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BIOSAFETY IN IN VIVO AND IN VITRO STUDIES OF HUMAN MALARIA

*blood*

by

*laboratory infection*

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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 recent implementation of global studies using in vivo and in vitro test systems for determining the sensitivity of human Plasmodium spp. to antimalarials, has focused attention on these dangers. This paper aims at highlighting these hazards and suggests ways of greatly reducing or eliminating them.

## 2. THE PROBLEM

There can be little doubt that the most commonly recorded blood-related infection to which laboratory staff working on human malaria are exposed is that caused by the hepatitis viruses. In studies made in the United States of America out of a total of 2168 laboratory associated infections, 268 (12%) were due to hepatitis viruses and of the 48 deaths recorded 3 (6%) were related to these organisms.<sup>1</sup> Moreover, there is every reason to believe that other viruses, including the exotic Lassa and Ebola, as well as other pathogens such as bacteria (Mycobacterium tuberculosis) and parasites (Plasmodium spp.) could be transmitted in the same way. As it is inherent to most in vivo and in vitro tests on Plasmodium spp. that they be made in the field under considerably less controlled and controllable conditions, it inevitably means that these hazards are considerably increased. Therefore, the need for the identification and elimination of potential biological hazards is accordingly more important.

## 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: defective personal hygiene; accidents; aerosols; mishandling of pipettes and syringes; inoculation with instruments contaminated by the blood of other persons; fomites; and waste material.

### (a) Defective 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 broken glassware and fractured centrifuge tubes, particularly haematocrit tubes (see below); hypodermic needles; 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.

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<sup>1</sup> Pike, R. M. (1979) Laboratory associated infections: Incidence, fatalities, causes and prevention. Annual review of microbiology, 33: 41-46.

(d) Mishandling of syringes or pipettes

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.

(e) Inoculation with instruments contaminated with the blood of infective persons

The simple procedure of the finger prick can effectively transmit pathogens from an infected to a healthy 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.

(f) Fomites

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

(g) Waste material

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

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.

Hands should be washed with soap and water immediately after any procedure which might cause contamination. The use of abrasive 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.

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.

#### 4.3 Handling of syringes and pipettes

The use of one-time disposable 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 and the needle. The clearing of blocked needles presents a particular danger in the production of leakages and aerosols.

Unshielded needles 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 reesterilization. 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 re-usable 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

Potentially infected or infective material must be disposed of in a systematic and safe way. The use of disposable laboratory ware greatly simplifies the elimination of hazardous material but care must be taken to ensure that these procedures are not compromised by unauthorized recycling of used material

However, as a result of economic restraints much nominally disposable material is in actual fact retained for recycling. Whilst this practice should be strongly resisted in the case of disposable syringes and needles which are not strictly reesterilizable using acceptable and readily available techniques, most other plastic-, glass- and metalware can be safely neutralized if the appropriate reagents are available and the correct procedures are followed.

### 5. STERILIZATION PROCEDURES

Many potential sterilants are both toxic and deleterious in various ways to laboratory equipment. Unfortunately, the extremely versatile possibilities of the autoclave are not always available at field level.

The following notes are restricted to procedures that are both safe, efficacious and adaptable to field conditions.

### 5.1 Autoclaving

When autoclaving is possible (and it should be remembered that in untutored hands the autoclave is a very dangerous instrument indeed), almost all laboratory material can be sterilized or decontaminated at 121°C (250°F) with 115 KN/m<sup>2</sup> gauge pressure (15 lbs/in<sup>2</sup>) over 30-60 minutes in a gravity displacement autoclave or at 132°C (270°F) with 207 KN/m<sup>2</sup> gauge pressure (27 lbs/in<sup>2</sup>) for 4-10 minutes in a pre-vacuum (or high temperature) autoclave. The variable timings depend on the nature of the material to be sterilized and timing should be determined by consulting the operating instructions for the apparatus. When the facilities are available, autoclaving is the method of choice in almost all circumstances.

### 5.2 Boiling

Although reasonably effective if actual boiling is maintained for at least 20 minutes, the effective temperature will depend on altitude: the higher the altitude the lower the boiling temperature. Many plastics will not withstand boiling.

### 5.3 Dry heat

Treatment of glassware and metals at 160°C to 180°C for two hours is very effective for sterilization and decontamination providing penetration of the material being sterilized is not impeded and thus the required temperatures not attained. Most plastics will be destroyed at these temperatures and may produce highly toxic and corrosive gases and smoke.

### 5.4 Incineration

This is the method of choice for the disposal of contaminated material. However, complete combustion must be assured and provision made for the safe dispersion of toxic gases and smoke. Incineration of infected material in an open space may result in the release of microbe-contaminated aerosols and smoke.

### 5.5 Chemical germicides

Of the many commercially available chemical germicides the most appropriate for purposes discussed in this document are: halogens, phenolics and alcohols.

#### 5.5.1 Halogens

Ideally, halogens are chlorine in the form of a bleach - 5.25% NaOCl - or of a 70-72% powder-Ca (OCl)<sub>2</sub>.

The former is more stable and is easier to store and handle. Dilution ratio in water is 8:1 for the NaOCl, and for the Ca (OCl) that quantity of chlorine carrier which will give 1000 parts per million available free chlorine for relatively clean material and 2500 ppm for grossly infected material. The solution can be used for:

- sterilizing equipment (which should be pre-washed)
- cleaning work surfaces
- neutralizing contaminated glass-, plastic- and metalware before disposal or prewashing prior to sterilization.

Sterilization is almost instantaneous when little or no organic matter is present but normally a soakage period of immersible items of 60 minutes would be standard procedure.

Chlorine solutions degrade quickly when diluted and have a limited effective life. They should be replaced at least daily.

#### 5.5.2 Phenolics

Used at a 1-3% concentration, phenolics are ideal for the sterilization of work surfaces and the neutralization of contaminated apparatus before disposal.

In concentrated form they are highly toxic and even at working concentrations may stain certain materials.

#### 5.5.3 Alcohols

Ethanol (at 70-90%) and isopropyl alcohol (50%) are both excellent antiseptics; at 100% concentration they are less effective.

Applications of the alcohols are ideal for antiseptic cleansing of the skin prior to withdrawal of blood and for cleaning the hands after possible exposure to pathogens.

They are also extremely useful for cleaning surfaces and apparatus which may be damaged by other sterilants.

### 6. APPROPRIATE BIOSAFETY MEASURES

Annex 1 lists the appropriate biosafety measures relating to the principal in vivo/in vitro activities.

### 7. CONCLUSIONS

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 most effective barrier against laboratory acquired infections.

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RESUME

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

Les risques associés à la manipulation de sang humain sont bien connus. Le présent article a pour but de mettre en relief les dangers découlant de la mise en oeuvre récente, à l'échelle mondiale, des épreuves de sensibilité in vivo et in vitro des plasmodies humaines aux anti-paludéens; 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 s'abstenir de manger, boire ou fumer sur les lieux de travail, se laver les mains à l'eau et au savon, puis à l'éthanol à 70 %, porter des vêtements appropriés, limiter les déplacements du personnel et du matériel dans la zone de travail, employer des instruments à usage unique - seringues, aiguilles, pipettes, etc., jetables.

Pourtant, les contraintes économiques font qu'une bonne partie du matériel dit à usage unique est en fait conservé pour être recyclé. A l'exception des seringues et des aiguilles, il est possible de décontaminer sans risque la majeure partie du 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 phénoliques et les alcools. L'annexe 1 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, où 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.

ANNEX 1

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. <u>In vivo</u> or <u>in vitro</u>	1. Withdrawal of blood from finger prick.	(a) Infection of donor through dirty skin. (b) Infection of donor through infected lancet. (c) Infection of investigator by hand-to-mouth contact. (d) Infection of investigator by blood spray from puncture.	(a) Clean puncture site with 70% ethanol. (b) Use one-time sterile lancets or lancets resterilized in autoclave or chlorine solution. (c) Wash hands before and after with soap and water followed by 70% ethanol. (d) Do not make puncture deeper than required and manipulate hand of donor gently so as to avoid spray.
2. <u>In vitro</u> macrotest	1. Venepuncture.	(a) Infection of donor through dirty skin. (b) Infection of donor through infected syringe and/or needle. (c) Infection of investigator by blood of patient. (d) Infection of third parties by blood or apparatus.	(a) Clean venepuncture site with 70% ethanol. (b) Use one-time sterile syringe needle or reusables sterilized in autoclave and sterile-packed for transportation. (c) Avoid all contact with blood of patient by good syringe and needle handling technique. Ensure that no droplets escape from syringe/needle seal and during discharge of contents of syringe. Neutralize syringe and needle and other material after use with appropriate sterilant: chlorine or phenol germicides for disposables, 70% ethanol for reusables. Wash hands with soap and water and 70% ethanol when transfer is completed. (d) Neutralize working surfaces with chlorine or phenol germicidal solutions. Destroy by incineration all disposable material; non-disposables to be washed and autoclaved.
	2. Pipetting	(a) Contamination through mouth pipetting or poor handling technique. (b) Infection of third parties by blood or blood containers.	(a) Use appropriate pipette aid and when possible disposable pipettes; neutralize all used pipettes and working surfaces with appropriate germicide. Do not drop pipettes into sterilizing fluid as this may produce contaminated air-borne particles or aerosols. Incinerate disposable materials; non-disposable materials to be washed and autoclaved. (b) Neutralize working surfaces and all containers with appropriate germicide. Destroy by incineration all disposable material; wash and autoclave reusables.
	3. Harvesting test vials using orange sticks.	Contamination by hand-to-mouth or poor handling technique.	(a) Do not agitate the contents of test vials too vigorously as this will produce droplet sprays. (b) Immediately dispose of used orange stick into a chlorine or phenol neutralizing solution. (c) Protect the hands from contamination by the use of rubber gloves and/or careful technique. (d) Ensure that all contaminated glassware, especially the test vials containing the residual blood samples are neutralized with chlorine or phenol germicides (or autoclaved) and then safely disposed of. (e) Wash hands with soap and water, followed by 70% ethanol.

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

Type of study	Technique	Hazard(s)	Preventive measures
3. <u>In vitro</u> microtest	1. Withdrawal of blood from finger prick.	As in 1.1 above.	As in 1.1 above.
	2. Transfer of blood in microcapillary tube	Hand-to-mouth infection of investigator and contamination of working surfaces by poor technique.	Use appropriate micropipette aid and disposable micropipettes; neutralize all used pipettes and working surfaces with appropriate germicide. Incinerate disposables. Wash and autoclave reusables. Wash hands with soap and water, followed by 70% ethanol.
	3. 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.	Neutralize all working surfaces and disposable pipette tips with appropriate germicide. Incinerate disposable material, wash and autoclave reusables. Wash hands with soap and water, followed by 70% ethanol.
	4. 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 cells to microscope slides and contamination of hands and working surfaces.	Neutralize all working surfaces, microcapillaries and harvested plates with appropriate germicide. Incinerate disposable material, wash and autoclave all reusables. Wash hands with soap and water, followed by 70% ethanol.