LABORATORY SAFETY MANUAL

OF THE

Department of Veterinary Tropical Diseases

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

	Name:	Date:
Compiled by:	J Gouws	September 2011
Reviewed by:	E Kapp	September 2011
Approved by:	L Taljaard	September 2011

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1. POLICY STATEMENT

1. The Department of Veterinary Tropical Diseases (DVTD) recognizes that the safety of all its employees and other persons on its premises is of paramount importance and accepts its responsibility for providing a safe and healthy workplace. The DVTD will meet its responsibility as far as is achievable, paying particular attention to the provision and maintenance of:

I. a safe work place with safe access to and from the laboratories
II. a healthy working environment
III. equipment and systems which are safe
IV. safe arrangements for the use, handling, storage and transport of articles and substances
V. sufficient information, instruction, training and supervision to enable employees to avoid hazards and contribute positively to their own health and safety at work

2. The DVTD undertakes the systematic identification of hazards, the recording of any significant risks arising from them, the establishment of arrangements to eliminate, reduce or control risks and procedures for the review and revision of these arrangements.

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3. The DVTD will appoint safety officers competent to provide health and safety advice and assistance to deal with emergencies and situations of imminent danger to health and safety.
4. The Faculty of Veterinary Science co-operates fully in the appointment of a Laboratory Safety Officers and will provide them where necessary, with sufficient facilities and training to carry out this task.
5. The University of Pretoria ensures the existence of a Health and Safety Committee.
6. A copy of this statement will be brought to the attention of all employees in the DVTD. It will be reviewed or modified from time to time and may be supplemented in appropriate cases by further statements relating to the work of employees in the Department.

2. GENERAL SAFETY OF LABORATORY PERSONNEL

1. Eating, drinking and smoking are prohibited in the laboratory.
2. Food must not be placed in a refrigerator used for specimens or reagents.
3. Personal items such as handbags and spectacles must be kept away from work areas.
4. Laboratory coats and gloves worn in the laboratory whilst processing specimens should not be removed from the laboratory and should not be worn outside the laboratory area.
5. Lab coats and gloves must be worn when handling all specimens.

6. Safety glasses or a full-face shield must be worn when working with chemicals such as acids or cold liquids such as liquid nitrogen. Face/eye protection must also be worn when there is a risk of splashes.
7. To avoid contamination, gloves should be removed when answering the telephone, using the computer keyboard, opening the door etc.
8. Hands must be washed efficiently with antiseptic soap after handling infectious specimens and prior to leaving the laboratory.
9. All specimens processed in the laboratory must be handled as if they are infectious in order to reduce the risk of transmission of infectious organisms.
10. All staff should read this safety manual and sign that they have done so. The list of signatories is kept at the back of the safety manual, which is kept in the laboratory or the First Technologist's office.
11. Eye wash equipment (bottles) is kept in all laboratories. This must be used in case of eye contact with any known or unknown liquid or substance. The distilled water in the bottles must be changed every 3 months and the expiry date must be clearly written on the bottle.

3. LABORATORY PRECAUTIONS:

1. Specimens collected in syringes should have the needle removed or have the needle capped before submitting the specimen to the laboratory.

2. Needles must be disposed of into a sharps container. Do not remove needles by hand.
3. Disposable pipettes are disposed of in a bio-hazard container.
4. During centrifugation of specimens, the centrifuge cover is kept closed until the centrifuge comes to a complete stop.
5. Specimens to be discarded are placed into bio-hazard bags and/or boxes.
6. All contaminated disposable items used in the laboratory are placed in bio-hazard bags.
7. Broken glass is disposed of into a sharps container.
8. Work surfaces, counter tops and instruments are cleaned and disinfected daily, using a commercially purchased chlorine-based product or equivalent that is diluted with water. This dilution will depend on the manufacturer's specifications and the purpose for which the disinfectant will be used.
9. All laboratory procedures should be performed according to the guidelines in the safety manual and the relevant SOPs.
10. Pipetting by mouth is strictly prohibited. Use a bulb when necessary or an automatic dispensing pipette if available.
11. Safety data sheets are available in the laboratory for all chemicals used in the laboratory and for all chemicals incorporated into reagent mixtures. It is essential that all staff is aware of any potential dangers associated with these chemicals and the safe handling thereof.

12. Disposable pipettes and/or disposable pipette tips are to be used when pipetting corrosive and infective materials. These pipette tips and pipettes must then be disposed of into bio-hazard bags/boxes or containers with antiseptic or disinfectant.
13. The outside surface of the automatic dispensing pipette must be wiped using a suitable disinfectant.

4. FIRE HAZARD

4.1. GENERAL CAUSES OF FIRES IN THE LABORATORY ARE:

1. Electrical overloading
2. Poor electrical maintenance
3. Apparatus unnecessarily switched on (e.g. hot plates)
4. Open flames (e.g. Bunsen burners)
5. Inferior gas piping
6. Recklessness with flammable chemicals/materials
7. Flammable and explosive chemicals that are stored in refrigerators
8. Matches and lighters

4.2. PREVENTATIVE MEASURES

4.2.1. Bunsen burners:

1. Always turn off the gas when not in use or when the room is unoccupied.

2. Check the gas taps regularly for leaks with a weak soap solution.
3. Report any gas leaks immediately to the official in charge of maintenance after the gas to the room concerned has been turned off. Personnel must be informed of the location of the gas line stop valves. The latter should be clearly marked.
4. Tubing to Bunsen burners must by inspected regularly and replaced where necessary.
5. Do not handle flammable substances near an open flame. Make sure that bottles containing volatile chemicals are tightly closed.

4.2.2. Gas cylinders

1. Do not place gas cylinders near heat emitting sources.
2. Keep the number of gas cylinders (with explosive gases) to the minimum.
3. Gas cylinders must be secured to the wall or cylinder stand if located in the laboratory.
4. It is against regulations to have butane/propane gas cylinders inside a laboratory. These should be stored in designated locked gas cages outside the building.

4.2.3. Flammable chemicals and strong acids

1. Store flammable chemicals and strong acids in volumes not exceeding 5 litres in a fire proof cabinet or room. Examples are ether, benzene, tolueen, xylol, acetone and alcohol. Always handle with care.
2. Information about the chemicals is available in the laboratories chemical file and/or on the internet website www.sciencelab.com/msdsList.php.
3. Flammable chemicals and strong acids should be stored separately.

4.2.4. Electrical apparatus /wiring

1. Defective electrical apparatus should be reported immediately. Only a qualified electrician must carry out electrical repairs.
2. Do not overload the electrical current by plugging in more than one appliance to a power point.
3. Switch off heating apparatus when not in use, e.g. hot plates.
4. Worn electrical cords of apparatus must be replaced.
5. All electrical cords must be secured.
6. Document incidents of short circuits in the electrical system and have the system checked regularly by the maintenance contractor.

4.2.5. Smoking in any of the laboratories is prohibited.

4.2.6. Rooms and passages must be kept free of litter. Prevent the accumulation of rubbish in the building.

4.3. FIRE FIGHTING EQUIPMENT AND USE

The following is available in the Department:

	Number	Location
Fire hose reels	5	1 North wing passage at lift
		1 East wing passage at dept. safe
Nearest fire hose reels		1 East wing passage near HOD office
		1 South wing passage at entrance
		1 West wing passage next to 2-41
Fire extinguishers (dry powder)	10	In all the passages
Asbestos blankets	None	
Asbestos gloves	2	At liquid nitrogen system
These items must never be removed unless required for a fire!		

Class	Type or fire	Example	Recommended
A	Combustible substances	Wood, paper, materials	Water, Dry powder
B	Flammable liquids	Alcohol, ether, etc.	CO_2, Dry powder Extinguisher
C	Electrical short circuits		CO_2 , Dry powder Extinguisher

4.4. HANDLING OF FIRE FIGHTING EQUIPMENT:

4.4.1. Fire hose reels

NB. Water is a good conductor of electricity and the person handling the fire hose could receive a fatal electric shock. Turn the main electricity supply off.

1. Unroll the fire hose.
2. Open the stopcock of the water mains.
3. Make sure that there is a water supply (by means of the control tap on the nozzle) before running to the fire.
4. Direct the nozzle to the base of the fire and open the control valve.
5. After the fire has been extinguished, close the tap and neatly roll up the fire hose so that it is ready for use again.

4.4.2. Carbon dioxide or dry powder fire extinguishers

CO_2 or dry powder extinguishers are especially useful in extinguishing small fires caused by flammable chemicals and electrical shorts. It does not cause pollution and can be safely used on burning apparatus.

1. Remove pin.
2. Direct the nozzle at the base of the flames.
3. Spray the fire after squeezing the lever fully open with the thumb.

4.4.3. Asbestos gloves

The gloves are specifically for the use of those handling the fire fighting equipment.

4.4.4. Asbestos blanket

The blanket is used to wrap around a person who is on fire or to cover burning material.

Showers are available at the chemical store to extinguish burning clothes.

4.5. EMERGENCY PROCEDURE

4.5.1. Small fires

1. Use the dry powder fire extinguishers.
2. Raise alarm in the building and call for help.
3. If the fire cannot be extinguished follow the steps under ***Big fires***
4. Avoid crowding of onlookers.

4.5.2. Big fires

It includes fires that also originated in other parts of the building

1. Raise alarm immediately.
2. Do not contact the emergency services yourself.

3. Dial 012 4202310 and inform the emergency section of the location, extent and nature of the fire. The fire brigade will be summoned.
4. Emergency numbers must be visible in every laboratory.
5. Close all doors and windows. Keep them closed!!!
6. Breathe through a wet handkerchief/towel and crawl low down on the floor when smoke is present.
7. Evacuate the area and use the fire escape (west wing i.e. Serology), if possible to leave the building.
8. Do not panic and avoid crowding at the exits.
9. If trapped, attract attention from the windows. Hose down all those trapped with water.
10. Members of the fire team and management of the department will see to:

I. Turning off of the main gas supply in gas cage.
II. Switching off of the main electricity supply to the department.
III. The evacuation of and first aid to the injured.
IV. Communication aspects.

Note:

The telephone might not be working and help must be sought by other means. As a result of the lack of electricity and smoke, passages may be shrouded in darkness. Torches and a chopper stored in the fire reel cabinet may be of use.

4.6. INSPECTION ROUTINE

Electrical equipment, e.g. wiring, power points, etc.	- 2 monthly
Gas tubing to Bunsen burners	- 2 monthly
Fire fighting equipment	- annually

REFERENCES:

Laboratory Biosafety Manual, Second Edition, WHO

5. CHEMICAL HAZARDS

5.1. INTRODUCTION

All chemicals must be regarded as potentially dangerous.

International Hazard warning symbols are clearly indicated on the label of most chemicals (example follows). Sometimes the directions for safe storage are also indicated. These symbols are:

5.1.1. Oxidising:

Reagents that cause highly exothermic reactions when it comes into contact with organic material (or other oxidising chemicals), e.g. potassium permanganate.

5.1.2. Explosive:

Unstable chemicals that can explode when they dry out or when exposed to friction, shock or heat e.g. ammonium dichromate

5.1.3. Flammable:

I. Chemicals with a flash point of under 66ºC (150ºF).
II. Chemicals that spontaneously ignite, e.g. phosphorus.

III. Chemicals that give off flammable gases when in contact with water, e.g. lithium.

5.1.4. Toxic:

Chemicals with a serious danger of acute poisoning, irrespective of the way they come in contact with the body I.e. inhalation, swallowing or skin contact.

5.1.5. Caustic (Corrosive):

Chemicals that can destroy living tissue or damage apparatus e.g. sulphuric acid

5.1.6. Irritant:

Chemicals with an irritant or harmful effects on the skin, eyes and respiratory organs, e.g. ammonia.

5.1.7. Radio-active:

Chemicals with a radiation risk.

5.2. SAFETY MEASURES FOR CHEMICALS

Always read the label on the container and or MSDS, making sure that all the instructions with regards to the use of the chemical are strictly adhered to

5.2.1. Storage

1. Chemicals should be stored according to instructions on the label and the material safety data sheets
2. Store in a well ventilated room, preferably at room temperature, protected against big temperature fluctuations.
3. All chemicals should be store in a controlled environment, where unauthorised entrance is prohibited. Poisonous chemicals e.g. potassium cyanide must be kept under strict control e.g. locked and a register must be signed with full details of its use.
4. Organic solvents, e.g. ethyl alcohol must be kept in a lockable cabinet and separate from acids. Smoking is prohibited where flammable chemicals are stored.
5. Oxidative acids must be stored in a well-ventilated room, as close as possible to the floor, where the containers cannot spill unto other chemicals when accidentally knocked over.

6. Some chemicals must not be stored in the fridge as there is a danger that they will ignite when the fridge switches on. Others chemicals e.g. picric acid can explode on contact with moisture.

5.2.2. Handling:

1. Check the identity of the chemical in the container.
2. Wear protective clothing and eye or face protection where necessary.
3. Open container carefully in a well-ventilated area or in a fume cabinet.
4. Guard against ingestion, inhalation and contact with the skin, eyes and clothes. Never pipette by mouth.
5. Eating, drinking or smoking is prohibited, when working with chemicals.
6. Take precautions against static electricity and avoid friction by not jolting containers.
7. Do not add water carelessly, especially when using acids.
8. Add acid to water and not the other way around.
9. Some chemicals, e.g. sodium hydroxide react exothermically when water is added to it. Such flasks may become very hot.
10. Use appropriate procedures to clean up spilt chemicals. Refer to 5.5 in Safety manual and appropriate MSDS.

11. Should exposure to a chemical substance affect you in any way, immediately seek first aid until medical assistance is available.
12. Take note of the first aid box in the passage or media room, as well as the wall chart indicating the relevant information about chemicals.

5.3. INCOMPATIBLE CHEMICALS NOT TO BE STORED TOGETHER OR NEXT TO ANOTHER

CHEMICAL	INCOMPATIBILITY
Acetic acid	Chromic acid, nitric acid, hydroxyl compounds, ethylene glycol, perchloric acid, peroxides, permanganates
Acetone	Concentrated nitric and sulfuric acid mixtures
Acetylene	Chlorine, bromine, copper, fluorine, silver, mercury
Alkali and alkaline earth metals (lithium, sodium, potassium)	Water, carbon tetrachloride or other chlorinated hydrocarbons, carbon dioxide, halogens, powdered metals (e.g., aluminum or magnesium)
Ammonia(anhydrous)	Mercury (e.g., in manometers), chlorine, calcium hypochlorite, iodine, bromine, hydrofluoric acid (anhydrous)
Ammonium nitrate	Acids, powdered metals, flammable liquids, chlorates, nitrates, sulfur, finely divided organic or combustible materials
Aniline	Nitric acid, hydrogen peroxide
Arsenical materials	Any reducing agent
Azides	Acids
Bromine	See Chlorine
Calcium oxide	Water
Carbon (activated)	Calcium hypochlorite, all oxidizing agents

CHEMICAL	INCOMPATIBILITY
Carbon tetrachloride	Sodium, Chlorates, Ammonium salts, acids, powdered metals, sulfur, finely divided organic or combustible materials
Chlorine	Ammonia, acetylene, butadiene, butane, methane, propane (or other petroleum gases), hydrogen, sodium carbide, benzene, finely divided metals, turpentine
Chlorine dioxide	Ammonia, methane, phosphine, hydrogen sulfide
Chromic acid and chromium	Acetic acid, naphthalene, camphor, glycerol, alcohol, flammable liquids in general
Copper	Acetylene, hydrogen peroxide
Cumene hydro peroxide	Acids (organic or inorganic)
Cyanides	Acids
Flammable liquids	Ammonium nitrate, chromatic acid, hydrogen peroxide, nitric acid, sodium peroxide, halogens
Fluorine	Isolate from everything
Hydrocarbons (e.g.,butane,propane, benzene)	Fluorine, chlorine, bromine, chromic acid, sodium peroxide
Hydrocyanic acid	Nitric acid, alkali
Hydrofluoric acid (anhydrous)	Ammonia (aqueous or anhydrous)

CHEMICAL	INCOMPATIBILITY
Hydrogen peroxide	Copper, chromium, iron, most metals or their salts, alcohols, acetone, organic materials, aniline, nitro methane, combustible materials
Hydrogen sulfide	Fuming nitric acid, oxidizing gases
Hypochlorite	Acids, activated carbon
Iodine	Acetylene, ammonia (aqueous or anhydrous), hydrogen
Mercury	Acetylene, fulminic acid, ammonia
Nitrates	Sulfuric acid
Nitric acid (concentrated)	Acetic acid, aniline, chromic acid, hydrocyanic acid, hydrogen sulfide, flammable liquids, flammable gases, copper, brass, any heavy metals
Nitrites	Potassium or sodium cyanide.
Nitro-paraffin	Inorganic bases, amines
Oxalic acid	Silver, mercury
Oxygen	Oils, grease, hydrogen, flammable: liquids, solids, or gases
Perchloric acid	Acetic anhydride, bismuth and its alloys, alcohol, paper, wood, grease, oils
Peroxides, Organic	Acids (organic or mineral), avoid friction, store cold
Phosphorus (white)	Air, oxygen, alkalis, reducing agents
Phosphorus pentoxide	Water

CHEMICAL	INCOMPATIBILITY
Potassium	Carbon tetrachloride, carbon dioxide, water
Potassium chlorate	Sulfuric and other acids
Potassium perchlorate	(see Sulfuric and other acids also chlorates)
Potassium permanganate	Glycerol, ethylene glycol, benzaldehyde, sulfuric acid
Selenides	Reducing agents
Silver	Acetylene, oxalic acid, tartaric acid, ammonium compounds, fulminic acid
Sodium	Carbon tetrachloride, carbon dioxide, water
Sodium Chlorate	Acids, ammonium salts, oxidizable materials, sulfur
Sodium nitrite	Ammonium nitrate and other ammonium salts
Sodium peroxide	Ethyl or methyl alcohol, glacial acetic acid, acetic anhydride, benzaldehyde, carbon disulfide, glycerin, ethylene glycol, ethyl acetate, methyl acetate, furfural
Sulfides	Acids
Sulfuric acid	Potassium chlorate, potassium perchlorate, potassium permanganate (similar compounds of light metals, such as sodium, lithium)
Telluride	Reducing agents
Water	Acetyl chloride, alkaline and alkaline earth metals, their hydrides and oxides, barium peroxide, carbides, chromic acid, phosphorous oxychloride, phosphorous

CHEMICAL	INCOMPATIBILITY
	pentachloride, phosphorous pentoxide, sulfuric acid, sulfur trioxide

5.4. TOXIC EFFECT OF CHEMICAL SUBSTANCES

Apart from poisonous chemicals it is well known that many chemicals are detrimental to the body. The respiratory tract, blood, lungs, liver and kidneys as well as other organs can be affected and seriously damaged. Some substances are carcinogenic or teratogenic.

Take note of the following chemicals that are sometimes in use.

Chemical	Acute	Chronic
Acetone	Slight eye, nose and throat irritation, anaesthetic	
Ammonia	Eye irritation	Pulmonary oedema
Benzene	Anaesthetic	Leukaemia, aplastic anaemia; liver damage
Benzidine	Abdominal pain, nausea, skin irritation	Carcinogenic
Chloroform	Head ache, nausea, mild jaundice, loss of appetite, anaesthetic	Liver and kidney damage; intestinal disturbances.

Chemical	Acute	Chronic
Cyanogen bromide	Abdominal pain, nausea, diarrhoea, visual disturbances	Pulmonary oedema
Di-ethyl ether	Vomiting, eye irritation	Addiction
Formaldehyde (Formalin)	Respiratory, skin and mucous membrane irritation.	Pulmonary oedema; carcinogenic
Gluteraldehyde	Respiratory and mucous membrane irritation	
Methanol	Anaesthetic Respiratory- and mucous membrane irritation	Retina- and optic nerve damage
a-Naphtylamine		Probably carcinogenic
Phenol	Abdominal pain, vomiting, diarrhoea, eye pain	CNS disturbances, coma
Toluene	Anaesthetic	Non-specific neurological disturbances. Probably addictive
Xylene	Anaesthetic; headache, nausea, dizziness, fatigue	Non-specific neurological disturbances

5.5. CLEANING UP OF CHEMICAL SPILLS

The following procedure must be adhered to when dangerous chemicals are spilt.

1. Notify the Safety Officer and evacuate non-essential staff from the area.
2. Give attention to persons who are contaminated. Avoid any fumes and ensure good ventilation (if this is advisable).
3. If the substance is flammable extinguish any open flames using the sand provide, located in the chemical store room. Close the gas supply in the room and surrounding area as well as any electrical equipment that may ignite the fire or flammable chemical.
4. Wear protective clothing, including heavy-duty gloves and rubber boots during cleaning up operation.
5. In the case of an acid spill, neutralise it with sodium bicarbonate.
6. In the case of an alkali spill, cover it with dry sand.
7. Remove glass pieces with tweezers. Remove the remainder by mopping up with paper hand towel and/or broom and dustpan.
8. Any other spillages should be covered by absorbent material e.g. paper towel and or shavings, located in close proximity and should be discarded using the method described in point 6.5

9. Additional protective equipment are situated in each chemical storeroom and consist of an acid fast apron, when using acids, protective eyewear, when using volatile gasses, gloves, when using poisonous and corrosive chemicals, sand and shavings, in case of spillages and must be used appropriately.

Chemical hazard signs

	**Poisonous** The poison symbol is self-explanatory. Whereas most chemicals are fairly dangerous if ingested or inhaled, many of these are dangerous even on contact.		**Stow away from foodstuffs** Harmful material to be kept away from edible material.
	**Environmental hazard** Relatively rare with laboratory chemicals (most of which pose some environmental hazard if not disposed of correctly), these require *particular* care to be taken on disposal.		**Dangerous when wet** This generally means that it will react fairly violently with water.
	**Corrosive** Avoid contact with the skin. Bear in mind that these can (under some circumstances) corrode chemical cupboards.		**Flammable Gas** Safety symbol used for the transport or storage of a flammable gas.
	**Explosive** Again, fairly self-explanatory, though fairly seldom seen in the average lab. Bear in mind that noise and movement can also trigger explosion (not just sparks/flames!).		**Non flammable gas** Safety symbol used in the transport of non flammable (and hence often non hazardous, at least out in the open) gases.

	Flammable or extremely flammable Chemicals to be stored in flame-resistant cupboards. Volatile solvents can be a particular problem as they are prone to spread around from unsealed containers. This also covers pyrophoric materials (that catch fire spontaneously on exposure to air).		Organic Peroxide Chemical safety symbol used in the transport and storage of organic peroxides.
	Irritant or Harmful This symbol covers a wide range of (sometimes relatively minor) hazards - with precautions such as avoid contact with the skin, do not breathe, etc. - best to refer to relevant data sheet for details.		Corrosive The corrosive symbol is used in the transport of corrosive materials - again, avoid contact with the skin.
	Oxidizing chemical Oxidizing chemicals are materials that spontaneously evolve oxygen at room temperature or with slight heating, or that promote combustion. To be kept away from flammable chemicals at all costs!		Inhalation Hazard Inhalation hazard transport/storage symbol.

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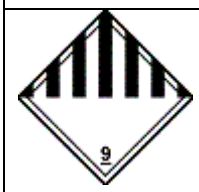
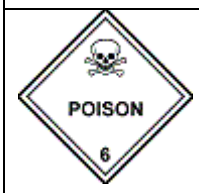
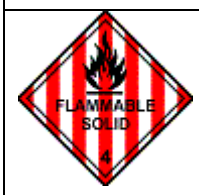
	Poisonous Gas Used for transport of a poisonous gas - on gas cylinders, or sometimes as an indicator on vehicles. Larger image >		Marine Pollutant Marine pollutant - do not dispose of in sewer system. Larger image >
	Miscellaneous danger Catch-all symbol for all other dangers (usually specified in the space). Larger image >		Explosive Used in the transport of explosive materials. Larger image >
	Poison More general symbol for the transport of poisonous materials (not necessarily a gas). Larger image >		Spontaneously Combustible Spontaneously combustible material (treat with great caution!). Larger image >
	Flammable Solid Flammable solid. Larger image >		Flammable Liquid Used in the transport of flammable liquids. Larger image >

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6. BIOSAFETY MEASURES

During the manipulations with micro-organisms some of them, may be released into the environment and can be the cause of laboratory-acquired infections. Such release may be entirely accidental or it may be intrinsic in the technique or equipment used. Even the most careful worker using the best methods and the correct equipment, is not immune from accidents and errors.

Over 4500 such infections have been reported during the last century.

Accidents that release micro-organisms include spillages and breakages. Activities that frequently release micro-organisms include opening of cultures, using inoculating needles, loops, hypodermic needles, pipetting, mixing, homogenising and centrifugation[1].

Micro-organisms released into the environment may enter the bodies of workers and other people, in and around the laboratory and initiate infections. Those most at risk are clinical laboratory and research staff.

Even in industry, e.g. in food testing laboratories, pathogens that are present in small numbers, in samples submitted for examination, may be concentrated by culture into infectious doses.

6.1. ROUTES OF INFECTION

Micro-organisms may enter the human body by any of the following routes: the respiratory tract, the alimentary tract, the skin and the conjunctivae.

6.1.1. The Respiratory Tract - Inhalation

Very small droplets of liquids (aerosols) that may contain micro-organisms are generated when films of liquids are broken, e.g. when cultures are opened or broken, liquids are pipetted violently, bursting bubbles, splashes, falling drops impacting on surfaces, and breakages in centrifuges. The smallest of these droplets, those less than 5um in size, remain suspended in the air and dry rapidly. The organisms they contain then become ``droplet nuclei'' and are moved around the room or to other parts of the building by air currents. If they are inhaled, they are small enough to reach the alveoli, where they may initiate an infection. Larger droplets sediment rapidly under the influence of gravity and may contaminate benches, equipment and or the hands. If they are inhaled they are trapped and removed in the upper air passages.

6.1.2. The Alimentary Tract - Ingestion

Workers' hands may be contaminated by spillage and by the larger aerosol droplets. The organisms may then be transferred to the mouth by the fingers, or by contaminated pencils, pipettes, food etc.

6.1.3. The Skin

Although the intact skin is a good barrier against micro-organisms, the exposed parts, e.g. the hands and face, are frequently damaged by small cuts and abrasions, many of which may not be visible to the naked eye. These are portals of entry for micro-organisms. In addition, ``sharps" injuries are not uncommon in laboratories. Pricks and cuts with needles, knives, broken glass, etc. will allow the entry of micro-organisms.

6.1.4. The Conjunctivae

The very thin membranes surrounding the eyes are readily penetrable by micro-organisms during splashes, or from contaminated fingers.

6.2. CLASSIFICATION OF MICRO-ORGANISMS ON THE BASIS OF HAZARD

It is obvious that not all micro-organisms have the same capacity to cause infections, and that infections vary in their incidence, their severity, and the availability of prophylaxis and therapy. By international agreement micro-organisms are now classified into groups or classes according to the hazard they pose to workers and the community.

There are four groups, ranging from the relatively harmless to the very hazardous. The wording varies slightly from region to region and that used in Europe is shown in Table 1.

Lists of bacteria, viruses, fungi and parasites in Groups 2, 3 and 4 have been published by various national and international agencies, e.g. the European Union.

Micro-organisms not listed in these groups are assumed to be in Group 1, although some of them may be responsible for allergies. There are inevitable disagreements, globally, because of differences in the geographical distribution, incidence, and local significance.

TABLE 1

Classification of micro-organisms on the basis of hazard and laboratory containment level		
Class	**Description**	**Laboratory**
1	Unlikely to cause human disease.	Level 1
2	May cause human disease; might be a hazard to laboratory workers; unlikely.	Level 2
3	May cause serious human disease; may be serious hazard to laboratory workers; may spread in the community; effective prophylaxis and therapy available.	Level 3
4	Causes severe human disease; serious threat to laboratory workers; high risk of spread in the community; no effective prophylaxis and therapy. Class 4 contains only viruses.	Level 4

TABLE 2 APPARATUS NEEDED FOR LABORATORY CONTAINMENT LEVELS

	Containment Level			
	1	**2**	**3**	**4**
Laboratory isolated and sealable for decontamination	**±**	**±**	**+**	**+**
Directional ventilation (inward)	**±**	**D**	**+**	**+**
Filtered air exhaust	**±**	**±**	**+**	**+**
Double door entry	**±**	**±**	**O**	**+**
Airlock with shower	**±**	**±**	**±**	**+**
Autoclave on site	**+**	**+**	**+**	**+**
Autoclave in workroom	**±**	**±**	**O**	**+**
Autoclave double ended	**±**	**±**	**±**	**+**
Microbiological safety cabinets Class I or II available	**±**	**+**	**+**	**+**
in workroom	**±**	**±**	**+**	**+**
Class III safety cabinet	**±**	**±**	**O**	**+**

Based on WHO Laboratory Biosafety Manual

Key: **±** not required **+** essential **D** desirable **O** optional

6.3. CLASSIFICATION OF LABORATORIES ACCORDING TO HAZARD GROUP

It follows from the classification of micro-organisms on the basis of hazard that precautions against laboratory-acquired infections should vary from minimal for those in Group 1 to maximum security for those in Group 4. Such precautions and safety requirements have been codified as Containment or Biosafety Levels

These are outlined in Table 2.

General precautions are considered below. Where there are disagreements in classifications the local system should be regarded as the minimum, but there is no reason why microbiologists, if they think fit, should not use higher levels of precautions than those prescribed nationally.

6.4. GENERAL PRECAUTIONS AGAINST LABORATORY-ACQUIRED INFECTIONS (BSL2+)

There are several international and national guidelines and codes of practice.

Only outlines can be given here.

6.4.1. Personal Protection

Protective clothing should be worn at all times in the laboratory. Gowns, coats and overalls should be fastened at the sides or back, cover the chest and neck

areas and fit closely at the wrists. Workers should remove this clothing before leaving the laboratory and not wear it in rest rooms, offices, libraries etc. Gloves should be worn if there is a risk of contaminating the hands, especially with blood.

Disposable (latex) gloves should be worn once only and then autoclaved with other laboratory wastes. Re-usable gloves should be washed while still on the hands and then disinfected before re-use. Safety spectacles should be worn during microbiological and hazardous chemical manipulations. Hands should be washed often and always before leaving the laboratory.

6.4.2. Inoculating Loops

Long wires vibrate and shed droplets, as do large and poorly made loops. The wires should be no longer than 6cm and the loops not more than 2mm in diameter and completely closed. Plastic disposable loops are preferred, as no flaming is required and they may be placed in a disinfectant immediately after use.

6.4.3. Glassware

Chipped and scratched glassware is hazardous and should never be used.

6.4.4. Pasteur Pipettes

Glass Pasteur pipettes should not be used as they are often responsible for cuts and punctures of the skin. Soft plastic pipettes are a safer option.

6.4.5. Hypodermic Needles

To avoid needle stick accidents, rather use pipettes and cannulas instead of hypodermic needles. Opening devices for vaccine and septum-capped bottles are available.

6.4.6. Centrifuges

1. Accidents with centrifuges, which are often the result of improper handling, may release massive amounts of aerosols. Centrifuges should be placed on low benches so that all operators can see the inside of the bowl when loading them.
2. Buckets and trunnions should be inspected regularly for evidence of corrosion and hairline cracks and any suspect parts should be discarded.
3. When not in use buckets should be placed upside down in racks to drain the fluid used in balancing.
4. Buckets should be paired by weight and labelled accordingly. Paired buckets should be placed opposite one another when loading the centrifuge.
5. When filling the tube At least 2cm space should be allowed between the top of the fluid in a centrifuge tube and its rim.

6. Use stoppered tubes and sealed buckets during centrifugation of any potentially infectious material.
7. Paired buckets, with tubes in situ, should be balanced by adding 70% alcohol (NOT saline, which may corrode metal and may lead to mechanical failure) to the space between the tube and the bucket.
8. Instructions for use of centrifuges and precautions should be documented and available.

6.4.7. Water Baths

Water in water baths may become contaminated from the outsides of culture tubes or leakage of their contents. These baths, even those operated at temperatures >60ºC should be emptied when not in use. If not, a deposit may form in which micro-organisms can grow. A disinfectant that does not attack metals may be added to the water in baths that are in continuous use (hypochlorite should not be used; see below).

6.4.8. Homogenisers and Shakers

Bench-mounted models may generate aerosols and should be covered, (e.g. by clear plastic boxes) when in use. These covers should be disinfected after use. Hand-held homogenisers should be held in a wad of cotton wool in case of breakage. Homogenisers and containers from shakers should be opened in a microbiological safety cabinet.

6.4.9. Pipetting

Pipetting by mouth, even of water, is prohibited. Pipetting devices should be provided. Pipettes should not be blown out vigorously; otherwise bubbles and aerosols may be formed.

6.4.10. Microbiological Safety Cabinets

These should conform to national standards and should regularly be tested independently by an accredited company to ensure that their performance is in accordance with the requirements of the standard. These cabinets are designed to protect the user from the inhalation of infectious aerosols and air-borne particles. They give no protection against spillages of cultures or against chemicals. Class I only protects the operator. Class II and Class III cabinets also protect the test or product from external air-borne contamination.

Microbiological safety cabinets should be used only by experienced, competent personnel who have received proper instructions about their limitations. They must not be used as fume cupboards or for work with flammable or toxic substances.

They should be decontaminated regularly by qualified staff, who follow manufacturers', or other recognised procedures.

6.4.11. Laminar Flow (clean air) Cabinets

These are NOT microbiological safety cabinets. They are designed to protect the work from external air-borne contamination and do not protect the worker, whose face and respiratory system are exposed to air that has passed directly over the work area. (See Cell and Tissue culture, below).

6.4.12. Fume Cupboards

Fume cupboards are designed to protect workers and the environment from toxic chemical fumes and gases. They should not be used for micro-organisms or other living material.

6.5. SPILLAGE AND BREAKAGE

Spillage, due to cultures or breakage of vessels containing them, must be reported immediately to the supervisor or local safety officer. In case of considerable spillage, the room should be vacated pending decontamination by qualified staff (see below).

Instructions for dealing with small-scale spillages and breakages should be posted in each laboratory, and should include the following:

1. Wear heavy-duty gloves.
2. Cover the spillage/breakage with absorbent material, e.g. large paper towels.
3. Pour disinfectant over the paper towels and leave for at least 15 minutes.

4. Scoop up the paper towels with a dust pan or stiff cardboard and place them along with the dust pan or cardboard and any broken glass into a laboratory discard container/sharps container.
5. Pick up any residual broken glass with forceps and add it to the sharps container.
6. Cover the area again with paper towels and pour on more disinfectant. Leave for 30 minutes before any further cleaning up.
7. Autoclave the discard container.

6.6. PRECAUTIONS AGAINST BLOOD-BORNE INFECTIONS

In addition to the precautions listed above, personnel who handle blood specimens or blood-stained material should wear high quality disposable gloves and also plastic disposable aprons over their normal protective clothing.

PRECAUTIONS WITH CELL AND TISSUE CULTURE

Separate accommodation should be provided to minimise contamination of cultures. Some cells and tissue cultures may contain unknown micro-organisms or viruses from which the operator must be protected.

All isolation work using cells and cell lines should therefore be conducted in a Class II microbiological safety cabinet.

Laminar flow cabinets (see above) must NOT be used.

6.7. STERILIZATION, DISINFECTION AND DECONTAMINATION

These terms are not interchangeable. In microbiology:

6.7.1. Sterilization:

Implies the complete destruction of all micro-organisms.

6.7.2. Disinfection:

Is the destruction or inactivation, usually by chemicals, of the vegetative forms of micro-organisms and spores. Note that not all spores are inactivated by chemical disinfectants.

6.7.3. Decontamination:

Means cleaning equipment, materials and waste from infectious agents.

6.7.4. Sterilization

This is restricted to autoclaving in this department. For other methods, e.g. hot air, standard textbooks should be consulted.

The hazard most frequently encountered in autoclaving is failure to sterilize, i.e. to achieve and maintain the temperature/time ratio that is known to kill micro-organisms.

1. Autoclaves should only be operated by personnel specifically trained and declared competent.
2. Infected materials and “clean" items should be treated in separate loads and preferably separate autoclaves.
3. Autoclaves should not be tightly packed: space must be allowed between items in the load to enable steam to circulate freely.
4. The ``Holding time at temperature" (HTAT) for steam sterilization is 20 minutes at 121ºC.
5. The time begins when the temperature in the load has reached 121ºC in the chamber as indicated by the recorder of the thermocouple.
6. Higher temperatures are required for the treatment of material containing “unconventional agents"

6.7.5. Control of Sterilization

In modern autoclaves this is achieved by instrumentation (thermocouple probes and recorders).

It is advisable, however, to include some form of indicator, e.g. “autoclave tape" in each load, and to check the HTAT independently at regular intervals.

Alternatively, or in addition, biological indicators may be used in the form of strips or ampoules that contain *Bacillus stearothermophilus* spores.

6.7.6. Chemical Disinfection

Disinfectants vary in their action against bacteria, spores, fungi and viruses and should be chosen in accordance with the intended use. Most disinfectants are toxic, in varying degrees, and precautions, e.g. eye protection, should be taken when stock solutions are diluted.

Disinfectants should be diluted according to the manufacturers' instructions. It is best to prepare dilutions daily as some deteriorate if working dilutions are stored. For most purposes hypochlorites are adequate and should be diluted to contain 1,000 - 2,500 ppm available chlorine for normal work and 10,000 ppm for blood and high concentrations of protein.

Industrial hypochlorite solutions usually contain 100,000 ppm available chlorine and should be diluted 10%.

6.7.7. Bench discard jars and containers

A discard jar containing an appropriate disinfectant should be provided at every work station to receive small items such as slides, Pasteur pipettes and plastic loops.

Large jars, for pipettes are also needed. Plastic containers are safer than glass. Items placed in these containers should be completely submerged in the disinfectant.

Discard containers should be emptied and replaced daily.

6.7.8. Decontamination of Work Surfaces, Equipment and Rooms

Work surfaces should be wiped with a suitable disinfectant at the end or beginning of the working day (gloves should be worn). The accessible parts of equipment may similarly be disinfected but not with hypochlorite as they may corrode metals.

Equipment to be serviced must also be decontaminated in this way and clearly labelled to indicate that this has been done and that it should not be used until after servicing.

The work surfaces and inner walls of microbiological safety cabinets should be swabbed with a suitable disinfectant and the cabinets should be fumigated with formalin, as indicated above before filters are changed or maintenance carried out.

Rooms rarely need disinfection unless a accident released aerosols. Formerly this was done by formalin fumigation, but this is now regarded as hazardous and uncertain. Spraying or washing with disinfectant/detergent mixtures is safer and more effective.

6.8. DISPOSAL OF INFECTED WASTE

Infected laboratory waste is included in the definitions of clinical waste and must ultimately be incinerated. As these are likely to be the most heavily infected of

all waste and may have to travel along the public highways, often for long distances, it is prudent to have it autoclaved first.

6.9. CONCLUSIONS

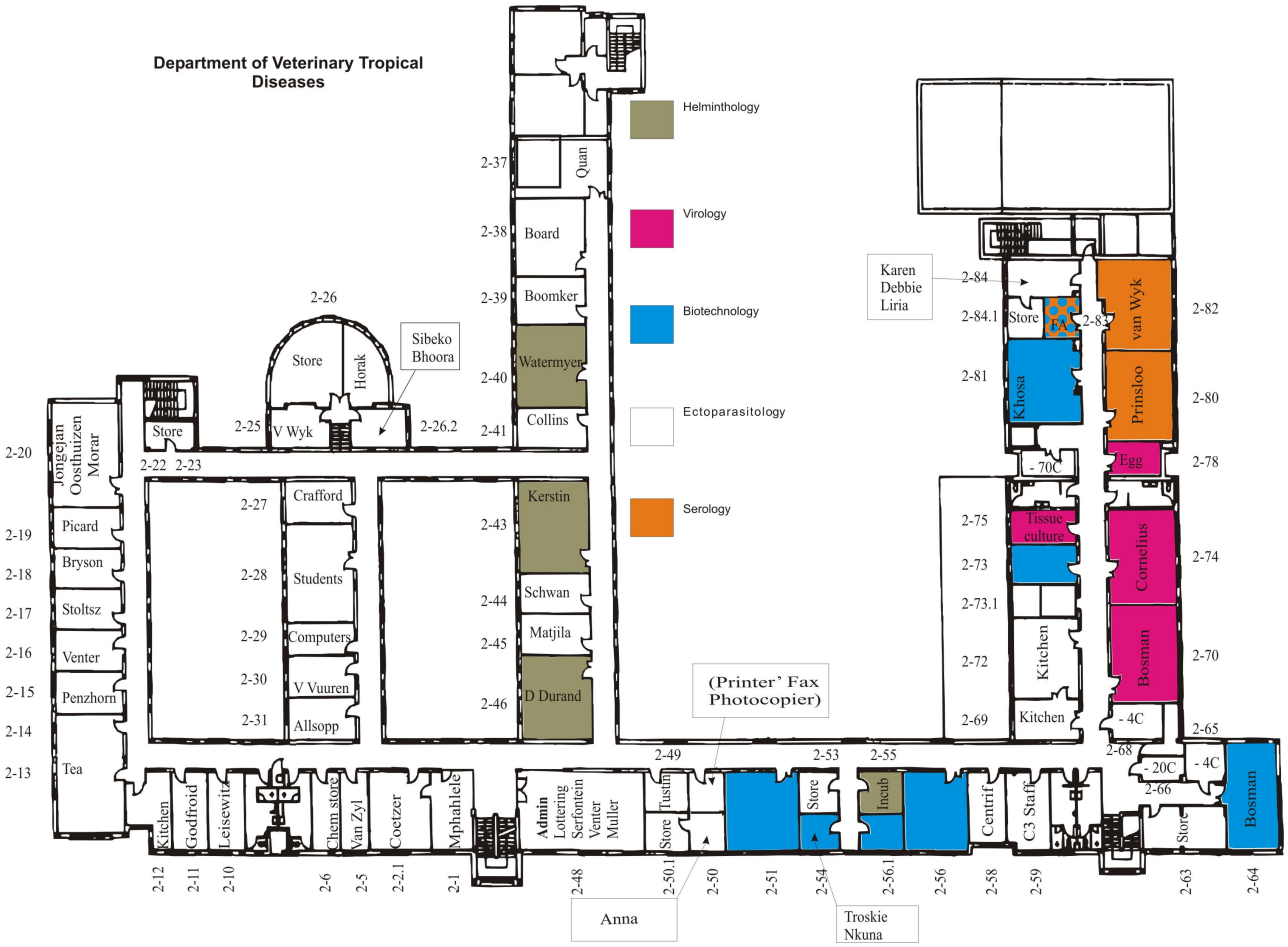
All members of staff should be aware of the approved procedures for containing and destroying micro-organisms. A schedule of regular microbiological cleaning or disinfection should be maintained for all working surfaces and adjacent areas.

See appendix 27 of the Quality Manual for waste disposal guidelines.

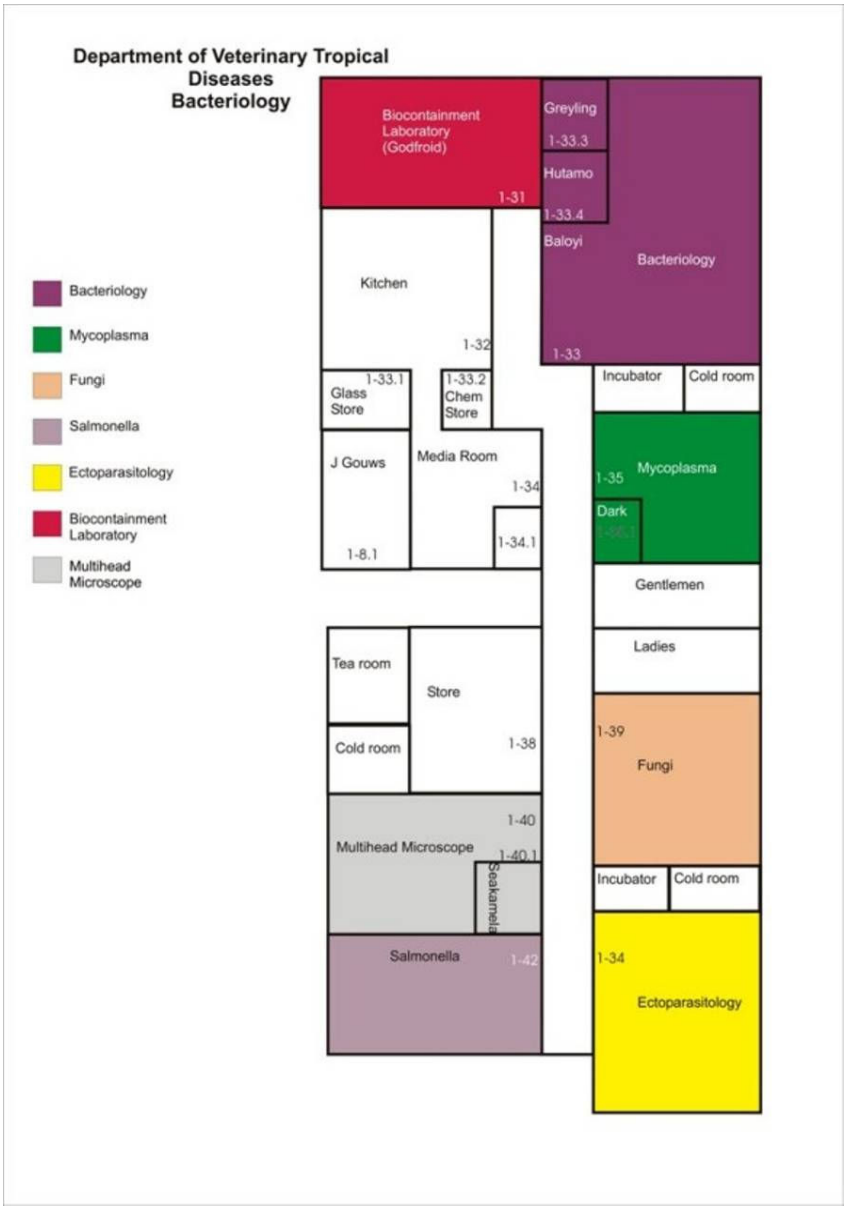
7. REFERENCES:

Laboratory Bio-safety Manual, Second Edition, WHO.

8. FLOORPLAN OF THE DEPARTMENT OF VETERINARY TROPICAL DISEASES



	Name:	Date:
Compiled by:	J Gouws	September 2011
Reviewed by:	E Kapp	September 2011
Approved by:	L Taljaard	September 2011



	Name:	**Date:**
Compiled by:	J Gouws	September 2011
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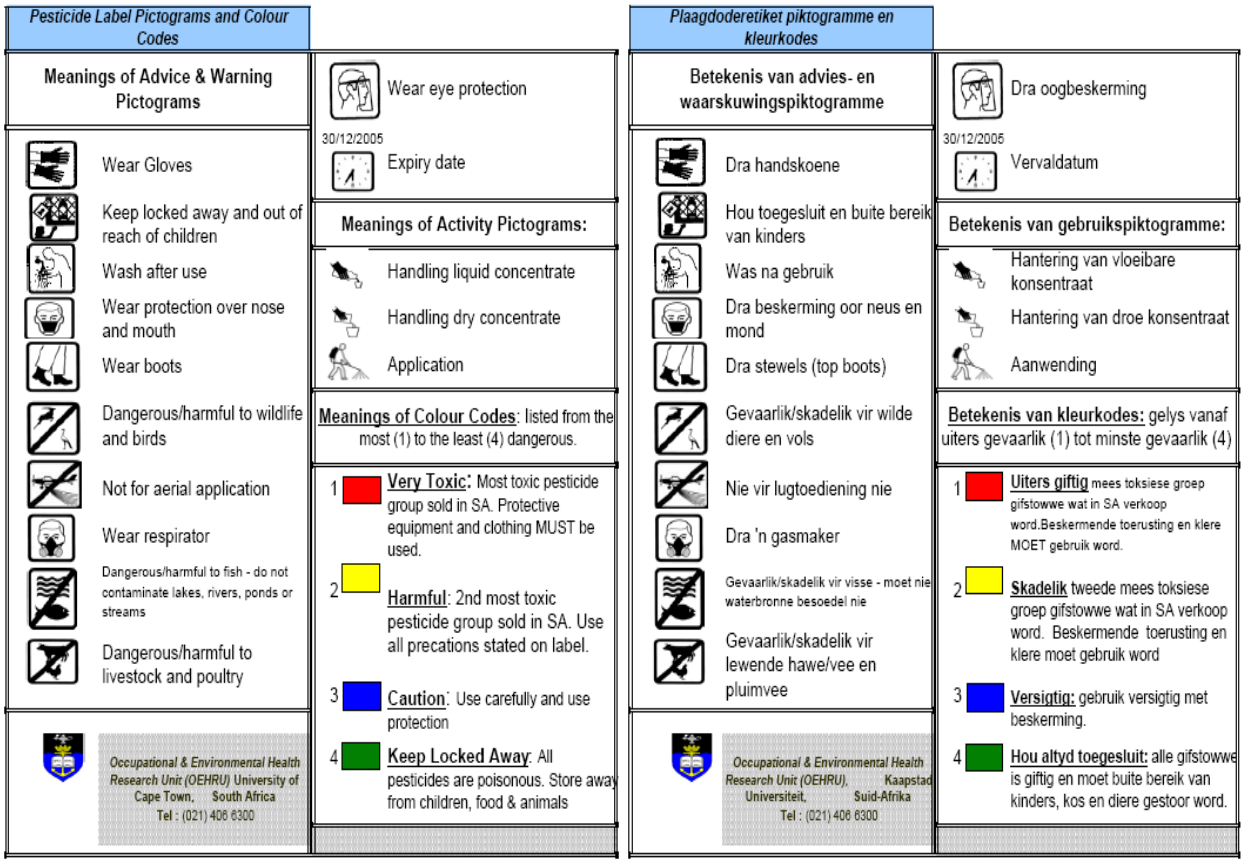
9. PESTICIDE LABEL INFORMATION



	Name:	Date:
Compiled by:	J Gouws	September 2011
Reviewed by:	E Kapp	September 2011
Approved by:	L Taljaard	September 2011

10. CONFIRMATION LIST OF STAFF THAT HAVE READ THIS DOCUMENT

Date	Name	Signature	Version

	Name:	Date:
Compiled by:	J Gouws	September 2011
Reviewed by:	E Kapp	September 2011
Approved by:	L Taljaard	September 2011