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BIOSAFETY IN *IN VIVO* AND *IN VITRO* STUDIES OF HUMAN MALARIA

by

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1. INTRODUCTION

The potential dangers concomitant with the handling of human blood are well documented. The increased risk of direct contact with human blood, stemming from the implementation of general research studies in malaria, including the use of in vivo and in vitro test systems for the determination of the sensitivity of human *Plasmodium* spp. to antimalarials, makes it imperative that attention is drawn to these dangers and that appropriate action is always taken to ensure the potential for infection is eliminated as far as it is humanly possible to do so.

Foremost among these concerns is the consideration that every effort must be made to avoid accidental infection of the persons from whom blood samples are taken for malaria studies.

This revision of the original MAP document on biosafety has been stimulated by the increased awareness of the global problem of AIDS which is caused by the human immunodeficiency virus (HIV). The original document, when written in 1983, certainly had in mind the possibility of accidental HIV infection as a potential risk, but, of course, at that time the aetiology of the disease was not well understood so the problem was included, without specific mention, in the general section on viral infections. However, with the information now available about HIV, it has become apparent that the general sterilization techniques given in the original publication are certainly effective against HIV and so any worker who followed those recommendations for handling material potentially infective for viruses has adequately protected himself/herself, and the subject from whom the blood sample was taken, against infection with HIV.

2. THE PROBLEM

Despite all the publicity given to HIV, the most commonly recorded blood-related infection to which laboratory staff working on human malaria are exposed remains that caused by the hepatitis viruses. Studies made in the United States(1) indicate that between 500 - 600 health workers, who handle blood in the course of their duties, are hospitalized every year with hepatitis B (HBV). By comparison, for the whole period up of recorded HIV cases up to September 1988, less than a third of that number had thus far been found positive for HIV with a history which indicated that their infection *might* have been due to accidental exposure to the virus in the course of their duties.

There is, moreover, albeit much more remotely, the risk of infection with other viruses, including the exotic Lassa and Ebola, as well as other pathogenic organisms (e.g. *Mycobacterium tuberculosis* and *Plasmodium* spp.): a danger which is often significantly enhanced in the less controllable field setting.

It is now widely acknowledged (1,2) that by far the most likely means by which the malaria health worker in the field, or the laboratory setting, is liable to be infected by HIV, HBV or other human pathogens found in the blood, is by contamination of the hands and mucous membranes of the eye, nose and mouth with blood from a person infected with these diseases. The most usual way that this happens is when a sharp object, such as a hypodermic syringe needle, or a blood lancet, infected with the blood of a person with the disease, accidentally pierces the skin of the malaria health worker, or his/her skin or mucous membranes is exposed to direct contact with the blood of the infected person. Of course, it is imperative that the malaria health worker also clearly understands that he/she can also infect a third person, i.e. if a needle or lancet used on an infected person is then RE-USED on a healthy person, or the skin or mucous membranes of the healthy person are contaminated with the blood of this infected person. Figures currently available (1,2) indicate the actual risk from a single exposure with an infected sharp instrument to blood infected with hepatitis B is between 6 and 30%, whilst that for HIV inoculation is about 0.5%, i.e. 45 to 120 times less.

Evidently then, whilst the risk of infection of malaria workers, malaria patients and healthy persons with HIV and other diseases of the blood is low in routine malaria field and laboratory activities, the results of infection can be very serious indeed and must not be underrated (1,2).

3. SOURCES OF INFECTION

While it has been estimated that the sources of 80% of laboratory-acquired infections are unknown, the principal potential sources of hazard in the study of malaria in humans are probably the following: poor personal hygiene; accidents; aerosols; mishandling of pipettes and syringes; inoculation with instruments contaminated by the blood of other persons; fomites; and waste material.

(a) Poor personal hygiene

Undoubtedly the most common route of infection is the "hands to mouth route" but infection may also occur through inhalation, the mucous membranes and superficial injuries to the skin. In particular, the ingestion of food and drink and smoking in the laboratory are common sources of infection.

(b) Accidents

Typically, these are auto-inoculations through contaminated hypodermic needles and blood lancets, broken glassware and fractured centrifuge tubes, particularly haematocrit tubes (see below); residues from inadequate decontamination procedures after spillage of pathogenic material; and wearing of contaminated clothing.

(c) Aerosols

Most liquids when violently agitated, produce almost invisible clouds of minute droplets called aerosols. Improperly operated mixers and centrifuges may throw out an aerosol to a radius of two or three metres and subsequently, by diffusion, totally contaminate a whole room.

Even pipettes or syringes can produce quite small and far-propelled particles which can be inhaled or which impinge on mucous membranes. Working surfaces can be contaminated in this way and serve as fomites.

(d) Mishandling of syringes or pipettes

The simple procedure of the finger prick with a lancet can effectively transmit pathogens from an infected to a health person, unless sterile procedures are followed. Syringes and needles are also a notorious route of infection. Careless handling of drinking water vessels can similarly set up a chain of infection.

Syringes may produce microparticles as described in (c) by over-vigorous expulsion of the contents through the needle, as a result of leakages in the contact points between the syringe tip and the needle, or by the clearing of a blocked needle. Mouth pipetting is a prime source of infection and even pipettes which contain cotton plugs to prevent inadvertent swallowing of the pipetted fluids may not be fully efficacious when wet. Moreover, hands may often contaminate the pipette mouthpiece during the pipetting process. Blowing out of pipettes may produce violently moving particles or aerosols.

The simple procedure of the finger prick can effectively transmit pathogens from an infected to a healthy person, unless sterile procedures are followed. Blood lancets, syringes and needles are also a notorious route of infection. Careless handling of drinking- water vessels can similarly set up a chain of infection.

(e) Fomites

These are any surfaces or materials which can be contaminated and pass on a pathogen through contact.

(f) Waste material

Discarded materials of all kinds can be highly hazardous in the hands of the uninformed. Disposable glass- and plastic-ware from the in vitro test kits are particularly dangerous as these are frequently recovered and re-used without proper resterilization.

(g) Transportation of potentially infective samples

Research protocols for malaria research studies increasingly include a requirement for the taking of additional samples of blood, urine, plasma or serum for sophisticated test procedures which cannot be conducted in the field setting. Therefore, these samples must be safely transported to the collaborating laboratory where these additional studies are to be carried out. Frequently these laboratories are located far from the field location, or in a second country, necessitating transportation by air. During the process of transportation these shipments of samples frequently pass through the normal communications network and, potentially, through the hands of persons who are not informed of the potential dangers involved.

There is, therefore, an over-riding obligation on all concerned to ensure that any potentially infective material is properly collected, packaged, labelled and only despatched through the appropriate mode of transportation. These procedures should be based on 'the worst case' contingency; that is assuming that the shipment could be damaged or delayed in transit and ensuring that additional protective measures are employed to eliminate, as far as is possible, any dangerous situation which might develop as a result of these events.

If the desired degree of safety during transportation cannot be assured, special arrangements may have to be made for the samples to be hand carried by a suitably informed person who will be responsible for the safe transportation and delivery of the samples to a responsible person in the collaborating institution.

4. PRECAUTIONARY MEASURES

4.1 Personal hygiene

To break the chain of infection from hands to mouth requires the establishment of a conscious and continuous self-discipline and the total avoidance of eating, drinking and smoking within the work area.

Gloves should be worn whenever potential infection is possible and the hands washed thoroughly with soap and water before donning and after removing the gloves. The use of abrasive cleansing material and harsh chemicals should be avoided as these may well exacerbate the problem by causing, or aggravating, superficial injuries to the skin which then more readily permit the access of pathogens.

Whenever possible, appropriate clothing should be worn; outside clothes should always be protected by the use of laboratory coats.

The disagreeable sensation arising from the long-term wearing of rubber surgical gloves in tropical climates can be greatly alleviated by first donning thin cotton gloves before putting on the rubber gloves.

All wounds should be liberally washed with soap and water immediately. Bleeding should be encouraged to flush out the wound. All such injuries should be reported immediately to the supervisor and professional advice and further possible treatment sought.

4.2 Accidents

It is almost inevitable that from time to time accidents will occur. The dangers arising from such incidents can be greatly minimized with a little prior planning and action: limitation of the movement of people and material within the work area as far as possible; ensuring that all the stipulated safety practices are strictly followed and that suitable materials are available to neutralize any spillage or dissemination of hazardous material; reporting of all accidents and ensuring that medical advice is sought when this is indicated; practising all procedures with "dry runs" before the procedure is adopted as standard technique; and, if an existing procedure requires the use of hazardous materials, trying to develop safer methodologies.

Spillage should be cleaned up using paper towels saturated with a suitable chemical sterilant, e.g. sodium hypochlorite, with 1% available chlorine (see para. 5.5.2). Gloves MUST be worn and sharp-edged material should not be handled directly.

4.3 Handling of dispoable lancets, syringes and pipettes

The use of one-time disposable lancets, syringes/needles and pipettes greatly reduces the possibility of contamination. Wherever feasible, needles should be of the screw-lock type to eliminate any leakage between the tip of the syringe. The clearing of blocked needles presents a particular danger in the production of leakages and aerosols.

Unshielded needles and carelessly discarded lancets are the most common cause of accidental contamination and should be handled with extreme care. Used syringes should have their needles recovered with the plastic shield provided and placed in durable protective containers until disposal or resterilization. Lancets should be discarded into sealed containers or put into a suitable vessel containing a chemical sterilant (see 5.5). Pipettes should have cotton plugs in the neck to reduce the possibility of contamination and mouth pipetting should be strictly prohibited and an appropriate pipetting aid provided. Pipettes of the "to deliver" type are to be preferred as these eliminate the need for "blow out" with its attendant serious potential for the production of air-borne droplets and aerosols. For the same reasons, pipettes should be emptied down the side of the container or below the surface of the liquid.

4.4 Avoidance of cross-contamination

Instruments that have been used to withdraw human blood are potentially contaminated and must not be used on a second person unless properly sterilized. Classic examples of this are the repeated use of lancets for drawing finger-prick samples and injections/withdrawals with needles and syringes that have not been properly sterilized.

4.5 Fomites

Any surface that can support contaminated material will serve as a fomite. An absorptive surface is not as efficient a fomite as non-absorptive material but the former is usually more difficult to sterilize.

All work surfaces should be neutralized with a suitable sterilant before and after handling of potential biohazardous material and particular care should be taken with reusable materials such as dusters, towels, laboratory protective clothing.

If particularly hazardous exposures are envisaged, the advice of a specialist on biosafety procedure should be sought before work begins or is permitted to be restarted after an incident has occurred.

4.6 Waste material

By far the greatest danger arising from the disposal of potentially infected material is the ever present possibility of its being recycled or falling into the hands of the uninformed general public and, in particular, children for whom this material seemed to have an almost magical attraction.

Of course, in the ideal world all disposable material such as lancets, syringes, needles, cloth, plastic plates and measuring equipment should be used only once and then disposed of in a safe manner. In the real world of chronic shortages and high cost which exists in some developing countries, such 'wastefulness' is deplored and considerable efforts may be exerted to thwart any methods of routine safe disposal by destruction or sterilization. So, whilst every effort should be made at safe disposal, when conditions are such that this is not likely to be reliable the alternative approach of effective sterilization should be instituted. In either event the aim is complete and ensured safety.

4.7 Transportation of potentially infective samples

The recommended procedures for the transportation of dangerous materials are set out in various regulations at the national and international level and all those responsible for the transportation of potentially infective material should obtain copies of the current relevant documents from agencies of: the International Air Transport Association (through the national airline); the Universal Postal Union (through the national Post Office) and the World Health Organization (through the office of the country WHO Representative).

A summary of these regulations, in so far as they concern the transmission of material potentially infective for HIV, are given in Section 6, Guidelines for the Handling, Transfer and Shipment of Specimens in Global Programme of Aids, cited previously(2).

However, to provide a background to this problem, below are summarized the salient points which are relevant to all blood samples taken for malaria research purposes:

- Containers for the collection of samples should be leak-proof and break-resistant with a seal that can be verified as tight: accordingly screw cap closures are preferable to the snap closure type.

- If it is planned to store the sample at sub-zero temperatures the containers should be of the cryopreservation type since glass may fracture, due to expansion of the contents upon freezing, and most kinds of plastic become brittle at low temperatures.

- After closing and sealing the container the outside should be carefully wiped with a strong sterilant such as a 1,000 ppm chlorine solution and then wiped dry.

- Details of the contents of the sample vials should be written directly onto the container with a permanent pen: labels tend to become brittle and glues lose their adhesive power at low temperatures.

- Excess moisture will have similar deleterious effects. A detailed list of the contents of the shipment should be sent under separate cover to the recipient.

- The sample containers should be wrapped, or packed, with sufficient absorptive material (cotton wool, paper tissue etc), to ensure that if the containers leak all the contents will be absorbed into this packing, and finally packed into suitable transportation carriers.

- Conditions where secondary containers and special packing are stipulated in the various regulations mentioned previously should be carefully observed and all other pertinent national and international requirements strictly adhered to.

- The recipient should be informed in advance of the shipment so that appropriate reception arrangements are made in good time. This will eliminate the possibility that the shipment inadvertently fall into uninformed hands or be otherwise incorrectly handled.

- Upon delivery the recipient should ensure that the material is handled with the appropriate biosafety precautions and that spilt, or otherwise contaminated material, is disposed of in a safe manner.

5. STERILIZATION PROCEDURES

Heat is the most effective method of inactivating HIV and HBV (and most other pathogens found in the blood), and therefore, sterilization based on heat is the method of choice (2).

Chemical sterilization is also generally effective, but is less reliable than heat unless very strictly controlled (2).

With the above information as guidance, the problem for laboratory worker, and, perhaps even more importantly the field worker, is to find a method which is both efficient and practical for the conditions under which he/she routinely has to work. Since penetration of the sterilant is essential for efficacy, all items should be so placed to ensure proper penetration: syringes should be dismantled, liquid sterilants should completely cover the objects to be sterilized and cloth should be loosely packed. Below are listed some of the more feasible options with comment as to applicability and efficacy.

5.1 Autoclaving

When autoclaving is possible (and it should be remembered that in inexperienced hands the autoclave is a very dangerous instrument indeed with the explosive potential of quite a large bomb), almost all kinds of laboratory material can be sterilized (decontaminated), at 121 degrees Celsius (250 degrees Farenheit) with 115KN/m2 gauge pressure (15lb/in2) for 30 to 60 minutes in a gravity displacement autoclave. Or, alternatively, at 132 degrees Celsius (270 degrees Farenheit) with 207KN/m2 gauge pressure (27 lb/in2) for 4 to 10 minutes in a pre-vacuum (high temperature) autoclave. The instructions for the autoclave should be carefully consulted to determine the correct procedures for the various material to be sterilized with due care taken to exclude non-autoclavable items.

Whilst it is unlikely that an autoclaving facilities will be available at the most peripheral levels of the health service, and certainly, not at all in the real field setting, with careful prior planning arrangements can usually be made to use the autoclaving service of small peripheral hospitals and clinics where these are available particularly for the decontamination of sharp instruments such as lancets. However, it should be realized that autoclaving does not CLEAN an object but only sterilizes it and whatever may be contaminating it. If it is proposed to reuse material it should be first thoroughly cleaned using soap and hot water and then sterilized remembering to take all the necessary precautions to prevent accidents during the cleaning process. (Also see Chemical Sterilization).

5.2 Boiling

The efficacy of boiling is often overlooked, or under rated, as a routine sterilization technique.

Providing that: the water really boils; all the objects to be sterilized have been dissembled and are fully immersed, and the boiling is continued for a full twenty minutes, then almost all pathogenic organisms found in the blood are safely sterilized.

In view of its simplicity and applicability to almost all situations boiling is the method of choice for field work.

5.3 Dry Heat

Dry heat is an efficacious sterilant under ideal conditions but it is difficult to ensure uniform penetration. Because of the high temperatures required (170 degrees Celsius, 340 degrees Farenheit) most plastic ware will be destroyed. The minimum period for adequate sterilization with dry heat is two hours. An ordinary household type oven, if properly calibrated for the operational temperature of 170 degrees Celsius, can provide a satisfactory alternative to the laboratory type oven.

5.4 Incineration

When proper facilities for complete and properly supervised incineration is available (e.g. the fully enclosed medical incinerators found in hospitals or dispensaries) then incineration ensures complete destruction of both contaminated material and the pathogens which may infect it: even syringe needles are rendered completely useless in this way. However, if incineration is incomplete, or incompetently carried out, the danger of a sense of false security is very real. Apart from the obvious danger of inadequately sterilized material being scattered about, the incomplete combustion of the infected material can produce infective smoke or aerosols.

5.5 Chemical germicides

Whilst chemical sterilization is a very convenient way of neutralizing potentially contaminated material as an intermediatory step to complete sterilization by heat it is not, by itself, as routinely efficient as sterilization by heat. But because of its ready adaptability to field work and emergency action, it is a useful sterilization method in the armamentarium of the laboratory and field worker when dealing with potentially contaminated material.

Unfortunately, the most effective chemical sterilants are not very stable and are subject to rapid deterioration if improperly stored.

The more commonly used, and most readily available, chemical sterilants are:

5.5.1 Alcohol

Ethanol (ethyl alcohol) and 2-propanol (isopropyl alcohol) are relatively cheap and effective against bacteria (but not spores) mycobacteria, fungi and viruses after a few minutes exposure. So they are ideal for sterilizing the skin of patients before taking blood samples and for cleaning, by wiping, surfaces that may have been contaminated.

Strange though it may seem, they are more biologically active at 70% v/v (with water) than at full strength.

Alcohol is not recommended for the sterilization of material which has been heavily contaminated and should never be used with material, such as syringes, needles and lancets, if these are to be reused.

5.5.2 Chlorine-releasing compounds

Sodium hypochlorite solutions (more commonly known as liquid household bleach, Chlorox, eau de Javel etc.) are excellent disinfectants and are usually inexpensive and widely available in public retail outlets such as supermarkets and pharmacies.

Unfortunately, they are corrosive to some metals and deteriorate rapidly in storage particularly when exposed to heat and light. The amount of chlorine in these preparations varies from country to country and manufacturer to manufacturer so the labelling should be carefully studied.

All these chloroquine-releasing compounds are gauged in efficiency in terms of the available (free) chlorine which is released by the concentrate.

For solid compounds this is expressed as the percentage of available chlorine and for liquids parts per million (ppm) of available chlorine. For example:

- Household liquid bleach usually contains 50,000 ppm; eau de Javel the same, but extrait de Javel 150,000 ppm. The solid calcium hypochlorite contains 70%, chlorinated lime 35%; sodium dichloroisocyanurate 60% and chloramine 25%. The RECOMMENDED EFFECTIVE CONCENTRATION of available chlorine for both these forms is 0.1%/1,000 ppm for general use in cleaning work surfaces, gloves, etc. and 1.0%/10,000 ppm for disinfecting used material such as swabs, lancets, syringes, etc. Neither of these groups of chlorine is very stable but the cheaper forms of chlorinated lime and bleaching powder are particularly less reliable in activity.

- Sodium dichloroisocyanurate forms hypochlorite when dissolved in water. It is usually marketed in tablet form and is quite stable and stores well. The RECOMMENDED EFFECTIVE CONCENTRATION of this compound is also 0.1% and 1.0% as detailed above.

- Chloramine (sodium tosylchloramide; chloramine T) is another efficient sterilant, available as tablets or a powder and, whilst more stable than sodium or calcium hypochlorite, it is rather sensitive to humidity, light and excessive heat. The RECOMMENDED EFFECTIVE CONCENTRATION is 2% and 4% using the criteria detailed above.

It is most important that the manufacturer's guidance on the use of these compounds is followed to the letter and that instructions concerning storage and shelf life are adhered to.

5.5.3 Other sterilants

Depending on availability and considerations of costs, other recommended sterilants are:

- Polyvidone iodine: 10% solution used at 2.5%; (very expensive).

- Formaldehyde : 35-45% formaldehyde, 10% methanol solution used at 4% (formaldehyde). The solution and vapour are both highly toxic and irritant.

- Glutaraldehyde: 2% solution activated before use with powder or liquid supplied by the manufacturer

As mentioned earlier, these chemical sterilants are effective but not so reliable as sterilization by heat. They are extremely useful in keeping the workplace clean and safely removing spillages. In the field they are extremely useful in providing safe temporary disposal measures for potentially contaminated material such as lancets, swabs, used glass and plastic wear etc. until some more reliable method such as sterilization can be carried out.

REMEMBER: ALL THESE SUBSTANCES ARE HIGHLY TOXIC AND THE MANUFACTURERS INSTRUCTIONS FOR THEIR USE AND STORAGE MUST BE CLOSELY FOLLOWED.

6. APPROPRIATE BIOSAFETY MEASURES

Annex I lists the appropriate biosafety measures relating to the principal *in vivo/in vitro* activities.

7. CONCLUSIONS

The one-time use of disposable lancets and syringes will effectively eliminate the most potentially obvious route of the transmission of diseases of the blood from one person to another. In those situations where disposable material is not available it is imperative that an appropriate and effective means of cleaning and sterilization of such invasive equipment is used to ensure absolute safety. There can be no compromise: safety must come foremost.

Experience indicates that the risk of laboratory infection is not high when compared with the number of persons at risk, but that infections do occur, some of which may result in death or incapacity of varying degree. Work at field level, where many of the standard laboratory control procedures are lacking, greatly enhances the risk of infection and, accordingly, the need for vigilance and preventive action assumes even greater importance.

There are a whole range of effective measures that can be utilized to minimize the risk of infection and these methods should be carefully evaluated to ascertain those best suited to a particular application.

The stringent adoption of a code of routine personal hygiene will produce the second most effective barrier against laboratory-acquired infections and the accidental infection of subjects of blood sampling procedures.

Résumé en français

LA SECURITE BIOLOGIQUE DANS LES ETUDES *IN VIVO* ET *IN VITRO* DU PALUDISME HUMAIN

Les risques associés à la manipulation de sang humain, y compris le SIDA, sont bien connus. Le présent article a pour but de mettre en relief les dangers découlant de la mise en oeuvre, à l'échelle mondiale, des épreuves de sensibilité *in vivo* et *in vitro* des plasmodies humaines aux antipaludéens; il propose en outre des méthodes destinées à réduire considérablement ou à éliminer ces risques.

Les principales causes de risque dans l'étude du paludisme chez l'homme sont probablement le manque d'hygiène individuelle, les accidents, la formation d'aérosols, la manipulation défectueuse des pipettes et des seringues, l'inoculation par l'intermédiaire d'instruments contaminés avec du sang infesté, les contages matériels et les déchets. Afin de réduire ou d'éliminer ces risques, plusieurs précautions sont proposées, par exemple employer des instruments a usage unique - seringues, aiquilles, pipettes, etc., jetables - s'abstenir de manger, boire ou fumer sur les lieux de travail, porter les gants chirurgicaux et se laver les mains à l'eau et au savon, puis à l'éthanol a 70%, porter des vêtements appropriés, limiter les déplacements du personnel et du matériel dans la zone de travail.

Pourtant, les contraintes économiques font qu'une bonne partie du matériel dit à usage unique est en fait conservé pour être recyclé. Il est possible de décontaminer sans risque la majeure partie de matériel en matière plastique, en verre et en métal. La stérilisation peut s'effectuer par passage à l'autoclave, ébullition, chaleur sèche, incinération et à l'aide de germicides chimiques, en particulier les germicides halogénés et les alcools. L'annexe I de cet article donne la liste des mesures de sécurité biologique appropriées pour les principales techniques *in vivo*/*in vitro*.

En conclusion, on soulignera le fait que les activités sur le terrain, ou la plupart des moyens de contrôle habituels des laboratoires font défaut, comportent des risques d'infection beaucoup plus élevés et qu'en conséquence la vigilance et les mesures de prévention indispensables revêtent une importance encore accrue. Il existe une large gamme de mesures efficaces permettant de réduire au minimum les risques d'infection; elles devront être évaluées soigneusement en vue de déterminer celles qui conviennent le mieux à une application donnée. Le respect rigoureux d'un ensemble de règles d'hygiène individuelles systématiques constituera la barrière la plus efficace contre les infections acquises au laboratoire et sur le terrain.

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- Special Programme on Safety Measures in Microbiology, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland.
- Global Programme on AIDS, World Health Organization, Geneva, Switzerland.

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ANNEX I

PRINCIPAL ACTIVITIES OF IN VIVO AND IN VITRO MALARIA STUDIES WHERE BIOHAZARDS OCCUR AND SUGGESTED PREVENTIVE MEASURES TO DEAL WITH THE PROBLEM

Type of study	Technique	Hazard(s)	Preventive measures
1. In vivo or in vitro	1. Withdrawal of blood from finger prick	(a) Infection of donor through dirty skin	(a) Clean puncture site with 70% alcohol
		(b) Infection of donor through infected lancet	(b) Use one-time sterile lancets or lancets resterilized by boiling, auto-claving or chlorine solution (10,000ppm)
		(c) Infection of investigator by hand-to-mouth contact	(c) Use gloves. Wash hands before and after with soap and water followed by 70% alcohol
		(d) Infection of investigator by blood spray from puncture	(d) Do not make puncture deeper than required and manipulate hand of donor gently so as to avoid spray
2. In vitro microtest	1. Withdrawal of blood from finger prick	As in 1.1 above	As in 1.1 above
	2. Venepuncture	(a) Infection of donor through dirty skin	(a) Clean venepuncture site with 70% alcohol

Type of study	Technique	Hazard(s)	Preventive measures
		(b) Infection of donor through infected syringe and/or needle	(b) Use one-time sterile syringe needle or reusables sterilized by boiling or autoclaving and sterile-packed for transportation
		(c) Infection of investitor by blood of patient	(c) Use gloves provided in <u>in vitro</u> test kit. Avoid all contact with blood of patient by good syringe and needle-handling technique. Ensure that no droplets escape from syringe/needle seal or during discharge of contents of syringe. Sterilize syringe and needle and other material after use with appropriate chemical sterilants. Wash hands with soap and water and 70% alcohol when transfer is completed.
		(d) Infection of third parties by blood or apparatus	(d) Sterilize working surfaces with appropriate chemical sterilant. Destroy by incineration all disposable material; non-disposables washed and sterilized by boiling or autoclaving.

Type of study	Technique	Hazard(s)	Preventive measures
	3. Transfer of blood in microcapillary tube	Hand-to-mouth infection of investigator and contamination of working surfaces by poor technique	Use gloves provided with <u>in vitro</u> test kit. Use appropriate micropipette aid and disposable micropipettes: sterilize all used pipettes and working surfaces with appropriate chemical sterilant. Incinerate disposables. Wash and boil or autoclave reusables. Wash hands before and after with soap and water followed by 70% alcohol
	4. Dosing microtitre plates	Although blood is now considerably diluted hand-to-mouth infection and contamination of working surfaces by poor technique is still possible. Tips of the dosing pipette can become contaminated.	Use gloves provided with <u>in vitro</u> test kit. Sterilize all working surfaces and disposable pipette tips with appropriate chemical sterilant. Incinerate disposable material, wash, boil or autoclave reusables. Wash hands before and after with soap and water followed by 70% alcohol.
	5. Harvesting microtitre plates	Even after 24 or more hours of incubation, the contents of the microtitre plate wells may still be infective. Contamination of the microcapillary tubes used for transferring blood to microscope slides and contamination of hands	Use gloves provided with <u>in vitro</u> test kit. Sterilize all working surfaces, microcapillaries and harvested plates with appropriate chemical sterilant. Incinerate disposable material, wash, boil or autoclave all reusables Wash hands before and after followed by 70% alcohol.