).

Removing brain, spinal cord, eye, and temporal bone
There is no consensus on the safest method for opening the skull and removing the brain of an HIV-positive cadaver. Aerosols from sawed bone have not been shown to be infective, and inhalation has not been shown to be a route of HIV infection. The handsaw or electric oscillating saw are used according to local preference. When opening the skull of a young child, an oscillating saw can throw a jet of blood toward an assistant holding the head, because of the plentiful haemopoietic marrow. Some pathologists have recommended opening the skull of high risk cases under a transparent plastic bag *(Bell and Ironside, 1993)*, but most do not do this. Modern electric saws can be fitted with remote exhaust and filter attachments to remove aerosols, but these engender a problem with subsequent cleaning. Similarly, either hand or electrical saw may be used to obtain the spinal cord and temporal bone if those organs are required. The eyes are readily removed by chiselling through the roof of the orbit.

Closing the body after the autopsy
Some pathologists and assistants prefer clips to the routine needle-and-thread to close a HIV-positive cadaver after

autopsy. There is no consensus on this, nor observational data available on the relative safety of each methods, although, in time, there may be a move toward clipping. Meanwhile it is important to stress that HIV-positive cadavers are not appropriate cases on which to evaluate a qualitatively new methodology.

Autopsies on fetuses and perinates do not involve saws, but otherwise the hazards are the same as for adult cases. The placenta is particularly blood-rich and may be fixed before cutting and trimming.

Other infections in HIV-positive cadavers
Hepatitis B and C virus infections are common in HIV-positive patients *(Li et al, 1993)*. Precautions against inoculation will minimise infection, but all pathologists and assistants performing autopsies should be vaccinated against HBV.

Whilst *M. avium* complex and *Pneumocystis carinii* infection are not a risk for prosectors who themselves are not immunocompromised, *M. tuberculosis* is. Previously undiagnosed tuberculosis can infect susceptible autopsy staff *(Kantor et al, 1988; Menzies et al, 1995)*. Moreover, the tuberculous lesions in untreated HIV-infected cadavers are heavily bacillated *(Lucas and Nelson, 1994)*. The recent rise in incidence of multi-drug resistant tuberculosis involves HIV-infected persons, and is a further cause for concern *(Pearson et al, 1992)*. In UK, the proportion of HIV-positive patients coming to autopsy with tuberculosis is low but may rise, as already noted in the USA *(Klatt et al, 1994)*. In one London centre it is 2/100 cadavers *(Lucas, personal observations)*.

The standard guidelines for minimising transmission of tuberculosis in the mortuary should be followed *(HSAC, 1991b)*. Transmission may occur in mortuaries with acceptable ventilation standards *(Kantor et al, 1988)*. The efficacy of downdraft ventilation autopsy tables in preventing transmission is not known, and it is virtually impossible to decontaminate mortuaries after dissecting an infective cadaver. For personal protection, a HEPA (high-efficiency particular air-filter) respirator may be worn *(Adal et al, 1994)*. Alternatively a face visor with a down-draft ventilation is effective, but this is cumbersome, noisy and inhibits communication among staff. The CDC report on tuberculosis in health centres *(CDC, 1994)* suggests a higher standard of personal respiratory protection against tuberculosis in the autopsy room compared with hospital wards.

Infections such as *Histoplasma* or *Coccidioides spp*, that are more prevalent in HIV-positive cadavers in other countries *(Klatt et al, 1994)*, may be encountered in UK *(Miller et al, 1994)*. These may often be recognised only after the histology has been performed. Advice should be sought on post-exposure management from the local occupational health physician.

Occupational health physicians should be aware of the risk of infection from agents such as tuberculosis to pathologists and mortuary technical staff as a consequence of general autopsy work irrespective of HIV.

Practice in resource-poor countries
Standards of hygiene in mortuaries are generally lower in resource-poor countries and the infectious hazards are different from those in industrialised countries *(Lucas, 1995)*. Where possible within the resources available, hazardous practices should be eliminated (for example, wearing gloves when handling cadavers that leak blood). As well as HIV infection, tuberculosis is associated with poverty. In many countries in Africa, local population HIV seroprevalence among adults often exceeds 10% *(Mann et al, 1992)*, and tuberculosis is ubiquitous *(Raviglione et al, 1995)*. One consequence is that mortuary staff may be co-infected with HIV and *M. tuberculosis* and suffer high morbidity.

Embalming
Many cadavers are embalmed prior to burial. Workers in funeral services are therefore at risk of acquiring infection with HIV (and other blood borne viruses) when inserting needles into cadavers for fluid removal and injection. The same standards of hygiene and safety precautions as pertain for anatomical pathology technicians should be applied by such workers *(Healing et al, 1995)*. Similarly, there is a potential risk to embalmers when treating the cadavers used by medical students for anatomical dissection *(de Craemer, 1994)*. The usual fluids used for embalming (ethanol, formalin, phenol) are effective against HIV. It should be noted that ACDP (1995) states that embalming of HIV positive cadavers should only be undertaken if essential.

The Cytogenetics Laboratory

The handling of specimens that are known to be, or are likely to be, infected with HIV

Cytogenetic investigations include prenatal and postnatal studies of constitutional chromosome abnormalities and also those related to malignancies and acquired chromosome abnormalities. The tissues involved include blood, bone marrow, amniotic fluid cells, chorionic villus samples, and a variety of post-mortem tissues. The chromosomes can only be seen in cells that are actively dividing so that, with the exception of chorionic villus samples and some bone marrow specimens, all other tissues require some degree of culturing to provide adequate numbers of dividing cells. The length of time in culture varies from two to three days for lymphocytes to a week or longer for amniotic fluid cells; cultured fibroblasts derived from solid tissue biopsies may require up to several weeks. The problem, therefore, arises as to the possible inadvertent culturing of HIV during such procedures. In order to reduce the risk of unexpected exposure to infection from the propagation of HIV, the ADCP Guidelines (1995) require that the culture of all cells from known or suspected cases of HIV must be conducted at containment level 3. A number of cytogenetic laboratories

do not have such facilities, and in these cases, arrangements should be made for specimens from HIV positive patients to be processed elsewhere. Where containment level 3 conditions do not exist, specimens *suspected* of being infected with HIV should not to be processed until the patient has been tested and confirmed as being antibody negative. Should the patient not consent to an HIV test, specimens should be treated as if HIV positive.

Constitutional Chromosome Studies - Prenatal

It is likely that a few HIV positive women will opt for termination and the need for prenatal testing for genetic disorders may well therefore be obviated. Each case, therefore, should be discussed fully with the referring physician and cultures established only when considered essential.

Amniotic Fluid Cells (AFC)

With current cytogenetic practice the culture time is often as short as seven days or even less. In some instances, however, the time taken to achieve an adequate cell density may be up to three weeks or more. Although the ability of amniotic fluid cells to support HIV replication is unclear, the virus has been isolated from amniotic fluid *(Mundy et al, 1987)* and culturing of samples from known or suspected HIV+ve patients should only be carried out using containment level 3.

Chorionic Villus (CV) samples

Chorionic villus sampling provides an alternative approach to prenatal testing and allows sampling at approximately 10 weeks gestation as opposed to AFC sampling which is usually carried out at 16 weeks. The technique can provide a very rapid result as chorionic villi contain a relatively high proportion of dividing cells and it is possible to make "direct" preparations without the need to culture. It is appropriate to handle samples of CV for direct preparations in containment level 2 provided the extra precautions required by the ADCP (1995) are implemented ("2+"). However, in most laboratories, there is a preference for culturing CV samples as the quality of the chromosome preparations is improved. As with amniotic fluid cell cultures, the possibility of CV cultures supporting the propagation of the virus cannot be excluded, and culturing of samples from known or suspected HIV infected patients should only be carried out using containment level 3.

Fetal Blood

Fetal blood sampling is possible from 18 weeks of gestation onwards, and provides large numbers of dividing cells requiring only a short incubation time (3 days) thus permitting its use within containment level 2 facilities. It is useful as a backup method where, for example, a previous AFC culture has failed and a rapid result is essential. At one time, prior to the introduction of DNA screening, it was the method of choice for Fragile-X chromosome studies, where relatively large numbers of chromosome spreads are necessary. The conditions for handling fetal blood samples are as for other samples.

Constitutional Chromosome Studies - Postnatal

Although most postnatal chromosome studies are carried out on peripheral blood lymphocytes, solid tissue cultures are also used. These may be from skin biopsies in cases, for example, where it may be important to look for chromosome mosaicism. However, a large proportion of solid tissue culture work in the cytogenetics laboratories is concerned with chromosome investigations on tissues from spontaneously aborted foetuses. As with prenatal samples, it is appropriate to consider whether chromosome studies on specimens from individuals known to carry HIV are appropriate.

Blood Cultures

Since macrophages and T "helper" lymphocytes express CD4 there is a risk of inadvertent propagation of HIV, although it is generally considered unlikely that the virus will reach significant titres when culturing for the maximum of three to four days required. Nevertheless, if a specimen is known or suspected to be from an HIV infected patient the ADCP guidelines require the use of containment level 3 facilities.

Where the blood samples are from individuals *not* known or suspected to be infected with HIV then the cultures may be handled at containment level 2 but with the extra precautions laid down by the ADCP, 1995 (containment level "2+").

Solid Tissue Cultures

Cells cultured from solid tissue biopsies are often grown for a number of passages. However, although it is considered unlikely that fibroblasts will propagate HIV, containment level 3 is required if the samples are from an HIV infected patient.

Acquired Chromosome Changes

Although a certain amount of solid tumour work is carried out in a number of cytogenetic laboratories, the largest proportion of investigations on acquired chromosome changes make use of bone marrow samples.

The processing of most bone marrow samples involves either direct preparations or minimal (24 hour) culture times and may therefore be performed in containment level "2+" facilities. However, some authorities take the view that bone marrow specimens should be treated in exactly the same way as blood cultures and, therefore, handled at containment level 3. As with other specimens, it is appropriate to assess each case individually, discussing it, where appropriate, with the referring physician.

Other cytogenetic studies - lymphoblastoid cell lines

Some laboratories may make continuous cell lines from cytogenetically interesting patients. Since these are often derived from peripheral CD4 expressing leukocytes, they must be handled at containment level 3 unless they have been tested and shown to be HIV negative. The informed consent of the donor will be required.

According to the guidelines provided by the United Kingdom Co-ordinating Committee on Cancer Research, and as also required under health and safety law, if HIV infected cell lines are passed from one laboratory to another, a written warning must be sent to the receiving laboratory. If attempts have proved unsuccessful in trying to establish whether or not the cell line is hazardous, then a cautionary note to this effect should be sent to the receiving laboratory.

Diagnosis of HIV infection

Counselling

The Department of Health has declared: *(i)* that patients must be counselled before being tested for HIV and be made aware of the consequences both of being tested and of the result being positive; and *(ii)* that testing will only follow explicit consent by the patient. It should be strictly understood that any discretion to depart from the practice of obtaining full consent applies only in exceptional circumstances and is only justified either when the interests of the patient are paramount *(MDU, 1990)* or where a test is "imperative in order to secure the safety of persons other than the patient" *(GMC, 1993)*.

The General Medical Council has made it quite clear that "it is reasonable for a doctor working in a laboratory to conclude, when a request for HIV testing is received, that the referring clinician has obtained the patient's consent".

Laboratory tests

(a) **HIV antibody tests**

In the UK, testing should be carried out by registered MLSOs in accredited laboratories which participate in NEQAS and use appropriate internal controls. Near patient testing is unacceptable. A consultant microbiologist/virologist should be available at all times for advice.

HIV infection is usually detected by the presence of specific antibody to HIV. The strategy used for testing depends on the prevalence of HIV in the population being tested, the cost of testing and the number of samples being tested. In low prevalence areas, the recommended strategy is to use an initial sensitive screening test (usually an EIA) followed by at least one if not two confirmatory tests of high specificity on reactive sera. Since the prevalence of HIV is generally low (<10%), most of the cost of testing is that of screening, and confirmation of positives contributes little.

In the USA, confirmation is by Western Blot, despite the expense and difficulties in standardisation of the test. In the UK, indirect or sandwich HIV-1 and HIV-2 antibody tests are preferred for screening, with a competitive EIA and a third test, which should be based on a different principle: *eg* an EIA method not previously used, particle agglutination, immunoblot, for confirmation. Tests should be selected to reflect differences in antigen preparation *ie* recombinant antigen, peptides or lysate. The diagnosis should be confirmed by testing of a second sample from the patient.

Blood donations are screened with a sensitive ELISA and any which are positive are rejected.

In countries with a high prevalence (> 10% of HIV in the population), the WHO recommend that a single confirmation of a positive screen is all that is necessary for diagnosis. Positive screens not confirmed should be considered as negative. The cost of screening blood donations and carrying out surveillance studies may be reduced by screening serum pools of up to five individuals. This approach is particularly cost effective where seroprevalence is low. The sensitivity and specificity of tests chosen should not be compromised by dilution of HIV positive samples. In addition, adequate quality assurance procedures should be maintained.

The recent discovery of divergent strains of HIV-1 (HIV-0) has once again highlighted the importance of reviewing detection strategies for HIV . These strains are, in general, best detected by current tests which are based on viral lysate antigens and indirect detection methods.

(b) **Antigen detection (p24)**

EIAs which detect the p24 core or nucleocapsid protein are useful for diagnosis of HIV infection before the appearance of specific antibody. (*ie* during the "window period" *see p 19*). In practice p24 is detectable in only 50-60% of such specimens. However, such window period testing, where feasible, may be important in detecting early infection amongst blood donors in countries where HIV spread is epidemic *(see p 20)*. It is essential that p24 reactivity is confirmed (by a blocking test).

(c) **Isolation of HIV**

Isolation of HIV from clinical material is not normally a routine diagnostic procedure as it must be conducted in containment level 3. In addition, the procedure is time consuming, labour intensive, and expensive. Virus isolation therefore tends to be used more as a research tool. Although virus isolation has now been superseded by PCR for diagnostic purposes, it may be indicated for samples from high risk patients which have not amplified by PCR.

Detection of HIV RNA

Detection of HIV RNA is only used routinely in the identification of vertically (mother to baby) transmitted HIV infection *(see p 16)*. Very rarely it may be necessary to detect the viral genome in diagnostically difficult cases. However, quantitative measurement of the amount of virus in the plasma (viral load) whilst not currently routinely

performed may soon become an important tool in the management of patients on antiviral therapy for HIV. The change in plasma viral load, as measured by genome copy number/ml, appears to be a sensitive and rapid indicator of short term drug efficacy as well as a way of monitoring drug-resistance. As such, viral RNA load is being increasingly used as the "gold standard" in clinical trials. The detection of point mutations in HIV DNA or RNA and *in vitro* tests of drug sensitivity may also become routine components of patient monitoring.

Methods for detecting HIV DNA and RNA

Three methods are available commercially for detection of HIV nucleic acid.

(a) **The Polymerase Chain Reaction (PCR)**

Until recently, most PCR was based on in-house assays. Commercial kits are now becoming available and results from a recent quality control exercise suggest that the use of one has improved the performance of the participating laboratories as compared with a previous exercise which involved a standardised in-house assay *(Bootman and Kitchin, 1994; Barlow et al, 1995).* More recently, concerns about the sensitivity of that kit have been raised*)*. PCR remains a specialised technique and should therefore only be routinely performed by laboratories with the relevant experience and expertise to identify and remedy technical problems. Viral load can be measured by reverse transcriptase PCR (RT-PCR).

A number of different protocols may be used for quantification. However, it is generally accepted that, for accuracy and to control for variation in the efficiency of each amplification reaction, an internal standard of known concentration must be included in each tube *(Sninsky and Kwok, 1993).*

(b) **Nucleic Acid Sequence-Based Amplification**

This is an isothermal, primer dependent, enzymatic amplification technique which, because it involves an RNA polymerase, is particularly suited to the detection of HIV RNA. The sensitivity of the method is similar to that of RT-PCR and a quantification method is now available. As with RT-PCR, the latter should be based on inclusion of internal controls *(van Gemen et al, 1994).*

(c) **Branch Chain DNA Assay**

This is a relatively new technique based on capturing HIV genomes onto a solid phase and detecting them with a branched DNA probe. Enzyme-labelled probes are then hybridised to the branched chain DNA and the signal measured by a chemiluminescent substrate. The advantages of the technique seems to be threefold: no extraction of nucleic acid is necessary, quantification based on the strength of signal is inbuilt, and the technique does not rely on an enzymatic amplification of the target nucleic acid. The last has been shown to improve the reproducibility of detection of HIV RNA and reduce the effect that natural inhibitors present in body fluids have on enzymatic amplification methods of detection *(Urdea et al, 1993).*

Diagnosis of HIV infection in the neonate

The early diagnosis of HIV infection in infants requires specialised investigations *(Table 9)* and it is therefore essential for paediatricians to liaise closely with laboratories which have the necessary expertise in order to ensure the appropriate specimens are collected and transported correctly, and that arrangements for follow-up specimens are made. Considerable experience is also required in the interpretation of results.

Maternal antibodies may persist for up to 18 months and therefore serological tests alone are of limited value for rapid diagnosis, although some infants who have escaped infection may exhibit declining levels of antibody over a period of a few months. Class specific antibody assays may, however, be of value as HIV infected infants may develop a virus specific IgA response during the first 3 months of life. If negative, retesting infants again at 3 to 6 months is advised. Some infants may also develop a virus specific IgM response although this is detected less reliably. However, as maternal IgA and IgM may cross the placenta *(Connell et al, 1992)* results must be interpreted with caution, especially in infants less than 3 months of age.

Isolation of virus from peripheral blood mononuclear cells or whole blood is the most reliable technique. However, it is labour intensive, expensive and requires specialised containment facilities. It may also take up to one month for results to be obtained. Detection of viral nucleic acid by PCR or NASBA is rapid and highly sensitive but requires considerable technical expertise and is prone to problems with contamination. Testing for p24 antigenaemia has been demonstrated to be useful in many cases, particularly if tests employing acid dissociation are used.

Although the US Center for Disease Control and Prevention requires only positive results on two separate determinations from one or more of PCR, HIV culture, or HIV (p24) antigen detection *(Caldwell et al, 1994)*, for added security, we recommend that a diagnosis of HIV in infancy resulting from intrauterine or perinatal transmission should only be made if two independent tests on a single sample are indicative of infection and a second sample is also positive.

If an infant remains well and, using the investigations described above there is no evidence of HIV infection by the age of 6 months, this suggests that the infant is uninfected. However, paediatricians should be advised to follow up the infant to ensure that seroreversion occurs. This may take up to 18 months. A second blood sample to confirm seroreversion is also advisable.

Staff need to be aware that the volume of serum required to complete all tests, including confirmatory assays, is often greater than can readily be obtained from babies.

Table 9
Diagnosis of paediatric HIV infection

Test	Comments
HIV antibody detection	Interference by maternal antibodies up to 18 months of age Tests at three monthly intervals from 6 months of age
HIV-specific IgA detection	Maternal IgA present in about 30% of children < 3 months If negative, repeat test at 3-6 months of age
p24 detection (with acid dissociation)	Sensitive and rapid Antigenaemia not always present Test at monthly intervals from birth
Virus isolation	Diagnostic, but: Labour intensive Results may take up to 28 days Specialised containment facilities required Test at monthly intervals from birth
Nucleic acid detection (RNA or DNA)	Highly sensitive and rapid Expertise required in conducting assay and interpreting results Test at monthly intervals from birth

Simple/rapid tests for the detection of anti-HIV

The development of simple/rapid tests for the detection of anti-HIV has been stimulated by the rapid spread of HIV infection and its high prevalence in regions where only limited laboratory facilities are available to screen the blood supply. In such areas the purchase and maintenance of the equipment required to perform EIA tests may be difficult and a simply performed, accurate test that gives a clear, visual end-point within 10-15m, and for which all the materials are supplied in kit form, is ideally suited.

Such simple/rapid tests should have the following characteristics: be easy to use; include all reagents and consumables; have a visually read end-point; provide a rapid result; and be of low cost. Tests currently available take several forms including: Rapid Test Devices or cartridges; dip-stick; or, latex agglutination.

Rapid Test Devices

Several rapid test devices are now available, most giving similar results to those of good EIAs but within 5-10 minutes. These assays are only suited to small scale testing (10-15 simultaneous tests) and tend to be expensive. Although the great majority of sera from HIV positive individuals give strong reactions a few give faint reactions which are difficult to evaluate and require further investigation.

Dip-stick tests

Only a few such tests are currently available which have a sensitivity approaching that of good EIAs. Up to 50 specimens may be processed simultaneously at a unit cost equivalent to that of EIAs. Assays are usually completed within 60 minutes. Problems with interpretation are similar to those for rapid test devices.

Latex agglutination test

One commercial test based on latex agglutination has recently been made available. This assay has a sensitivity similar to that of good EIAs and is used by some laboratories as a confirmatory assay *(see p 15)*.

Hazards of using such tests as "over the counter" tests

Problems associated with rapid tests being available "over the counter" include: lack of pre- and post- test counselling, particularly if the test proves reactive; subjectivity of the tests - especially with regard to weak reactions; lack of internal controls or confirmatory procedures; lack of quality control; low positive predictive value in low prevalence populations.

Same day testing

Introduction

The decision to operate a same-day testing service for HIV, and the organisation of that service, will be based on the locality and, to a large extent, the expectations of the patient population. This section is designed to identify some of the factors which should be considered, and some of the problems which might arise, when starting such a service.

The clinic

Local environment and population will be major considerations when siting the service. Examples of successful same day testing services currently operating in different hospitals are: from or adjacent to the virology department; from the GUM department; associated with specialist HIV clinics where such clinics are not GUM-based; or from independent sites. Decisions will have to be made about: whether the clinic will be walk in or appointment only; opening times (every day or specific days; latest time each day to ensure a same day result); and whether the same-day result will be negative only, reactive or confirmed positive. These last two points will require input from the testing laboratory.

Counselling

It is generally understood that counsellors should liaise closely with the laboratory performing the HIV tests and should be aware of the possible problems which may arise. This is especially important with same day testing as patients need to be made aware of such factors as: the status of the result (negative; reactive and requiring confirmation; or confirmed positive); the timings involved (latest time to ensure a same day result); and the potential lack of time to repeat tests should any technical problem arise.

Specimen transport

With time being of the essence it is essential that specimens arrive early at the laboratory to be checked, processed and tested, and results generated within the day. Secure and reliable transport arrangements are vital.

HIV testing

The testing laboratory will need to consider which test(s) to use (speed, accuracy, ease of use) and how confirmations of reactivity are to be performed (same or subsequent days; will a second blood sample be requested?). As with all serological assays it is essential to ensure that the primary sample is used (*ie* tests are performed on the patient's clotted sample). The laboratory will also have to consider whether same day samples are to be tested as a separate batch or together with other samples.

It should be noted that in some laboratories the routine HIV test employed can provide a result within 2-3 hours. In these locations the majority of results are "same day" but with the same constraints relating to confirmation of reactive sera.

Confidentiality and transmission of results

Of particular importance is the necessity for maintaining confidentiality while transmitting results rapidly. The identification of patients by numbers or soundex codes should be carefully considered. It is essential that counselling staff are informed as soon as possible of *(a)* any reactive sera and *(b)* any test failures likely to delay results.

Management

Experience in many locations has demonstrated the necessity for single identified individuals at requesting site and laboratory to deal with queries/problems.

The requirement to produce results both quickly and without the possibility of repeat testing should the test fail has been reported to cause stress in some laboratory staff. This should be monitored.

Recommendations

With such a wide variety of options there can be no detailed recommendations other than that the laboratory manager needs to be involved in all aspects of the design of the service.

Molecular epidemiology of HIV transmission

Introduction

Nucleotide sequence data have been employed recently in the investigation of several transmission events of HIV including the Florida dentist who infected six of his patients *(Ou et al, 1992; Albert et al, 1993; Holmes et al, 1993; Jaffe et al, 1994)*. Whilst the information from such data cannot on its own be construed as providing proof of transmission from one individual to another, it may indicate a link. Amplified proviral DNA is sequenced and analysed using statistical methods to determine the relatedness of the nucleic acid sequence *(Leigh-Brown and Simmonds, 1995)*. Three parameters should be considered when using these methods: the variability of the nucleotide sequence being analysed, the average level of relatedness among viral sequences from individuals who could reasonably have constituted an alternative source of the virus (*ie* the population of sequences amongst local controls, *Ou et al, 1992*) and the statistical analysis and interpretation of the data.

Sequence variability

The similarity (genetic distance) between two sequences depends on the time since they last shared a common ancestor (that is the number of replication cycles), and on the rate of evolution. The latter is different for different genes (such as *gag* and *env*), but is assumed to be fairly constant within a gene. Viruses of the same subtype from different patients may differ at about 10% of nucleotide sites in *env*; this figure may be as high as 20% if they are from different subtypes.

Both the C2-V3 regions of the *env*-gene *(Hills et al, 1994)* and the p17 coding region of the *gag*-gene *(Holmes et al, 1993; 1995)* have been successfully used in previous studies of transmission events. Where the analysis is concentrating on recent infections the more rapidly evolving *env*-gene will be more informative *(Hills et al, 1994)*. However, where the investigation takes place long after the transmission events, p17 is likely to be easier to analyse (Holmes et al, 1993).

Controls

In most investigations of putative transmission events it is necessary to establish an independent background comprising a reasonable number of unlinked patients *(Ou et al, 1992)*, unless a direct alternative source can be proposed, *(Rogers et al, 1993)*. The selection of individuals comprising the "local controls" should be carefully conducted, paying close attention to:

- geographical location, in case HIV strains are spatially clustered *(Holmes et al, 1995)*
- risk group, in case of strain differences between risk activities
- duration of infection, to allow for the evolution of the virus population during the infection

When the dataset has been assembled, the molecular data is examined to determine whether the individuals of interest form a cluster. If several individuals, identified by epidemiological investigation, are found to cluster, this is good evidence of a real association. If, however, there is only one patient involved, then the association with the source case has to be very strong to be considered significant.

Analysis and interpretation of data

The analysis of sequences in the dataset involves a phylogenetic approach. No one method can be considered optimal overall *(Leigh-Brown and Simmonds, 1995)*. Whichever is chosen, the statistical significance of the inferences relating to relationships among the sequences must be analysed.

In conclusion, the availability of molecular approaches for analysis of relationships among HIV sequences has added greatly to the power of investigations of transmission from health care workers. However, the complexity of the data generation and analysis necessitates the involvement of laboratories experienced in these methods.

Safety of Blood and Plasma Products

Introduction

The emergence of HIV in the 1980s, and the realisation that the virus was transmitted by blood and coagulation factor concentrates, provided a major driving force for the development of virus screening tests as well as virus-inactivation methods for use with plasma products. The inactivation methods that were developed also proved effective against other major plasma-transmitted viruses, *eg* hepatitis B and C.

In the UK plasma products are controlled by Medicines Control Agency who license each individual product as well as the manufacturing facilities. For plasma products, a batch release system operates with the National Institute for Biological Standards and Control as the control laboratory.

In England and Wales, plasma products are manufactured at BPL, Elstree, exclusively from plasma collected by the National Blood Service. In Scotland, plasma is collected by the Scottish National Blood Transfusion Service and fractionated at the Protein Fractionation Centre in Edinburgh.

In addition to the range of plasma products manufactured in the UK, others are available from "not for profit" fractionators using plasma from non-remunerated donors in Europe, or commercial fractionators who use plasma from remunerated donors in Europe and the USA.

Risk of acquiring HIV by transmission from blood or blood products

UK

Over a period of approximately ten years (September 1984 to August 1994) a cumulative total of 22,581 infections with HIV has been reported in England and Wales (PHLS, 1994). The proportion transmitted by blood or blood products is low (1380; 6.1%) and has shown a marked decrease following the institution of reliable screening procedures for antibody to HIV and heat treatment of blood products used, for example, in the management of haemophilia. The last two documented cases of HIV transmission associated with blood or blood products in the UK occurred in 1985 (England) and 1987 (Scotland). Up to 1994, 22 million blood donations had been screened, with a reassuringly low anti-HIV prevalence of 0.001% (1 in 100,000). In new (previously unscreened) donors the prevalence was higher at 0.004% (1 in 25,000). Newly detected HIV antibodies in a previously negative donor is indicative of seroconversion since the previous donation and the possibility that the donor was infectious, but anti-HIV negative, at the time of that donation (the window period, *see below*). Recipients of blood or blood products from those few donors in whom seroconversion is observed have been exhaustively followed up but, so far, only one case of a seronegative, yet infectious, "window period" donor has been observed.

Resource-poor countries

The testing of all blood for HIV-1 and HIV-2 prior to transfusion should be mandatory but, in resource-poor countries where HIV is highly prevalent, it is sometimes not universal. Under such circumstances blood transfusion is to be avoided, unless to save life. Because of the window period *(see below)* it is inevitable that, even with the best serology available, in zones where HIV is prevalent a small proportion of seronegative units will be HIV-infected. For example, in the largest city of the Ivory Coast, West Africa, the overall adult HIV prevalence is 11%. A detailed evaluation of the well-managed blood testing system showed that up to 1% of tested blood units might have been infected *(Savarit et al, 1992)*.

The Window Period

The HIV window period is defined as the period after infection during which an individual is infectious but where infection is undetectable by the tests used *(Figure 2)*. The risk of transmission associated with screened blood or blood

products is a function of the duration of this window and the incidence of seroconversion among the donor population.

Is there a requirement for window period testing?

The continuing improvements in HIV antibody tests have reduced the window period to an estimated 22 days *(Busch et al, 1995)*. As a result of the use of these third generation tests, in the US, the risk of HIV transmission associated with blood or blood products has dropped from 1:60,000 between 1985 and 1991 *(Donahue et al, 1990; Nelson et al, 1992)* to 1:420,000 *(Peterson, 1995)*. This translates to an estimated 50-60 transmissions per year. It has been estimated that the use of gene amplification technologies would reduce the window period to 11 days *(Busch et al, 1995)*. However manufacturers have estimated that such techniques will not be available for use as screening assays for some 3 to 5 years; furthermore the cost of introducing such assays into routine screening strategies would equate to some $10,000,000 per transmission prevented. In the UK, where the residual risk is much lower, the cost per transmission prevented would be much higher.

Possibly of more relevance are data which demonstrate that testing for p24 reduces the window period to 16 days *(Busch et al, 1995)* and reports from the US and elsewhere of p24 antigenaemia in HIV-negative blood and transmission from HIV-1 antibody negative/antigen positive donors *(Gilcher et al, 1990)*. However, again, the lower residual risk in the UK again calls into question the cost-effectiveness of such screening.

Although a case could be made for routine HIV-1 antigen screening in those parts of the world with high HIV prevalence or where HIV infection is spreading rapidly, many resource poor countries are finding it difficult to find resources for conventional HIV antibody testing let alone more sophisticated assays.

For low prevalence areas the following recommendations are appropriate:

- Self-exclusion of "at risk" donors
- HIV1/2 antibody screening of all donations by approved assay protocols
- Detailed follow-up of any evidence of seroconversions which may indicate infectivity of previous donations
- Routine use of HIV antigen (p24) and gene amplification assays not cost effective.

For areas of high or rapidly increasing prevalence the following recommendations are appropriate:

- Self-exclusion of "at risk" donors
- HIV1/2 antibody screening of all donations by approved assay protocols
- HIV-antigen screening *only when HIV antibody screening is universal.*

Screening

A number of factors contribute to the safety of both blood and plasma products. The high quality of blood donated by non-remunerated UK donors, donor selection and the testing of each donor for various viral markers, are the initial contributors to virus safety. Antibody to HIV-1 and HIV-2 is detected by highly sensitive third generation EIA tests. Repeatedly positive samples are sent to a reference laboratory for confirmation testing by Western-Blot, p24 antigen or PCR analysis as appropriate. As with any testing method the sensitivity of the procedure is influenced by the sample size, the existence of non-reactive strains or variant as well as the inherent properties of the test.

Products Derived from Plasma

The main products derived from plasma include the coagulation factors VIII and IX, and albumin immunoglobulin. Virus-inactivated plasma is being evaluated by some manufacturers and this should also be considered a derived product as it involves plasma pooling, treatment and manufacturing processes. The use of plasma pools of 10,000 or more donations increases the risks of the virus transmission. In addition to HIV screening on each donation, additional testing is also done on the plasma pool, process intermediates and final products. Currently, the use of more sensitive techniques, such as PCR, to detect HIV require further development, with issues such as accuracy, quantification, standardisation and automation needing to be addressed. In addition, PCR positivity does not necessarily equate with infectivity. Indeed laboratory studies have shown that a dry-heat treatment that can inactivated high levels of HIV infectivity has little effect on the PCR titre.

Coagulation Factors

Intermediate Purity Products

These products are prepared from plasma by various precipitation and adsorption steps. Although the manufacturing process itself may lead to some degree of reduction in the virus load, concentrates of this type were responsible for the transmission of HIV before screening tests were introduced and effective techniques for inactivating HIV had to be developed. Early attempts to heat-treat freeze dried coagulation factors resulted in considerable loss of product activity and temperatures below 70°C were not fully effective for preventing HIV transmission. However treatment at 80°C for 72h results in only a small loss (<10%) of clotting activity and no increased incidence of inhibitor development in recipients. One major advantage of using a terminal virus inactivation step on the product in its final container it is that there is no risk of product recontamination because there is no further processing. No cases of HIV transmission have been associated with the use of factor VIII inactivated in this way; similarly, such products have not been associated with hepatitis C transmission - a virus for which screening was only introduced in 1991. Laboratory studies have demonstrated inactivation of 5 logs HIV-1 in 4-8 hr at 80°C over a range of sugar, protein, and final moisture concentration. Virus inactivation by dry-heat treatment has also found to be

effective in factor IX production.

Pasteurisation, *ie* heat-treatment at 60° for 10 hr in the liquid state, may also be used in effect virus activation in coagulation products. Stabilisers, such as sodium citrate, are added to retain product activity, and removed after the virus inactivation step without any significant effect on virus-inactivation. With HIV-1, 5 logs were inactivated in 10 mins at 60°C. Terminal dry-heat treatment may also carried out.

Treatment with ß-propriolactone/UV has also been used in the past for the viral inactivation of human plasma and one manufacturer has used this to prepare factor IX. However, a single batch of this concentrate was found to transmit HIV. In laboratory studies the inactivation of HIV (>4-6 logs), as well as HBV and HCV, has been satisfactory. Also the product had been safe in chimpanzee trials and clinical use. Thus a technical error in carrying out the procedure may be the most likely explanation for HIV transmission.

High Purity Products

Coagulation factor concentrates of high purity incorporates an in-process virus inactivation step. The most widely used method is the solvent/detergent procedure using Tri-n-butyl phosphate and a non-ionic detergent such as Triton X 100 or Tween -80. This method is currently used for about 70% of all plasma products and has an unblemished safety record for non-enveloped viruses such as HIV. The method has no adverse effect on the product, although the chemicals must be removed after treatment. Other virus inactivation methods such as pasteurisation or moist heat-treatment are also used. Dry heat-treatment and virus-removing filters are being evaluated as additional virus-reducing steps in a effort to control more resistant non-enveloped viruses such as Hepatitis A and human parvovirus B19. Such procedures will also further increase the safety of such products with regards to HIV, since the virus is comparatively large and heat-sensitive. However the possible of neoantigens in highly purified products by severe heat treatment needs to be evaluated.

Laboratory studies have shown that the solvent/detergent step is able to inactivate up to >5 logs of HIV-1 or -2 in the first few minutes of treatment.

Subsequent purification steps involving chromatographic processes can also contribute to the virus safety of the product, particularly when affinity methods are used. For example, the use of a monoclonal antibody column to bind factor VIII followed by extensive washing reduces the level of HIV-1 by 4 logs. The use of a copper chelate column factor IX, although evaluated with HIV, has been shown to reduce the levels of other enveloped viruses such as Sindbis and vaccinia by 5-7 logs.

Albumin

Albumin is prepared from plasma by cold-ethanol fractionation, a process which is likely to reduce the virus load in the product. Pasteurisation can be carried out on both the bulk product (60°C for 10hr) and on the final product after bottling (to prevent any risk of recontamination). Under these conditions HIV is rapidly inactivated within 30m. Albumin prepared in this way was introduced in the 1940s and has an excellent virus safety record. There are no reported cases of HIV transmission.

Immunoglobulins

Intramuscular

Immunoglobulins are prepared from plasma by cold-ethanol fractionation. The reduction of HIV during the process has been estimated at >14 logs, largely due to partitioning of virus into the precipitate at each fractionation step. However, although there are no reports associating virus transmission with the use of intramuscular immunoglobulins, several reports attributed the transmission of hepatitis C to the use of intravenous immunoglobulins. These reports suggested that the conditions of cold-ethanol fractionation, by themselves, are not sufficient to render immunoglobulins completely safe from virus transmission. It has been conjectured that the viral safety of intramuscular immunoglobulin in comparison to intravenous immunoglobulin may be due to the route of administration, the smaller dose size, freeze drying in the presence of ethanol, or its storage in the liquid state prior to use.

Intravenous

Further processing is required to make an immunoglobulin product suitable for intravenous use. This is simply accomplished in some products by treating the intramuscular product with pepsin at pH4 for extended periods of time. This treatment also inactivates some viruses, including HIV, and such products have a good virus safety record in clinical use. Alternative approaches for the production of intravenous immunoglobulin involve chromatographic processes.

Although intravenous immunoglobulin products have been safe with regard to HIV transmission, there have been examples where high rates of hepatitis C virus transmission have occurred. The reasons for this are not clear, but may involve high virus load in the start plasma, variation in the fractionation conditions, or failure in good manufacturing practice. For instance, the segregation of material after virus inactivation from that which had not been treated, may not have been adequate. Because of these concerns, there has been increasing pressure for the incorporation of a specific inactivation step in such products and for the adoption of improved methods of segregating product after an in-process virus reduction step. For example, a solvent/detergent step using Tween-80 can inactivate >5 logs of HIV-1 within the first 30 minutes of a six hour process. In addition, the CM Sepharose ion-exchange chromatographic step can reduce the level of enveloped viruses such as Sindbis and HSV by 4 or 6 logs respectively. It is expected that these steps will contribute further to the safety of the product with regard to other enveloped viruses such as HIV and hepatitis C virus.

In conclusion, the safety of blood components and plasma products has greatly increased over the last 10 years with the introduction of virus screening and virus reduction methods. Residual risk estimates are in the order of 1 in $3.5x10^5$ per unit of blood and <1 in 10^6 per unit of solvent/detergent treated factor VIII. However, there has been pressure to introduce additional specific virus inactivation steps for plasma products. This has been in response to rare incidents of hepatitis B and C transmission and also the inability of current manufacturing processes to deal with the small non-enveloped and resistant viruses such as hepatitis A and human parvovirus B19. Laboratory virus reduction studies, the adequate qualification and validation of virus reduction steps used in manufacturing, in addition to quality control and good manufacturing practices, are essential for ensuring the success of the virus reduction procedure. Currently, many new techniques such as photochemical inactivation, iodine based chemicals, UV-C, virus filtration, and phase conversion induced by high pressure, are being evaluated as practical virus inactivation methods. Recognising the properties of HIV, such processes are expected to contribute further to the safety of plasma products with respect to this virus.

HIV Transmission and Organ Donation

Organs and tissues transplanted

These include kidneys, heart, lung, liver, spleen, pancreas, islets of Langerhans, bone marrow, cornea, and bone. A full list is included in Department of Health guidelines *(Department of Health, 1990).*

Screening of recipient

In cases where the recipient will be iatrogenically immunosuppressed after organ transplant it is recommended that the recipient should be tested for anti-HIV antibodies prior to transplant because, if the test proves positive, the further immunosuppression after transplantation may significantly shorten the life of that person. It is not considered necessary to screen recipients routinely for HIV antibodies after transplant. However, if this is undertaken, it should be noted that, whereas the antibody response is not significantly delayed in solid organ transplant recipients, it will certainly be greatly delayed in recipients of allogenic bone marrow transplants.

Screening of donors prior to acceptance

Specific guidelines are available from the DoH *(Department of Health, 1990).* Explicit consent from the donor should be obtained before any living donor is tested for HIV *(see p 15)*, and persons who have engaged in high risk behaviour and/or show signs and symptoms usually associated with HIV infection should be excluded from donation. There is no requirement for consent from next of kin after death, although, once the corpse is in the possession of the executors testing without their consent will not be possible *(MDU, 1990).*

Donor testing

For all prospective donors, a blood sample should be collected as close to the time of removal of tissue as possible. Bone marrow donors must provide samples long enough before marrow harvest to permit the test to be performed and results reported before the recipient commences the conditioning regimen for marrow ablation. Tissues from living donors which may be stored prior to use should not be stored with other tissues and should not be transplanted until a second negative test at least 90 days later is obtained *(see also sperm donation p 23).*

Blood transfusions and infusion of other fluids to the prospective donor might produce false negative results because of haemodilution. If possible, a specimen should be taken before such transfusion/infusion. Certainly blood should not be taken immediately downstream of an intravenous cannulation site because of the risk of dilution.

An exchange of one total blood volume following transfusion will reduce the intravascular concentration to 35% of initial levels if there is no replacement from the extravascular space. More than 50% of total body IgG is extravascular, and re-equilibration to normal levels of IgG should be nearly complete within 24 hours of a total blood volume exchange of albumin.

If the time available is short, *eg* organ from brain dead or cadaveric donor required for lifesaving organ transplantation, then the assay should be performed in at least duplicate. If the sample gives a reactive or indeterminate result then the organ should not be used. If there is more time available, *eg* live donor or corneal donor, then false positive reactions may be excluded by repeat testing and referral to a reference laboratory. In the meantime, the advice given should be not to use the organ until testing is completed.

The use of HIV p24 antigen assay is not recommended for exclusion of the rare donor who may give a positive test for antigen but a negative test for antibody since the population of donors by definition has a low prevalence of infection and thus the greater proportion of reactive results will be false positives. Heavy reliance must therefore be placed on donor exclusion criteria.

For both medico-legal reasons and research purposes, all sera from organ donors and recipients should be stored at -20^0C for as long as is practically possible; certainly for a minimum of two years.

Finally, the responsibility for obtaining patient consent to an HIV test rests with the transplant co-ordinator and the referring clinician and not the laboratory carrying out the test *(see also p 15).*

HIV transmission and assisted conception

Treatment of couples using gamete donation

Limitation of risks of transmission of any infection from donor to recipient is a major concern in overcoming infertility using donor gametes. Very specific guidelines have been issued by the RCOG, the American Fertility Society and endorsed by the code of practice issued by the Human Fertilisation and Embryology Authority (HFEA) in this country *(British Andrology Society, 1993; RCOG, 1992).*

Sperm donation

Donor insemination (DI: formerly AID, Artificial Insemination using Donor) has been an important, if somewhat controversial, technique for circumventing infertility in those couples where the male partner has been found to be severely oligozoospermic or azoospermic. A careful history is an important part of the screening of men wishing to be sperm donors. As with blood transfusion, careful attention is paid to a sexual history, recent travel to or sexual contact with persons living in those parts of the world where HIV is endemic, a history of blood transfusions and of intravenous drug use. After suitable counselling, all prospective donors are required to have an HIV test, in addition to screens for other STDs. In addition, in order to prevent transmission of infection, it is mandatory that sperm is quarantined by cryopreservation for a period before use. The exact period varies between countries but is set at a minimum of 3 months (6 months being preferable) with sperm only being released for use following a further negative HIV test on the donor after this time of quarantine. This rigorous programme deters many donors, follow up is difficult, and thus donor semen is in short supply nationally. The use of fresh donated semen samples for treatment is now banned in this country, and all donor insemination clinics have to be licensed and inspected by the HFEA. *(For storage of semen samples see p 24)*

Ovum donation

Ovum donation presents a particular problem as the equivalent technology for cryopreserving unfertilised oocytes has yet to be developed successfully. Fertilised oocytes (pronucleate stage-day 1 after insemination in vitro) can be frozen successfully as can 4-8cell cleavage stage embryos (day 2 and 3). However, pregnancy success rates using frozen embryos are lower than using fresh embryos. Although a similar screening protocol as that used for cryopreserved donor sperm would almost eliminate any likelihood of transmission, the physical rigours that a woman must to go through to be an egg donor (injectable ovarian hormone stimulation, serum and ultrasound monitoring, and transvaginal or laparoscopic egg retrieval) is likely to deter all those except the most committed, most of whom are in stable heterosexual relationships usually with their own children, and embark on egg donation for altruistic reasons. Thus they are a low risk population and the likelihood of HIV seroconversion following a negative HIV test is probably exceedingly small in this group. Currently fresh oocyte donation is condoned and permitted by the HFEA.

Treatment of couples using their own gametes

Intrauterine insemination

Intrauterine insemination using husband's or partner's sperm (AIH or IUI), is a treatment increasingly used in unexplained infertility where the female partner's fallopian tubes are patent and where there is no or only a minimal defect thought to be present in the semen. It is usually combined with ovulation induction to facilitate timing of insemination and to increase the likelihood of successful fertilisation of at least one oocyte. The semen is usually concentrated and enhanced for motile sperm by washing in an appropriate medium, or by separation of a Percoll gradient. A reduced volume (around 0.2 ml) is inserted into the uterine cavity using a specially designed transcervical catheter.

Screening for HIV is not mandatory, but in the interests of the child may be offered should the couple wish or where it is felt that the couple may be at risk.

IUI in couples where the male partner is known to be HIV positive

Many HIV-discordant couples in which the woman is HIV-negative wish to have children notwithstanding the risks associated with unprotected intercourse, estimated to be about 1:100 episodes of intercourse. Although some reduction in risk may be made by decreasing the frequency of unprotected intercourse by monitoring ovulation and timing intercourse, there has been recent interest in the use of artificial insemination (IUI) with semen processed to remove infectious virus. There are contradictory data on the distribution of HIV in semen and, more specifically, whether HIV is present in, or attached to, spermatozoa. Although HIV contaminated sperm were reduced during the Percoll separation to under 5%, and were said to be undetectable after a "swim-up" procedure, there is still doubt about the sensitivity of the assay used. Nevertheless, no seroconversion was seen in the 28 couples who participated in the study *(Semprini et al, 1992).* Further investigation is required before this type of technique is declared safe.

In vitro fertilisation (IVF)

The replacement of embryos into the host woman following fertilisation of her oocytes in the laboratory with spermatozoa prepared from her partners semen, has become an important part of the treatment spectrum for the alleviation of subfertility. This, and allied techniques are regulated strictly by the HFEA in terms of the Human Fertilisation and Embryology Act (1990). There is no requirement in the HFEA that all patients embarking on IVF are screened routinely for HIV in IVF units, the policy generally being decided upon by the individual licensed units. However, whilst some units will offer screening only for those patients who are felt to be in at risk group, in order to help the couple in their decision making, some units make it a condition of acceptance, either for the protection of the laboratory staff, or in the interests of "the welfare the child" in accordance with the Act, and will refuse treatment to HIV

affected couples. Processing of sperm (as above) may reduce transmission in discordant couples, although for the same reasons this therapy should not be seen as a means of protection.

Prevention of viral contamination during cryopreservation

Recent cases of hepatitis B infection resulting from contamination of cryo-preserved bone marrow and peripheral blood stem cells *(Tedder et al, 1995)* have demonstrated the need for security of cryo-preserved samples from viral contamination.

In principle, human material should be stored in the gaseous phase of cryo-preservation tanks, secondarily enclosed to prevent access of liquid nitrogen. One suggested method is storage in screw-top "Nunc" cryovials secondarily sealed in cryoflex tubing. However, if a system is used which guarantees storage in the gaseous phase only, there would be no requirement for a secondary seal.

Donors should be screened for any required virus markers prior to storage in the same vessel as other samples.

If a donor is found to have been infected at the time of a donation, should material from that donor have been stored with other samples, and should storage conditions not have been microbiologically secure, the potential risk of contamination must be considered. If the destruction of potentially infected samples is not possible, the hazards presented by the use of such materials must be discussed with the relevant patients.

It should be noted that the current practice of using semen quills is microbiologically hazardous.

Audit

Audit is recognised as an fundamental component of maintaining and improving standards. Procedures may be audited within the laboratory as well as at the clinical interface. A multidisciplinary approach should be encouraged. Although local issues may be audited, some aspects of HIV infection are appropriate for regional, or even national, audit.

Audit is directed towards achieving and maintaining high standards of laboratory practice as well as high levels of patient care. An essential part of audit is to ensure that established standards are met or, if such standards are unavailable, are set as a result of the exercise. Audit encourages communication and discussion between clinicians and laboratory personnel thereby ensuring that the various specialties make optimum use of laboratories and are satisfied with the service they receive.

The following topics may be considered:

Autopsies

In HIV infection, because of the multiplicity of opportunistic diseases, the discrepancies between pre-mortem and post-mortem diagnoses tend to be marked. For example, undiagnosed conditions were noted in 47% and 74% of cadavers in two large series *(Wilkes, et al, 1988; d'Arminio Monforte et al, 1992)*. In addition, infections of public health importance, such as previously undiagnosed pulmonary tuberculosis and histoplasmosis, may be discovered at autopsy among HIV-infected patients. Autopsies of HIV-positive patients continue to provide both individual case clinico-pathological feedback and accumulated data that influence strategies of patient management.

Occupational exposure to HIV and other blood-borne diseases

A number of departments should be involved including such departments as Occupational Health, Accident and Emergency, GUM, Operating Theatres, and Obstetrics and Gynaecology, as well as Pathology departments. The reporting of incidents and their causal factors should be identified and attempts made to ensure that they are managed appropriately. The results should be used to educate health care workers in order to ensure that high standards of infection control are practised, thereby minimising occupational exposure to HIV and other blood-borne viruses.

Significant occupational exposures to HIV, and their outcome, should be reported to the national, voluntary, confidential surveillance system at the PHLS, (CDSC), as should documented occupationally acquired hepatitis B and hepatitis C infection.

Efficiency of screening programmes (including same day testing)

Audit could involve antenatal clinics, drug dependency units, and GUM, examining, and, if possible, trying to improve uptake rates on anonymised and named patient testing.

Laboratory procedures

Examination of safety within the laboratory: collection, delivery and handling of samples; turn-round times; and screening and confirmatory assays. Internal and external quality assurance and methods of reporting should also be examined.

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