AN ASSESSMENT ON THE USE OF TISSUE CLEAR® VERSUS XYLENE IN DEPARAFFINIZING WAX CONTAINING SPECIMENS FOR ELECTRON MICROSCOPY

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Dedicated to the memory of my mother, Teodora.

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DECLARATION OF INDEPENDENT WORK

I, VALERICA NECSULESCU, do hereby declare that this project submitted for the degree MASTER TECHNOLOGIAE; BIOMEDICAL TECHNOLOGY in the SCHOOL OF HEALTH TECHNOLOGY at the CENTRAL UNIVERSITY OF TECHNOLOGY, FREE STATE, is my own independent work that has not been submitted before, to any institution by me or anyone else as part of any qualification.

_____ _____

Signature of student **Date**

VERKLARING TEN OPSIGTE VAN SELFSTANDIGE WERK

Ek, VALERICA NECSULESCU, verklaar hiermee dat die navorsingsprojek wat vir die verwerwing van die MAGISTER TECHNOLOGIAE: BIOMEDIESE TEGNOLOGIE in die SKOOL VIR GESONDHEIDSTEGNOLOGIE, aan die SENTRALE UNIVERSITEIT VIR TEGNOLOGIE, VRYSTAAT, deur my voorgelê word, my selfstandige werk is en nie voorheen deur myself of enige ander persoon, by enige ander instelling, ter verwerwing van enige kwalifikasie voorgelê is nie.

_____ _____

Handtekening van student **Datum**

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SUMMARY

Electron microscopy plays an important role in diagnostic histopathology. When this investigation is anticipated, extra tissue is submitted directly for electron microscopy. However, often it is decided only later in a problematic case to perform this investigation and then the only tissue available is embedded in the routine laboratory's paraffin block. This tissue has to be retrieved from the wax and this entails using a clearing agent before the rest of the electron microscopy method can be implemented. Traditionally xylene is the agent that is used but has the disadvantage of being extremely toxic.

This study compared the morphological effects of a relatively new and non toxic clearing agent, Tissueclear®, with that of xylene. Exposure of tissue to clearing agents for 30 minutes and overnight was performed to assess whether Tissueclear® gave better results in the long term than xylene, in the hope that the laboratory turn around time could be improved and the amount of toxic reagents used in the EM laboratory will be reduced. A second part of the study involved a questionnaire submitted to laboratory staff to assess their knowledge of xylene toxicity.

Of the 325 cases submitted for electron microscopy at Universitas Hospital between January 2004 and July 2005, 140 of these had to be retrieved from paraffin wax. Four specimens were prepared from each case. Two were processed in xylene for 30 minutes and overnight and two in Tissueclear® for 30 minutes and overnight. The specimens were evaluated for consistency and resin compaction as well as ultrastructural preservation of the cell membrane, cytoplasmic content and extracellular material.

The results showed that Tissueclear® and xylene gave comparable results after 30 minutes and that Tissueclear® was superior after overnight processing.

This meant that a specimen submitted for electron microscopy would be processed immediately without waiting for the following morning as was the case with xylene and that the processing time for such a specimen had been shortened from 3 to 2 days. It also meant that the laboratory staff was exposed to one less toxic reagent.

The results on the questionnaire showed that there were large areas of ignorance regarding toxicity as well as appropriate safety procedures that need to be followed in the laboratory. It is hoped that this study will improve awareness in this regard and encourage the use of other newer less toxic reagents.

OPSOMMING

Elektronmikroskopie speel ‘n belangrike rol in diagnostiese histopatologie. Indien hierdie ondersoek verwag word, word ekstra weefsel direk vir elektron mikroskopie ingestuur. Dikwels word daar eers later besluit, in ‘n probleem geval, om hierdie ondersoek uit te voer en dan is die enigste weefsel beskikbaar binne-in die roetine laboratorium se paraffinblok vasgelê. Hierdie weefsel moet van die paraffien herwin word en hierdie proses benodig die gebruik van ‘n ophelderingsmiddel voordat die volgende elektronmikroskopiese metode toegepas kan word. Tradisioneel was xileen die reagens wat gebruik was, maar het die nadeel dat dit baie toksies is.

Hierdie studie vergelyk die morfologiese uitkomste van ‘n relatief nuwe en nie toksiese ophelderingsmiddel, Tissueclear®, met die van xileen. Blootstelling van die weefsel vir 30 minute en oornag is uitgevoer om vas te stel of Tissueclear® beter resultate lewer as xileen en sodoende die laboratorium se voorbereidingstyd sou versnel. ‘n Tweede deel van die studie het ‘n vraelys ingesluit wat deur laboratoriumpersoneel beantwoord is om hul kennis met betrekking tot xileen se toksisiteit te toets.

Tussen Januarie 2004 en Julie 2005 is 325 gevalle vir elektronmikroskopie ingestuur. Van hierdie moes 140 van die paraffienblok herwin word. Vier monsters is van elke geval voorberei. Twee is in xileen geprosesseer vir 30 minute en oornag, en twee in Tissueclear® vir 30 minute en oornag. Die monsters is vir ooreenstemming en harskompaksie asook ultrastrukturele bewaring van die selmembraan, sitoplasmiese inhoud en ekstrasellulêre materiaal ge-evalueer.

Die uitslae het bewys dat Tissueclear® en xileen soortgelyke resultate na 30 minute blootstelling gelewer het, maar dat Tissueclear® beter in vergelyking met xileen na oornag blootstelling was.

Dit het beteken dat indien ‘n monster vir elektronmikroskopie ingestuur was, kon die prosessering onmiddellik begin sonder om vir die volgende oggend te wag soos in die geval van xileen en dat die prosesseringstyd vir ‘n monster van 3 na 2 dae verkort kan word. Dit beteken ook dat die laboratoriumpersoneel aan een minder toksiese reagens blootgestel word.

Die uitslae op die vraelys het groot areas van onkunde in verband met xileen se toksisiteit asook toepaslike veiligheidsmaatreëls wat in ‘n laboratorium gevolg moet word, getoon. Daar word gehoop dat hierdie studie bewustheid van laboratoriumveiligheid verbeter het en word die gebruik van ander nuwer en minder toksiese reagense aanbeveel.

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ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registration
CI	Confidence Interval
CNS	Central Nervous System
CEU	Continuous Educational Units
DER	Diglycidyl Ether of Polypropylene Glycol
EM	Electron Microscope
ERL	Vinylcyclohexene dioxide
GIT	Gastrointestinal Tract
H&E	Haematoxylin and Eosin
HCS	Hazardous Chemical Substances
MEL	Maximum Exposure Limits
NIOSH	National Institute for Occupational Safety and Health
NSA	Nonenil Succinic Anhydride
NTC	Tissueclear® for Normal Period of Time
NX	Xylene for Normal Period of Time
OES	Occupational Exposure Standard
TCO	Tissueclear® for Overnight
XO	Xylene for Overnight
RT	Room Temperature
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope

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AN ASSESSMENT ON THE USE OF TISSUE CLEAR® VERSUS XYLENE IN DEPARAFFINIZING WAX CONTAINING SPECIMENS FOR ELECTRON MICROSCOPY

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

The electron microscope plays an essential role in modern histopathological diagnosis. Electron microscopy (EM) can often provide the answer in diagnosing tumours when histology and immunohistochemistry have been unsuccessful. It is also an essential investigative tool in many other conditions including renal (Trump & Jones, 1980: 204), skin, blood (Bessis, 1972: 715), muscle and metabolic diseases (Mair & Tomé, 1972: 55-68).

Many different types of specimens are submitted to a histopathological laboratory for diagnostic purposes. These include tissue obtained during surgery and superficial and percutaneous biopsies, as well as cytology specimens obtained from fine needle aspirates of solid tumours, or body fluids (including effusions, peripheral blood and bone marrow). While some of these specimens are fixed in glutaraldehyde and sent directly to the EM laboratory, many of the tissue samples are initially fixed in formalin and embedded in paraffin wax as part of the routine light microscopy processing procedure. If, however, a diagnosis using light microscopy is not possible, EM is performed at a later stage and then the formalin fixed, paraffin embedded tissue is all that is

available. A special method is required to remove the paraffin wax from the tissue, which is then processed for EM.

Xylene, a clearing agent, is traditionally used in the routine histopathology laboratory (Bancroft & Stevens, 1982: 46) and is also used in the EM laboratory for deparaffinising tissue that has been embedded in paraffin wax. This reagent has toxic and inflammable properties (Wexler, 1998: 742-750) and, as with a number of other toxic substances used in the EM laboratory, requires careful handling and disposal, which is an expensive exercise (Bancroft & Stevens, 1982: 618). In recent years laboratories have become more safety conscious and aware of the risks when using certain chemicals. As a result, new and less harmful chemicals are being commercially produced, and need to be assessed as alternative reagents (Quinn, Fuller, Bello & Galligan, 2006).

A possible replacement for xylene is Tissueclear®. Tissueclear® was produced in the early 1990's (Sakura, 1998), in an attempt to overcome the many disadvantages associated with xylene use and other clearing agents commonly used in routine histopathology laboratories. It is non-toxic, non-carcinogenic, non-inflammable, virtually odourless and fully biodegradable, and as a result can be disposed of down a drain (Sakura, 1998).

This study compared the effect of Tissueclear® and xylene on tissue specimens' ultrastructure and morphology during paraffin wax removal. To our knowledge this was the first such examination performed (Sakura, 2001).

1.2 HYPOTHESIS

Tissueclear® does not affect the morphological results of EM specimens and can replace the xylene as a better alternative in the EM laboratory.

1.3 AIM

The aim was to compare the morphological effect of Tissueclear® with that of xylene when removing paraffin wax from specimens submitted for EM.

1.4 OBJECTIVES

The primary objective of the project was to replace xylene with the less toxic reagent Tissueclear®, but equally effective, which is used to remove paraffin wax from tissue examined by EM.

Sub-objectives were:

a) by comparing the effects of longer specimen exposure to Tissueclear®, so that preparation time may possibly be shortened, thus reducing laboratory turn around time and speeding up diagnoses.
b) to assess by means of a questionnaire the awareness amongst laboratory staff as to the potential dangers of xylene
c) to educate laboratory staff by providing feedback after completion of the questionnaire on the problems associated with xylene

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

Xylene is one of the reagents used in the electron microscopy (EM) laboratory. However, it has toxic properties and if a non toxic agent with similar clearing properties could be found it would be beneficial to the safety of the laboratory personnel (Bancroft & Stevens, 1982: 616-629; Wexler, 1998: 749).

In this review, the most important aspects of EM will be discussed, before briefly reviewing the current method of processing formalin fixed, paraffin embedded tissue for EM, and the problems associated with exposure to toxic chemicals including xylene in the laboratory.

The compound microscope pioneered by Galileo in the 16th century made an invaluable contribution towards understanding the normal human body and in diagnosis of diseases. In 1858, Virchow used a microscope to disprove the humoural theory where disease was attributed to an imbalance of humours, such as blood, black bile, yellow bile and phlegm. Using this instrument he showed that the body's cells and not its fluids were the most important components (Tildsley & Lakhani, 1992). It was not sufficient to study ultrastructural components using a compound microscope with X500 magnification, which is approximately 2 to 3 times the magnitude of cellular organelles, (Erlandson, 1994: 13).

The electron microscope, which was constructed in the 1900's and developed over a period of two decades has contributed to our ability to visualize cells and enables us to observe organelles at an ultrastructural

level. This instrument has become a very important tool in major medical centres throughout the world (Glauert, 1974: 39-57).

Two types of electron microscopes are commercially available. These are the Scanning Electron Microscope (SEM) and the Transmission Electron Microscope (TEM) (Koehler, 1973: 153-296).

With the SEM, the specimens are scanned with a focused beam of electrons which produce "secondary" electrons as the beam hits the specimens. These are detected and converted into an image on a screen, and a three-dimensional image of the surface of the specimen is produced. Three dimensional images of solid unsectioned specimens of complete biological units such as glomeruli, cilia and microvilli can be obtained (Koehler, 1973: 153-203), as well as cytoplasmic structures such as mitochondria. However, this technique has limited application in diagnostic histopathology especially when dealing with tumours (Erlandson, 1994: 20-21).

With TEM, sectioned specimens are examined by passing an electron beam of very short wavelength electrons, which are used for illumination through them. This results in a significant improvement in magnification. This is the instrument used in diagnostic histopathology (Wischnitzer, 1970: 6-8).

2.2 THE TRANSMISSION ELECTRON MICROSCOPE (TEM)

2.2.1 DESCRIPTION OF THE TEM

The TEM consists of an evacuated metal cylinder (the column) about 2 meters high with the source of illumination, a tungsten filament (the cathode), at the top, the anode plate, electromagnetic lenses, a fluorescent viewing screen and a photographic system (Bancroft & Stevens, 1982: 467-468). When the filament is heated and a high voltage

is passed between the cathode and anode, the filament will emit electrons. A high vacuum is always maintained in the column, than the electron's trajectory is not deviated by other particles. These negatively charged electrons are accelerated to the positive pole (the anode) placed just below the filament, some of which pass through a tiny hole in the anode, to form an electron beam. Electromagnets (condensers) placed at intervals in the column focus the electrons. By the use of condenser lenses and apertures the electron beam is focused onto the specimen that is clamped into the removable specimen holder. The specimen is on a copper grid and the grid is placed on the specimen holder. As the electrons pass through the specimen, they are focused by the objective lens onto a phosphorescent screen or photographic film to form an image. The enhancement of the image contrast is obtained by blocking the unfocused electrons with the objective aperture. The contrast of the image can be increased or lowered by reducing the size of this aperture. The remaining lenses on the TEM are the intermediate lens and the projector lens. The intermediate lens forms a real image on the fluorescent screen at the base of the microscope column (Wischnitzer, 1970: 8-108).

2.2.2 RESOLVING POWER OF THE TEM

The human eye can recognize two objects, if they are not closer than 0.1mm at a normal viewing distance of 25cm. This ability to optically separate two objects is called resolving power. Any finer detail than this can be seen by the eye only if the object is enlarged, by using optical instruments such as hand lenses, compound light microscopes and electron microscopes (Wischnitzer, 1970: 37-38; Weakley, 1972: 1-5).

2.2.3 SPECIMEN PREPARATION FOR TEM

The greatest obstacle in the examination of biological material with the electron microscope is the specific conditions to which specimens must

be exposed. Since the material must be exposed to a very high vacuum (1X100 000 mmHg), and electron beam, when being examined, it must be dried at some stage in its preparation. The specimen is treated with a complex series of techniques so that its ultrastructure is stabilized and is as close to that in the living material when exposed to the vacuum and electron beam. The limited penetrating power of electrons means that the specimen must be sliced into thin sections (50-100nm) to allow electrons to pass through it. The contrast is a very important aspect in EM and depends on the atomic number of the atoms in the specimen; the higher the atomic number, the more electrons are scattered and the greater the contrast. Biological molecules are composed of atoms of very low atomic number (carbon, hydrogen, nitrogen, phosphorus and sulphur). Thin sections of biological material are made visible by selective staining. Exposing the sections to salts of heavy metals such as uranium, lead and osmium, which have a high atomic number will increase the contrast and as a result the visibility of the tissue (Koehler, 1973: 2-63).

2.2.4 HISTORY, PERFORMANCE AND CURRENT USE

The first TEM was made by Max Knoll and Ernest Ruska at the Technical University of Berlin in 1931 and was capable of a magnification of only 17 times, and the era of ultra-structural and intracellular exploration began. As with light microscopy, the quest to investigate structures in even finer detail led to continual improvements, in electron optics. The 50nm resolution obtained in 1932 had been improved to 3nm by 1940, and now the modern transmission TEM can achieve a resolution of 0.14 nm (1.4 x $10^{-4}\mu m$) or better. The first electron micrograph of a biological specimen was published in 1934 (Erlandson, 1994: 1-2; Wischnitzer, 1970: 4-5).

Initially ultrastructural studies of specific neoplasms for diagnostic purposes were performed in a small number of cases in the United

States, Canada, and Europe but no significant articles specifically devoted to diagnostic TEM of tumours were published in the English literature. The first book / atlas devoted entirely to this subject was authored by Feroze Ghadially from the University of Saskatchewan, Saskatoon, Canada in 1980 (Erlandson, 1994:2-3).

Today, due to advanced technology, the transmission electron microscopes are sophisticated machines with most of the functions computerized and magnifications of up to 1 500 000 time are now possible (Arrgone National Laboratory, 2006). Electron microscopy has contributed to an understanding of the structural intricacies of normal and disordered cells and tissues and it has become an important and sometimes indispensable technique in the investigation and diagnosis of diseases including tumours, renal diseases, endomyocardial, skeletal and peripheral nerve diseases, certain skin diseases, storage diseases, viral diseases and the study of cilia. However, findings are always correlated with light microscopy, immunocytochemistry, other tests and the clinical history of the patient before a final diagnosis is made (Azar, 1988: 28-35; Erlandson, 1994: 43-52).

A new development in EM is nanotechnology, the technology of the very small (< 100 nm) and involves visualizing particles at an atomic level. This includes the ability to determine the structure of large proteins. Carbon nanotubes are being used as an alternative to tungsten as an electron source in high resolution electron microscopes, which enable smaller details to be visualized (De Jonge, Lamay & Schoots, 2002: 140-142). The technique is being used in industry (Miyagawa, Misra & Mohanty, 2005; Midgley & Wyland, 2003) and in medicine it is being applied in drug manufacture and viral studies (Zhao, Cao & Wan, 2002: 461-463), but at this stage has no place in diagnostic histopathology.

2.3 ELECTRON MICROSCOPY LABORATORY SAFETY

2.3.1 INTRODUCTION

Until recently the major emphasis in histopathology laboratories was on processing and reporting the specimens as rapidly and accurately as possible. However, recently there has emerged a new awareness in health care that hospital employees' health and as well as environmental safety are important issues (Quinn, Fuller, Bello & Galligan, 2006). These aspects are being addressed in the laboratory accreditation systems. Amongst the many hazards which are receiving attention are prevention of infection, exposure to radiation, the danger of fire and the handling, storage and disposal of chemicals (Bancroft & Stevens, 1982: 620-621). In contrast to earlier times there is a heightened awareness of occupational toxicology (Sheron, 2003; Wexler, 1998:743).

2.3.2 TOXICITY OF CHEMICALS USED IN THE ELECTRON MICROSCOPY LABORATORY

Toxicity refers to the negative effects of exposure to a toxin. These may vary from slightly to highly toxic (CHEC's HealtheHouse, 2002). The major routes of toxin exposure in a laboratory are by ingestion, through the skin and by inhalation (Schilling, 1973: 739).

The factors determining the harmful effects that occur following exposure to a toxic chemical are: the dose, period of exposure, pathway of exposure, other chemicals to which a person is also exposed, and individual characteristics such as age, gender, nutritional status, and health status (CHEC's HealtheHouse, 2002).

Xylene is only one of many toxic chemicals (fixatives, dehydrating agents, embedding materials, and staining reagents) that are used in processing tissue in the EM laboratory. Other adverse effects that may result from exposure to these chemicals range from allergies such as

asthma to malignant tumours (Parmeggiani, 1983: 2335-2336; Wexler, 1998: 749).

Osmium tetroxide and glutaraldehyde are the fixatives that are used in the EM laboratory. Short-term exposure to even low concentrations of osmium tetroxide vapours cause irritation to eyes resulting in weeping and conjunctivitis. They also irritate the mucous membranes. This may result in a sore throat or bronchitis, bronchial spasm and difficulty in breathing which can last for several hours. Inhalation may cause lung oedema. Long-term or repeated exposure may cause dermatitis, ulceration and discolours the skin black. It may damage the lungs and kidneys and cause blindness (Culling, 1974: 657-669; Parmeggiani, 1983: 2335; NIOSH, 1999b). Glutaraldehyde is a clear viscous colourless liquid with a pungent odour. On short-term exposure the substance irritates the mucous membranes. Long-term or repeated exposure may cause dermatitis and asthma (Curran, Burge & Wiley, 1996; NIOSH, 2000).

The dehydrating agents are ethanol and acetone. Ethanol and acetone vapours are not very toxic but may cause headaches. These substances are highly inflammable (Parmeggiani, 1983: 2336). The embedding agents used in EM are resins that contain reactive components which produce allergies on short-term exposure. The staining agents are lead citrate and uranyl acetate. Lead citrate is highly poisonous and a high concentration of airborne particles can be reached quickly. After short-term exposure, toxins affect the following: the gastrointestinal tract causing colic or constipation, blood causing anaemia, central nervous system (CNS) resulting in an encephalopathy clinically seen as headache, convulsions, delirium or even death, and in blood vessels causing hypertension with kidney damage. Long-term or repeated exposure to toxic agents results in symptoms of even greater severity

and may also result in paralysis of active muscle groups (Wexler, 1998: 747-748).

Uranyl acetate is uranium salt and is mildly radioactive and highly toxic. Although the amounts used are relatively small, both the chemical and radioactive toxicities of the compounds are significant. Toxicity affects kidneys and artery walls, and radiation may cause lung and bone cancer (Parmeggiani, 1983: 2335).

2.3.3 TOXICOLOGICAL PROFILE OF XYLENE

One of the two reagents involved in this study is xylene, also known as xylol. It is derived from petroleum and / or coal tar distillation and is an aromatic hydrocarbon. It is used in thinners, solvents for inks, rubbers, gums, resins, adhesives and lacquers, as paint removers and as intermediates in the production of plasticizer and polyester fibers. Xylenes are also extensively used as intermediates in the manufacture of perfumes, dyes, insecticides and pharmaceutical products. They easily dissolve hydrophobic compounds, especially fats, oils and waxes (ATSDR, 1995, 1996; Parmeggiani, 1983: 2336).

Xylene occurs in three isomers (ortho, metha and para xylene) and the xylene used in the laboratory is a mixture of all three. It does not mix with water but does with alcohol and acetone (Parmeggiani, 1983:2335). The reagent has traditionally been used in the routine histopathology laboratory as a clearing agent at the end of the preparation procedure. It has also been used as a solvent for removing paraffin wax from tissue for EM (Bancroft & Stevens, 1982: 616-629; ATSDR, 1995).

Unfortunately it is inflammable, associated with many toxic effects and requires careful disposal. It is a colourless liquid with a sweet smell at room temperature. Xylene is not apparatus friendly. It dissolves paint,

melts plastic containers, pens and pencils and destroys protective materials such as plastic goggles, plastic windows and gloves.

In the short-term, xylene irritates the eyes, respiratory and gastrointestinal tracts. Direct contact with the skin is irritating and will cause defatting which may lead to dryness, cracking, blistering and dermatitis. Central Nervous System depression resulting in drowsiness and confusion may also occur. It will cross the placenta and enter foetal tissue (Parmeggiani, 1983: 2335; Trujillo, Dang & Starck, 2003).

Long-term exposure result in similar symptoms, but are more severe in nature. Inhalation may result in CNS symptoms such as excitation followed by depression, parasthaesia, tremors, apprehension, impaired memory, weakness, vertigo, headache, anorexia, nausea and flatulence. Moderate but reversible bone marrow hypoplasia, liver enlargement and renal nephrosis may also occur (NIOSH, 1999a; Parmeggiani, 1983: 2336).

Animal studies have also produced CNS, renal and haematological changes as well as evidence of decreased foetal weight, increased numbers of foetuses per litter with skeletal variations, but no teratogenic effect (Saillenfait, Gallissot & Morel, 2003). Although there have been fears that xylene is carcinogenic (Quinn, Fuller, Bello & Galligan, 2006), no proof of this exists (ATSDR, 1996; D'Azevedo, Tannhauser & Tannhauser, 1996).

2.3.4 SAFETY EXPOSURE LIMITS AND REGULATIONS

The international occupational exposure standard (OES) level is not permitted to exceed 100 part of gas vapour per million [(ppm) of contaminated air by volume at 25°C and 760mmHg pressure] time weighted average over a ten hour period. The maximum exposure limits

(MEL) is 150ppm, within a maximum of 15 minutes period (Wexler, 1998: 744-745; Parmeggiani, 1983: 2335).

Xylene is classified under the category of Hazardous Chemical Substances (HCS) in the South African Safety Regulation Act. The toxic and inflammable characteristics make xylene a hazardous chemical waste, therefore the disposal is regulated by the Occupational Health and Safety Act (Schilling, 1973: 739-780, ATSDR, 1996).

Accredited medical laboratories are required to have a hazardous waste minimization plan in place (Cornell University, 2000). To reduce the risks associated with its use, xylene must be stored in metal fire-proof cabinets, which should be kept in purposely-built fire-resistant rooms with heavy metal fire-proof doors. The rooms should either be sunk below floor level or have a metal lip at the entrance to prevent the escape of burning liquid under the door. The disposal costs of xylene can be high since it is not permissible to discharge xylene into the environment *via* waste water. If xylene contaminates underground water it may remain there for years and be absorbed by plants, fish and birds. Discarded xylene needs to be stored in special drums or the laboratory has to have a special drainage system installed that is approved by the fire department. The stored xylene has to be disposed of by a licensed waste hauler. Highly corrosive or oxidizing agents like nitric acid must never be stored with xylene or other solvents. Fume cupboards and safety cabinets are required in the laboratories. Protective equipment is essential for personnel working with xylene. Activities such as eating, drinking or chewing gum are prohibited while handling this reagent to prevent accidental ingestion (Schilling, 1973: 744-756). The best waste minimization strategy is to replace highly hazardous chemicals with less hazardous or non-hazardous products (Cornell University, 2000; Maini, 1999; Quinn, Fuller, Bello & Galligan, 2006).

2.4 CHARACTERISTICS OF TISSUECLEAR®

Tissueclear® is a clearing agent with a similar function in the histology laboratory to xylene and was developed in the early 1990's (Sakura, 1998), as a less toxic alternative to xylene. It is non-toxic, non-carcinogenic, non-inflammable, virtually odourless and is fully biodegradable so that it can be disposed of *via* the drain. The chemical configuration is a long chain aliphatic hydrocarbon (Sakura, 2001).

No special measures have to be employed when handling and storing Tissueclear®. However, general protective equipment and hygienic measures are recommended as minor problems may result from exposure (Sakura, 1998).

A literature search has failed to trace any other information regarding Tissueclear®. Other clearing agents such as Micro-Clear, Slide-Brite, Shandon Xylene Substitute and Pro-Par (Maini, 1999), HistoSolve and ClearRite 3 (Pilot Study, 2003) do appear in the literature and have been tested in the routine laboratory. These are less toxic than xylene but not as innocuous as Tissueclear®. The first four reagents produced inferior morphological results (Maini, 1999) and the last two resulted in dry brittle tissue, difficult to handle.

2.4.1 TOXICOLOGICAL INFORMATION

Repeated or prolonged contact with xylene can degrease or desiccate the skin. It may irritate but not damage the eye tissues. If swallowed it may produce minimal toxicity. Small amounts if inhaled during swallowing or vomiting can cause bronchopneumonia or lung oedema. For that reason if any accidental inhaling or swallowing occurs, immediate medical attention is recommended (Sakura, 1998).

Tissueclear® is not hazardous for water but it is recommended that it is not disposed together with household garbage or reach sewage systems. It is not a marine pollutant (Sakura, 1998).

2.5 FINANCIAL IMPLICATIONS

Tissueclear® and xylene have a similar selling price, but Tissueclear® proves to be more cost effective when the storage and disposal costs are taken into account. Being less toxic the probability of an accident occurring is also reduced, with less chance of medical treatment being required (Sakura, 2001).

2.6 MOTIVATION FOR THE PROJECT

As a result of the obvious advantages of Tissueclear® and the knowledge that it has proved to be a good alternative clearing agent in the routine histopathology laboratory, this project was designed to assess whether the clearing properties could also be utilized in the EM laboratory.

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN

An experimental study was performed on specimens submitted for EM to compare the morphological results of tissues processed with xylene to those of Tissueclear®. A descriptive study comprised the second part of this project which involved the use of a questionnaire submitted to laboratory personnel concerning their awareness of the toxic properties of xylene.

3.2 MATERIALS

3.2.1 TISSUE SAMPLES EXAMINED BY ELECTRON MICROSCOPY

Between 1 January 2004 and 1 June 2005, 325 specimens were submitted to the EM laboratory at Universitas Hospital, Bloemfontein, South Africa (Figure 3.1). One hundred and forty (140) of these had to be retrieved from paraffin wax because there was no other tissue available. The types of tissue retrieved from the paraffin wax are shown in Figure 3.2. The specimens retrieved from paraffin wax were utilized in the study to compare the morphological quality of the tissue processed with xylene or Tissueclear®.

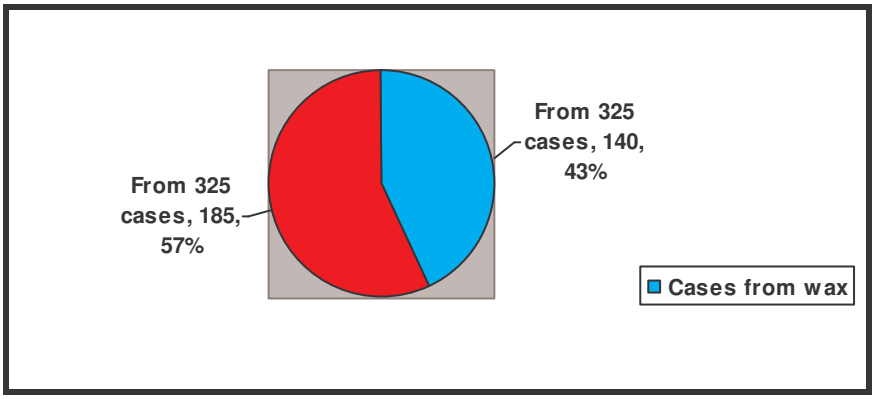

Figure 3.1: Specimens submitted to the EM laboratory at Universitas Hospital, Bloemfontein, South Africa (January 2004 to June 2005)

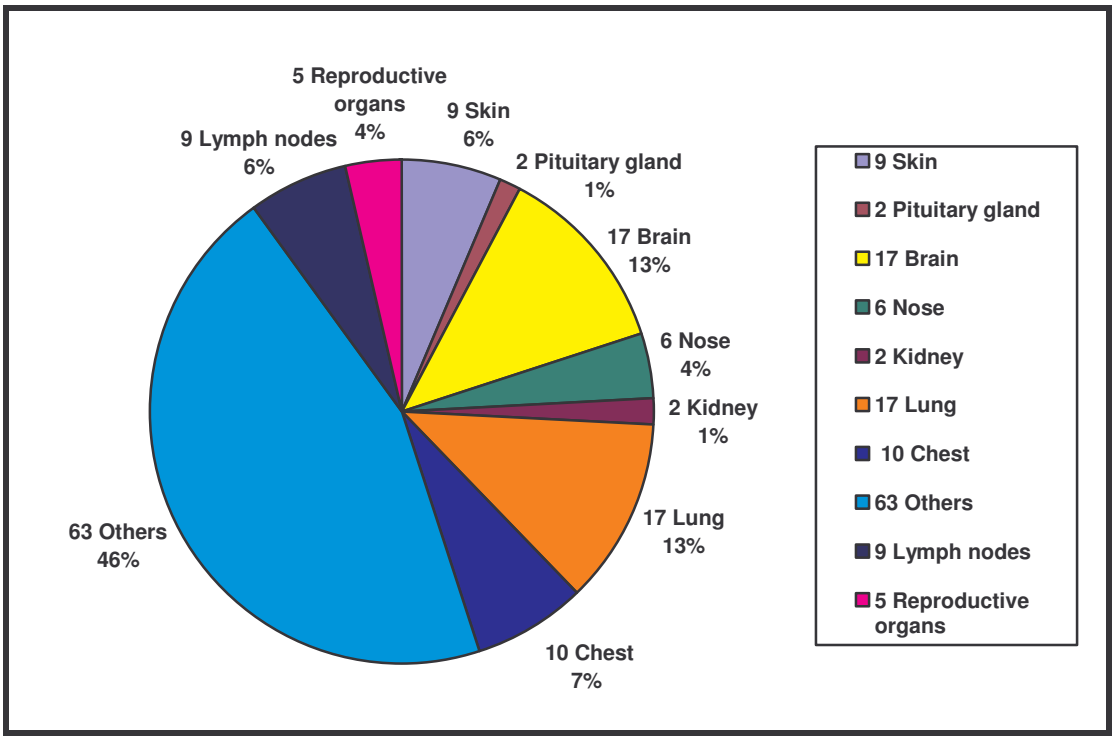

Figure 3.2: Types of tissue retrieved from paraffin wax (n=140)

3.2.2 QUESTIONNAIRE REGARDING XYLENE TOXICITY AMONG LABORATORY WORKERS

A questionnaire (Appendix B) was compiled regarding the toxicity of xylene and distributed among laboratory technologists working at the Anatomical Pathology, Cytology, Genetics, Haematology, Chemical Pathology and Microbiology Laboratories at Universitas Hospital Bloemfontein, South Africa (n=85). The questionnaire included demographics information, knowledge regarding xylene toxicity, symptoms, safety regulations, disposal and experience on xylene exposure. The technologists were requested to complete the questionnaire anonymously and were subsequently provided with feedback information on the topic. For taking part in this exercise they were awarded three Continuous Educational Units (CEU) points.

3.2.3 APPARATUS

The apparatus used during the procedures listed in the table 3.1 was in good working condition and accurately calibrated.

Table 3.1: Apparatus used

Apparatus	Description	Brand name	Suppliers
Rotator	Agar Scientific Multivoltage	Agar Scientific, England	Laboratory and Scientific Equipment Co (Pty)Ltd
Magnetic stirrer	IKE-Combimag-RCHD	IKE-Combimag-RCHD, Austria	Optolabor (Pty)Ltd
Incubation oven	Reichert	Reichert, Austria	Optolabor (Pty)Ltd
Knife Maker	Knife Maker 7800	LKB KnifeMaker 7800, Sweden	Laboratory and Scientific Equipment Co (Pty)Ltd
Pyramitome	LKB Pyramitome 11800 Ultramicrotoy System	LKB Pyramitome, Sweden	Optolabor (Pty)Ltd
Ultramicro-tome	Reichert Om U3 Ultramicrotome	Reichert, Austria	Optolabor (Pty)Ltd
Ultratome	Ultratome III LKB 8800	LKB Bromma, Sweden	Optolabor (Pty)Ltd
Dissecting microscope	Leica	Leica	Set Point Instruments
Light microscope	Zeiss	Zeiss, Germany	Optolabor (Pty)Ltd
Electron microscope	Philips Tecnai 10	Philips, Holland	Philips (Pty)Ltd
Digital camera	MegaView II	Philips, Holland	Philips (Pty)Ltd

3.2.4 REAGENTS

Reagents were obtained from the main suppliers of high grade chemicals in South Africa and are mentioned in Table 3.2.

Table 3.2: Reagents used

Reagent	Used for	Brand name	Suppliers in South Africa
Glutaraldehyde	Fixation	Merck	Merck Chemicals (Pty) Ltd. South Africa
Osmium tetroxide	Fixation	Merck	Merck Chemicals (Pty) Ltd. South Africa
Sodium dihydrogen orthophosphate	0.1M phosphate buffer	Merck	Merck Chemicals (Pty) Ltd. South Africa
Sodium citrate	0.1M phosphate buffer	Merck	Merck Chemicals (Pty) Ltd. South Africa
100% Alcohol	rehydration	Merck	Merck Chemicals (Pty) Ltd. South Africa
100% Acetone	dehydration	Merck	Merck Chemicals (Pty) Ltd. South Africa
Uranyl acetate	Staining EM	Merck	Merck Chemicals (Pty) Ltd. South Africa
Lead citrate	Staining EM	Merck	Merck Chemicals (Pty) Ltd. South Africa
Sodium hydroxide	Staining EM	Merck	Merck Chemicals (Pty) Ltd. South Africa
Sodium veronal	Palade's buffer	Merck	Merck Chemicals (Pty) Ltd. South Africa
Sodium acetate	Palade's buffer	Merck	Merck Chemicals (Pty) Ltd. South Africa
Toluidine blue	Staining LM	Merck	Merck Chemicals (Pty) Ltd. South Africa
Xylene	Dewaxing	Merck	Merck Chemicals (Pty) Ltd. South Africa
Tissueclear	Dewaxing	Sakura	Bayer
Spurr's epoxy resin	Embedding	Agar	Wersam Scientific(Pty) Ltd. South Africa
Gelatine capsules	Embedding	Merck	Merck Chemicals (Pty) Ltd. South Africa

3.2.5 GLASSWARE AND PLASTICS

Disposable plastics and high quality glassware that were used during procedures are found in Table 3.3.

Table 3.3: Disposable plastics and high quality glassware

Product	Description	Used for	Suppliers in South Africa
Glass vials	5ml	Processing	General Medical Supplies
Plastic pipettes	2.5ml	Processing	PlastPro Scientific
Petri dishes		Staining	General Medical Supplies
Pasteur pipettes	150ml	Staining	General Medical Supplies
Glass bars	410mm/25mm	Making knives	Wersam Scientific
Beakers	150ml	Preparation of resin	General Medical Supplies

3.2.6 ACCESSORIES AND INSTRUMENTS NECESSARY FOR ELECTRON MICROSCOPIC PROCESSING, CUTTING AND STAINING

Accessories used are listed in Table 3.4.

Table 3.4: Accessories used in electron microscopy

Accessory name	Use	Brand name
Razor blades	Cutting	General Medical Supplies
Tweezers	Cutting, staining	General Medical Supplies
Scalpel	Cutting, making the troughs	General Medical Supplies
Wax	Making the troughs	Merck
Glass knives	Cutting thin and ultra thin sections	Wersam Scientific (Pty) Ltd
Diamond knives	Cutting ultra thin sections	Wersam Scientific (Pty) Ltd
Allen key 3mm	Cutting	General Medical Supplies
Copper grids 200 mesh	Support the sections for EM	Wersam Scientific (Pty) Ltd

3.3 METHODS

3.3.1 INTRODUCTION

Standard methods were used to process and stain the specimens removed from paraffin wax (Culling, 1974:658-659; Hajibagheri & Nasser, 1999; 342-382), apart from the step in removing the wax from the paraffin block (see 3.3.4).

3.3.2 REMOVAL OF TISSUE FROM THE PARAFFIN BLOCK

In the 140 cases used for this study, the pathologist first marked the area to be examined on a slide stained with Haematoxylin and Eosin (H&E) for light microscopy with a pen. The EM staff then retrieved the paraffin block from the routine laboratory. The fragment of tissue was removed from wax block with a scalpel and divided in two fragments. One was placed in a vial with xylene and the other one in a vial with Tissueclear®.

3.3.3 CUTTING-UP

The tissue was cut using a dissecting microscope because the size of the tissue fragments were too small to handle without magnification and unwanted areas such as blood clot and collagen were dissected from the fragments. From each fragment, two pieces of tissue were cut, and placed in two vials with xylene and two vials with Tissueclear®. Each fragment was then sliced into five smaller ones, which were the final pieces of tissue to be used for EM examination. The tissue was sliced with a razor blade on a glass surface in one drop of either Tissueclear® or xylene. Razor blades were used to avoid mechanical damage to the tissue. Finally, there were four glass vials with the five small fragments in each, two containing xylene (one for the usual 30 minute processing time and one for overnight processing) and the other two containing Tissueclear® (processed as with xylene). Each was labelled with a registered number.

3.3.4 DEPARAFFINISING

Deparaffinising was performed four times for each case instead of the usual single procedure. Two vials, one containing 3ml of xylene and the other 3ml of Tissueclear® were kept in the oven to melt the wax at 45°C for 30 minutes and another two which also contained xylene and Tissueclear® were left overnight at the same temperature.

3.3.5 REHYDRATION

After cutting and deparaffinising, the tissue was rehydrated with decreasing concentrations of alcohol from 100% to 40% (see Table 3.5) to soften the tissue and to return it to as close to its original state as possible.

Table 3.5: Steps for processing specimens for EM (Hayat, 1070:15-26)

Solutions used	How many times	Temperature	Duration	Effects
Tissueclear®/xylene	2	45°C	15 min each	wax removal
100% alcohol	3	RT	5 min each	rehydration
90% alcohol	2	RT	5 min each	rehydration
70% alcohol	2	RT	5 min each	rehydration
40% alcohol	2	RT	5 min each	rehydration
Phosphate buffer	2	RT	5 min each	rehydration
Osmium tetroxide	1	RT	90 min	secondary fixation
70% acetone	2	RT	10 min each	dehydration
90% acetone	2	RT	10 min each	dehydration
100% acetone	3	RT	10 min each	dehydration
1:1, 100% acetone and Spurr's epoxy resin	1	RT	90 min	impregnation
Spurr's epoxy resin	1	RT	30 min	impregnation
Spurr's epoxy resin	1	45°C	30 min	impregnation
Spurr's epoxy resin	1	Over night	70°C	polymerization

RT-Room Temperature

3.3.6 PROCESSING

Routine processing for EM include: fixation, secondary fixation, dehydration, impregnation, and embedding. This procedure is

summarised in Table 3.5. Since the tissue specimens were very small, the processing involved removing the solutions with a pipette and immediately replacing them with the next solution. Great care was taken that the tip of the pipette did not damage the tissue specimen. The solution changes during processing had to be carried out with speed so that the specimens did not dry out. Specimens stayed in the original vial until the dehydration and impregnation process was finalized. After that the specimens were ready to be transferred into the gelatine capsules for embedding.

3.3.6.1 Secondary fixation

The specimens submitted directly for EM are fixed in 3% glutaraldehyde (primary fixation) (Hayat, 1970: 32). However, the specimens involved in this project had to be removed from wax. These had already been fixed in 10% buffered formalin when they were processed for light microscopy so the primary fixation step was omitted in this study. Secondary fixation was performed with 1% osmium tetroxide in distilled water and Palade's Buffer. Osmium tetroxide was prepared in a fume cupboard initially in a stock solution of 2% and then in a 1% working solution.

3.3.6.2 Dehydration

Water was removed from the fixed tissue and replaced with acetone in increasing concentrations from 70% to 100%, before the tissue was embedded in a water-insoluble resin.

3.3.6.3 Impregnation

Suitable embedding material penetrating the tissues is a prerequisite for satisfactory sectioning. Impregnation involves a gradual replacement of the dehydrating agent, acetone with the embedding medium, Spurr's epoxy resin (Hayat, 1970:30-45). First the tissue was treated with a mixture of 1:1 acetone and Spurr's epoxy resin for 90 minutes at room

temperature (RT), then for 30 minutes in pure Spurr's epoxy resin at RT and then for 90 minutes in the oven at 54ºC. To ensure good exposure to the medium a rotator was used.

3.3.6.4 Embedding

The embedding medium was Spurr's epoxy resin which was freshly prepared every day. The components were: Hardener (NSA), Plastic (ERL), Plasticizer (DER) that controls hardness, and Accelerator (S-1) that controls the rate of hardening (Hayat, 1970: 39-49). The amount of epoxy resin prepared depended on the number of specimens being processed in a particular batch (see Table 3.6).

Table 3.6: Quantities of Spurr's epoxy resin components required for different numbers of specimens

	1 SPECIMEN	2-3 SPECIMENS	3-4 SPECIMENS	5-6 SPECIMENS
Hardener (NSA)	6,5 g	13 g	19,5 g	26 g
Plastic (ERL)	2,5 g	5 g	7,5 g	10 g
Plasticizer (DER)	1,5 g	3 g	4,5 g	6 g
Accelerator (S-1)	0,1 g	0,2 g	0,3 g	0,4 g

The components were stirred continuously with a magnet on a magnetic stirrer, to be homogenously mixed and to prevent the formation of air bubbles. The specimens were placed in pre-dried gelatine capsules labelled with the appropriate registration number, filled with Spurr's epoxy resin and placed in a Reichert polymerisation oven.

3.3.6.5 Polymerisation

Polymerisation took place over 18-20 hours in the oven at a temperature of 70°C and resulted in a block suitable to be sectioned into ultra-thin sections.

3.3.7 MAKING GLASS KNIVES

Glass knives were made in our laboratory to trim the block and cut thick sections. A special glass strip was washed, rinsed and dried, to remove any dust and grease. Then the Knife Maker LKB 7800 B was used for the two steps in making the knife; first breaking squares and then making the glass knife. The cutting edge of the knife was examined under the microscope. Not all the glass knives prepared proved satisfactory, and some after evaluation under the microscope were not considered acceptable. A trough was mounted on a glass knife to hold the distilled water onto which the sections were cut.

3.3.8 CUTTING SECTIONS FOR ELECTRON MICROSCOPY

3.3.8.1 Trimming the block and cutting thick sections

For cutting thick sections and trimming the block glass knives were used in a LKB 11800 Pyramitome. The first step of trimming consisted of cutting away the resin until the specimen was seen. Sections were cut at 2μ thickness, placed on glass slides and stained with 1% Toluidine Blue. On these sections the representative area was selected and only that area was left on the block. The other parts were trimmed out because the ultra-thin sections were cut from the area with tissue (Figure 3.3). The size of the tissue surface was 0.1mm/0.1mm. The operation of trimming is one of the most important prerequisites for successful sectioning on the ultramicrotome, and the LKB 11800 Pyramitone is a combined histo-microtome and pyramid-maker intended for preparation of specimens for cutting ultra-thin sections for EM (Glauert, 1974: 41-46). The entire cutting routine was observed through a stereo-microscope with 10x and 20x magnification. One eyepiece has graticules (micrometer hair lines) for alignment purposes and section size determination.

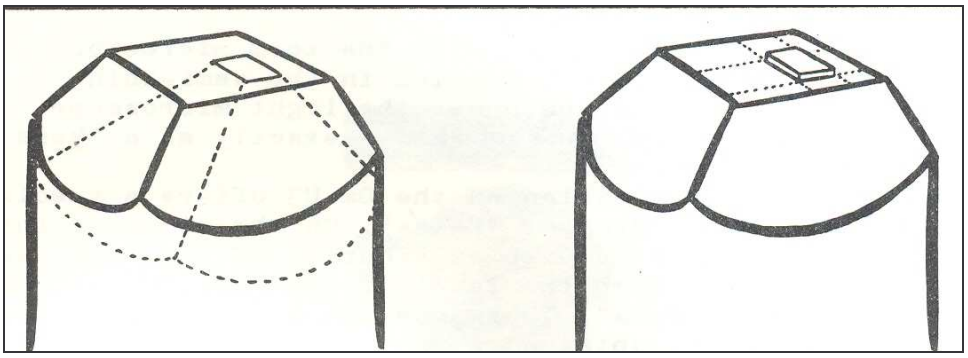

Figure 3.3: Identifying the specimen area and shaping the section through the trimming process (Bancroft & Stevens, 1982: 416)

3.3.8.2 Cutting ultra-thin sections

Ultra-thin sections were cut using a Reichert ultramicrotome, LKB ultratome, and a Diatome diamond knife. The ultratomes were protected from vibrations and air currents by placing them in cubicles especially built to protect the working area. The Diatome diamond knife was used following the instructions available in the specific manual (Diatome, 1989).

The sections were floated on a boat attached to the knife. These were assessed for the correct thickness by their colour (Figure 3.4). The thickness of the sections must be uniform and without chatters. The thickness of the optimal sections is indicated by their interference colour. The colour gold measuring between 90 and 100 nm was selected.

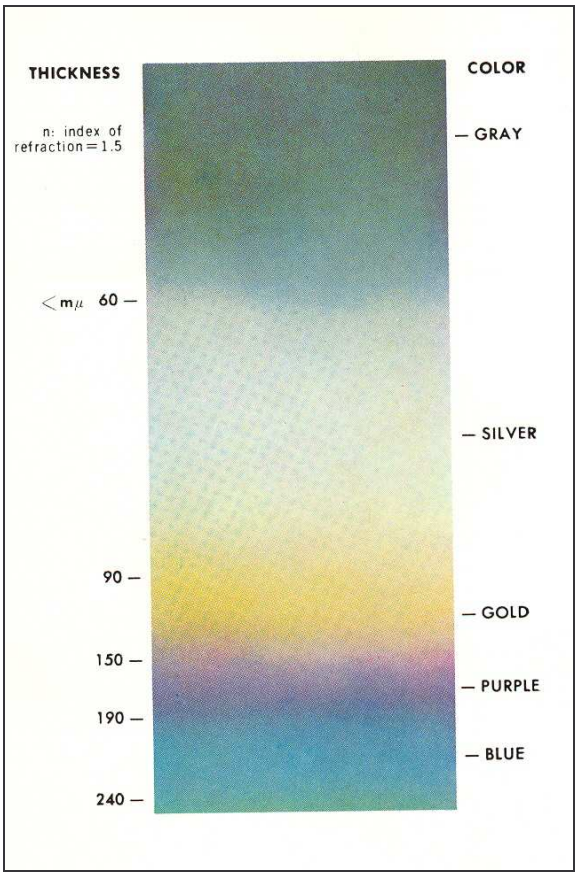

Figure 3.4: Continuous interference colour index and thickness scale for ultra-thin sections. The thickness of sections used for electron microscopy may be estimated within 90 to 100nm using this scale by noting the colours of the sections as they float in the collecting trough (Hayat, 1970: 1)

Before the sections were placed on copper grids, they were stretched while floating in the trough by being exposed to chloroform vapours (Figure 3.5). For each specimen, two grids were prepared with approximately 15 sections on each grid. The grids were left to dry in an oven at 45°C for 30 minutes. The blocks were always cut in numerical order because the grids could not be labelled. The grids were stored to enable them to dry and were stained in the same order.

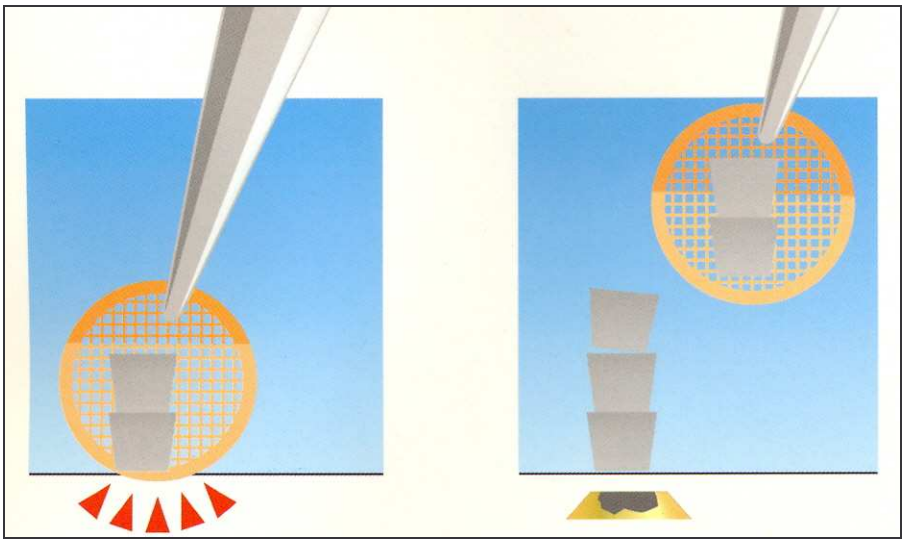

Figure 3.5: Picking-up the ultra-thin section from water onto copper grids (Diatome, 1989)

3.3.9 STAINING

3.3.9.1 Staining ultra-thin sections

Standard staining procedures as given in Hayat (1970: 13-318) were used. The staining procedure involved impregnation of the tissue with heavy metal salts like uranium, lead and osmium. Two Petri dishes partially filled with dental wax were the containers used for staining. With the aid of a clean glass pipette a small quantity of uranyl acetate was drawn up from below the surface of the staining solution. The first drop of solution was put aside and then two drops for each case were placed on the staining surface (one for each grid, Figure 3.6).

Case 1	Case 2	Case 3
O	O	O
O	O	O

Figure 3.6: The grid was floated with the section side down on a drop of staining solution

The ultra-thin sections were treated for 30 minutes with a saturated aqueous solution of uranyl acetate that resulted in increased contrast of the tissue that had been fixed with osmium tetroxide, which had stained all the structures a uniform grey. The sections were then washed with sterile distilled water in three beakers. The washing was accomplished by holding the grid at its edge with a forceps and dipping it rapidly under the surface of the fluid and by gentle agitation in the beaker. The grids with sections were dipped 10 times into the first two beakers and 20 times into the last one. The washed grids were blotted dry with filter paper making certain that the sections were not touched by the filter paper. The preparation of the second Petri dish was made immediately after the grids were placed in the first dish. In the second Petri dish several pellets of sodium hydroxide were placed at one side of the dish. This removed carbon dioxide from the solution, which was necessary when staining with lead citrate to prevent precipitations from forming on the sections. Two drops of lead citrate solution per specimen were placed on the other side of the dish, and the dish was then covered with a lid. The dried grids were placed in the drops of lead citrate by raising the lid of the dish just enough to be able to introduce the forceps with the grid. This was done to avoid carbon dioxide contamination. After five minutes, the grids were rinsed one by one, in the first beaker with a solution of 1N sodium hydroxide in sterile distilled water and then in two other beakers with sterile distilled water. These were then blotted dry with filter paper and placed in a grid box. The position of the grids and corresponding case number were recorded. The staining method is summarized in Table 3.7.

Table 3.7: Staining method summary (Hayat, 1970, 82-94)

Solution	Procedure	Time	Temperature	Special precautions
Uranyl acetate	Staining	30 minutes	RT	Clean
Sterile distilled water – 3 beakers	Rinse the grids	1 minute	RT	Gentle movement to not wash the sections from the grids
Lead citrate	Staining	5 minutes	RT	Avoid carbon dioxide contamination
Sterile distilled water – 3 beakers Two drops of 1N sodium hydroxide added to 1st beaker	Rinse the grids	1 minute		No talking and breathing during rinsing Gentle movement to not wash the sections from the grids

RT-Room Temperature

3.3.10 EXAMINATION AND ANALYSIS

3.3.10.1 Introduction

The specimens were examined, blinded with a Philips Tecnai 10 electron microscope. The preservation of the ultrastructural structures was evaluated, especially those structures important in making a diagnosis. As is the usual procedure in EM the morphological features were photographed as a record. The photomicrographs were taken with a digital camera type Mega View II.

3.3.10.2 Methods of analysis

Specimens were evaluated on grounds of light microscopic and electron microscopic appearance. The specimens' morphology was compared with the classical appearance described in atlases and books on ultrastructure (Henderson & Papadimitriou, 1982:128-355; Ghadially, 1985: 125-487; Rosai, 2004: 103-359). In each case, the 4 blinded specimens were assessed separately and the evaluation was noted on data sheets.

3.3.10.2.1 Light microscopy evaluation

A dissecting microscope was used to assess the consistency of the tissue (hard or soft, dry or wet, brittle or not,) and a light microscope to assess the tissue for cracks. The resin compaction was assessed by the presence of defects in the resin.

3.3.10.2.2 Electron microscopy evaluation

This involved the evaluation of: resin compaction and cracks, and the ultrastructural preservation of the tissue. Preservation was recorded on a scale of 1 to 3. The following structures were evaluated independently by two members of the EM staff: cell membrane including the basal lamina and intercellular junctions, cytoplasmatic content including granules, filaments, vacuoles, vesicles and specific structures (premelanosomes, neurosecretory granules, tonofibrils), and extracellular material.

The scale was as follows:

1 –poor - impossible to identify the structure

2 – partial - the structures were partially preserved, but identifiable

3 – good – structures preserved and easily identifiable.

3.4 STATISTICS

3.4.1 MORPHOLOGICAL ANALYSIS

Results of the tissue evaluation were recorded on coded data sheets. The groups were described by frequencies and percentages and compared by the chi-square or Fischer's exact test, whichever applicable. An alpha level of 0.05 was used to evaluate statistical significance.

3.4.2 QUESTIONNAIRE

The results from the questionnaires were recorded on coded data sheets. The SAS® System for Windows (version 8.2) was employed for

all statistical calculations. Continuous variables were described by the mean, standard deviation (std), minimum (min) and maximum (max). The percentages of subjects having the correct knowledge for applicable questions were calculated and 95% confidence intervals (CI) for these percentages were calculated using the method proposed by Altman(Altman, Machin, Bryant, & Gardner, 2000:117-203).

CHAPTER 4

RESULTS

4.1 INTRODUCTION

The results of 140 cases were divided into 560 specimens (S), which in turn were equally divided into 4 groups. Thus, each case was represented in each group assuring that the preservation was the same. Each of the 4 treatment groups were as follows: Tissueclear® for a normal period of 30min (NTC), overnight with Tissueclear® (OTC), xylene for a normal period of 30min (NX), and overnight with xylene (OX). The groups were described by frequencies and percentages and compared by the chi-square or Fischer's exact test, whichever applicable. An alpha level of 0.05 was used to evaluate statistical significance.

4.2 SPECIMEN RESULTS

4.2.1 NUMBER OF SPECIMENS FROM EACH REGION OF THE BODY

The specimens were organized into categories based on the region of the body from where they were removed and marked from 1 to 10 in the statistical analysis (Table 4.1).

Table 4.1: Number of specimens from each region of the body

Number	Region	NTC#	OTC#	NX#	OX#	Total	Percentage
1	Skin	9	9	9	9	36	6.43
2	Pituitary gland	2	2	2	2	8	1.43
3	Brain	17	17	17	17	68	12.14
4	Nose, trachea	6	6	6	6	24	4.29
5	Kidney	2	2	2	2	8	1.43
6	Lung	17	17	17	17	68	12.14
7	Chest wall	10	10	10	10	40	7.14
8	Other	63	63	63	63	252	45
9	Lymph node	9	9	9	9	36	6.43
10	Reproductive organs	5	5	5	5	20	3.57
	Total	140	140	140	140	560	100

#NTC: Tissueclear® for 30 minutes; OTC: Tissueclear® overnight, NX: xylene for 30 minutes, OX: xylene overnight

4.2.2 CONSISTENCY ASSESSMENT USING A DISSECTING MICROSCOPE

Consistency results are summarized in Table 4.2. The consistency all (n=140, 100%) the samples treated with Tissueclear® in both groups, NTC and OTC, stayed soft and were not brittle. The opposite was found during treatment with xylene, where all the samples in both groups (n=140, 100%) were dry and brittle. After treatment with Tissueclear® (NTC and OTC), all the samples stayed wet, whereas all the samples treated with xylene (NX and OX) were dry. Due to the obvious difference in all specimens when Tissueclear® was compared to xylene, statistically significant differences, as indicated by Fisher's exact test ($p<0.0001$), were found.

Table 4.2: Consistency results

Procedure#	Dissection microscope evaluation						
	Consistency				Dryness		
	Dry	Soft	Brittle	Not brittle	Dry	Wet	Total
NTC		140		140		140	140
OTC		140		140		140	140
NX	140		140		140		140
OX	140		140		140		140
Total							560 (100%)

NTC: Tissueclear® for 30 minutes; OTC: Tissueclear® overnight, NX: xylene for 30 minutes, OX: xylene overnight.

4.2.3 RESIN COMPACTION AND CRACK ASSESSMENT UNDER LIGHT AND ELECTRON MICROSCOPES

Table 4.3 summarises resin compaction and crack assessment as seen under light and electron microscopes. All tissues treated with Tissueclear® in both groups (NTC and OTC) resulted in good resin compaction and no cracks. The direct opposite was seen in tissues treated with xylene (NX and OX). Figures 4.1 and 4.2 are EM photographs

of tissue treated overnight with xylene and Tissueclear®, respectively. Similar results were obtained during assessment with light and electron microscopy. Statistically significant differences were found when Tissueclear® was compared with xylene, as indicated by Fisher's exact test ($p<0.0001$).

Table 4.3: Resin compaction and crack assessment

Procedure#	Light microscopy				Electron microscopy				Total
	Resin compaction		Cracks		Resin compaction		Cracks		
	Yes	No	Yes	No	Yes	No	Yes	No	
NTC	140			140	140			140	140
OTC	140			140	140			140	140
NX		140	140			140	140		140
OX		140	140			140	140		140
Total									560

NTC: Tissueclear® for 30 minutes; OTC: Tissueclear® overnight, NX: xylene for 30 minutes, OX: xylene overnight

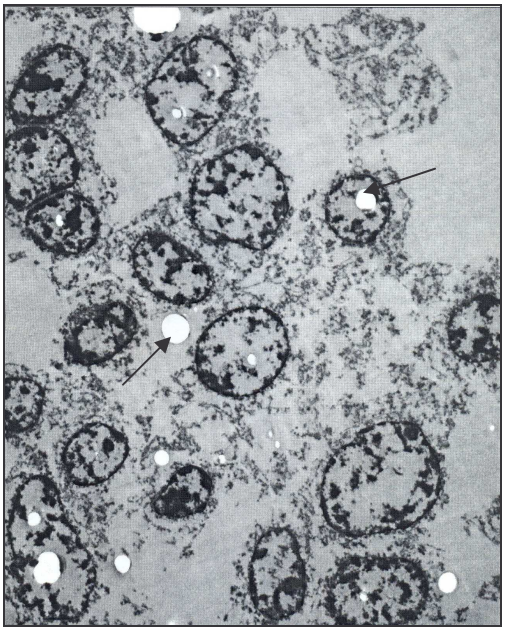

Figure 4.1: Holes (arrows) in the resin after treatment with xylene overnight (X2550)

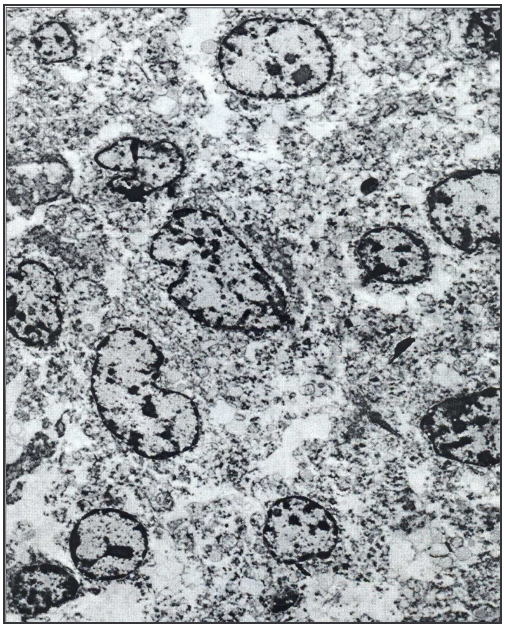

Figure 4.2: Compact resin after treatment with Tissueclear® overnight (X2550)

4.2.4 ULTRASTRUCTURAL PRESERVATION ASSESSMENT

The preservation of certain structures were evaluated independently and blind, by two members of the EM staff and then recorded on a scale of 1 to 3 representing good, partial and poor preservation (Table 4.4).

Table 4.4: Ultrastructural preservation of specimens treated with Tissueclear® and xylene over a normal period of time (NTC and NX, respectively) and overnight (OTC and OX, respectively)

Ultrastructure preservation	Scale	NTC n (%)	OTC n (%)	NX n (%)	OX n (%)
Cell membrane	Poor	28 (20)	28 (20)	28 (20)	39 (27.86)
	Partial	11 (7.86)	11 (7.86)	11 (7.86)	101 (72.14)
	Good	101 (72.14)	101 (72.14)	101 (72.14)	0 (0)
	Total	**140 (100)**	**140 100)**	**140 (100)**	**140 (100)**
Cytoplasmic content	Poor	1 (0.71)	1 (0.71)	1 (0.71)	27 (19.29)
	Partial	39 (27.86)	39 (27.86)	39 (27.86)	113 (80.71)
	Good	100 (71.43)	100 (71.43)	100 (71.43)	0 (0)
	Total	**140 (100)**	**140 (100)**	**140 (100)**	**140 (100)**
Extra cellular material	Poor	1 (0.71)	1 (0.71)	1 (0.71)	28 (20%)
	Partial	39 (27.86)	39 (27.86)	39 (27.86)	112 (80%)
	Good	100 (71.43)	100 (71.43)	100 (71.43)	0 (0)
	Total	**140 (100)**	**140 (100)**	**140 (100)**	**140 (100)**

4.2.4.1 Cell membranes

Cell membranes including the basal lamina and intercellular junctions were evaluated and scored as poor, partial or good. Figure 4.3 represents cell membrane preservation and Figures 4.4 and 4.5 are EM photographs of cell membrane preservation after overnight treatment with Tissueclear® and xylene, respectively.

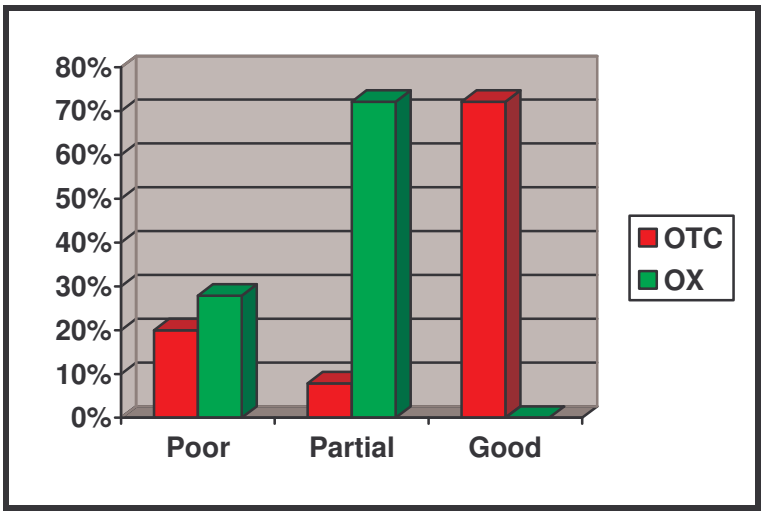

Figure 4.3: The preservation of cell membranes treated with xylene (OX) and Tissueclear® (OTC) overnight

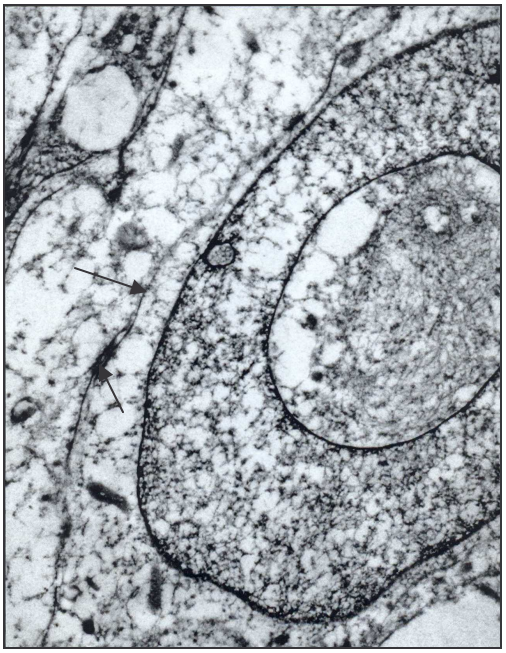

Figure 4.4: Preservation of cell membrane and cell junction (arrows) in tissue treated with Tissueclear® overnight (X8900)

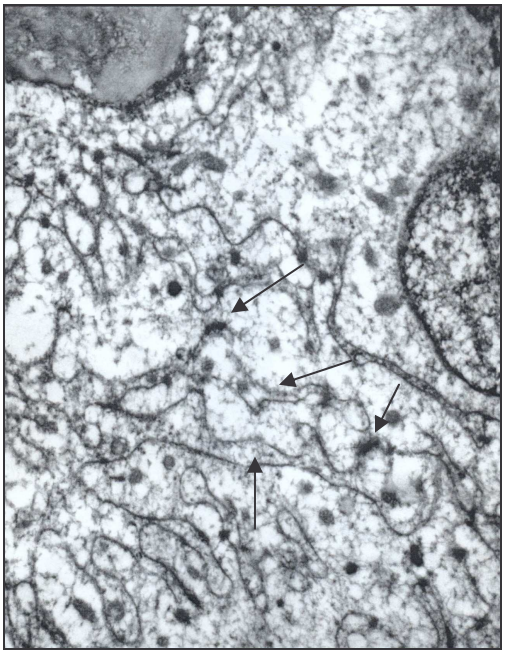

Figure 4.5: Preservation of cell membrane and cell junction (arrows) treated with xylene overnight (X8900)

As indicated in the figure 4.4 the tissue treated with Tissueclear® show better definition.

4.2.4.2 Cytoplasmic content

Cytoplasmic content including granules, filaments, vacuoles, vesicles and specific structures (premelanosomes, neurosecretory granules) were investigated. Figure 4.6 represents cytoplasmic content preservation and scored as poor partial or good. Figures 4.7 and 4.8 are EM photographs of cytoplasmic content preservation after overnight treatment with Tissueclear® and xylene, respectively.

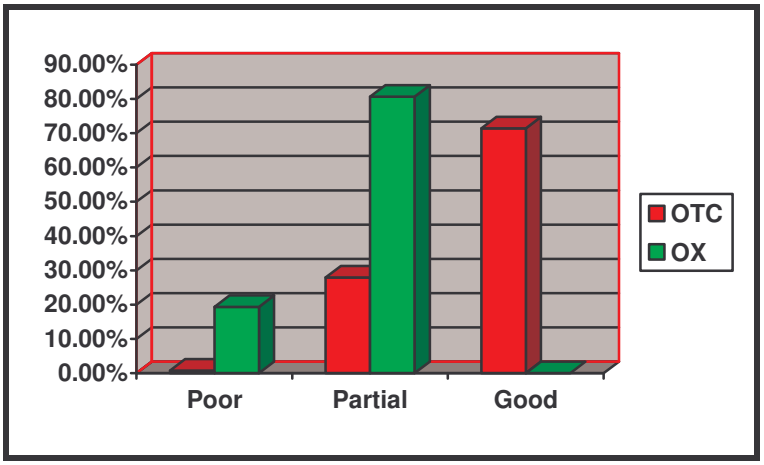

Figure 4.6: Preservation of cytoplasmic content after xylene and Tissueclear® overnight

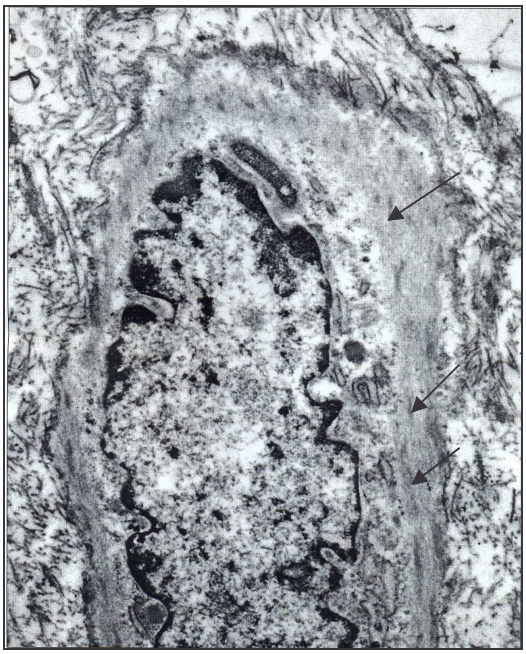

Figure 4.7: Cytoplasmic content of a tissue treated with Tissueclear® overnight (X8900)

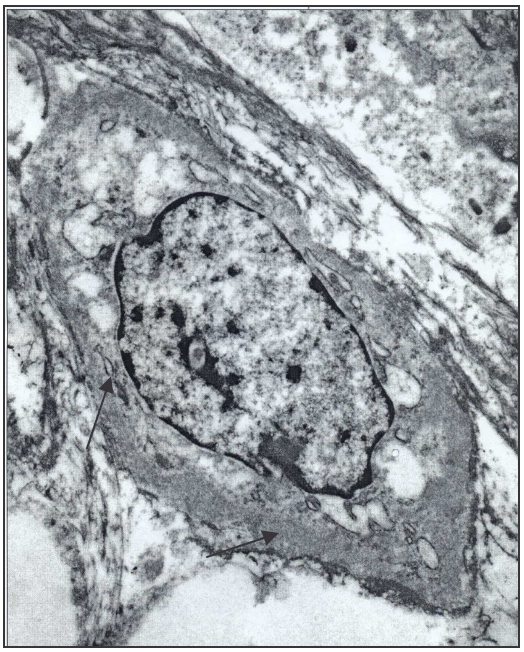

Figure 4.8: Cytoplasmic content of tissue treated with xylene overnight (X8900)

4.2.4.3 Extracellular material

Figure 4.9 represents extracellular material preservation and Figures 4.10 and 4.11 are EM photographs of extracellular material preservation after overnight treatment with Tissueclear® and xylene, respectively.

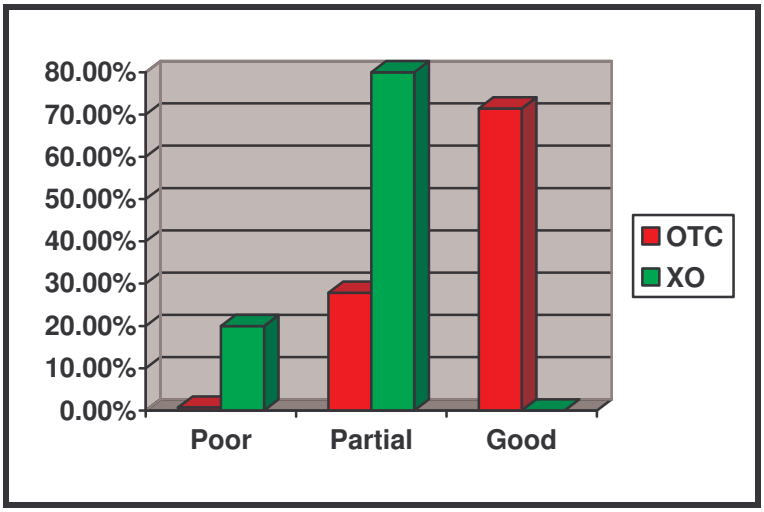

Figure 4.9: Graph showing extracellular material preservation

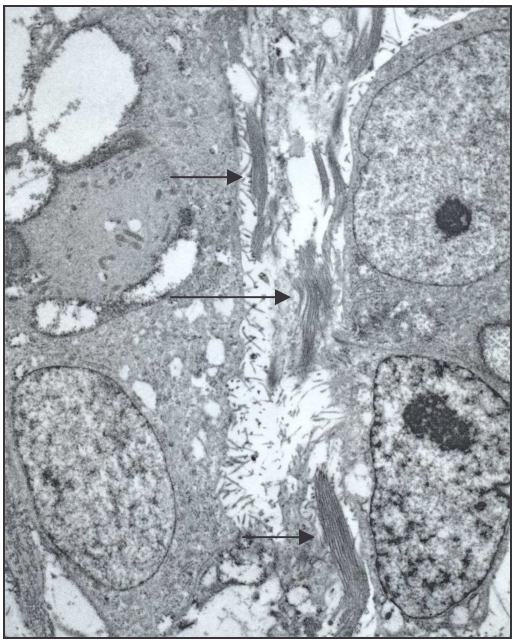

Figure 4.10: Extracellular material in tissue treated with Tissueclear® overnight (X3700)

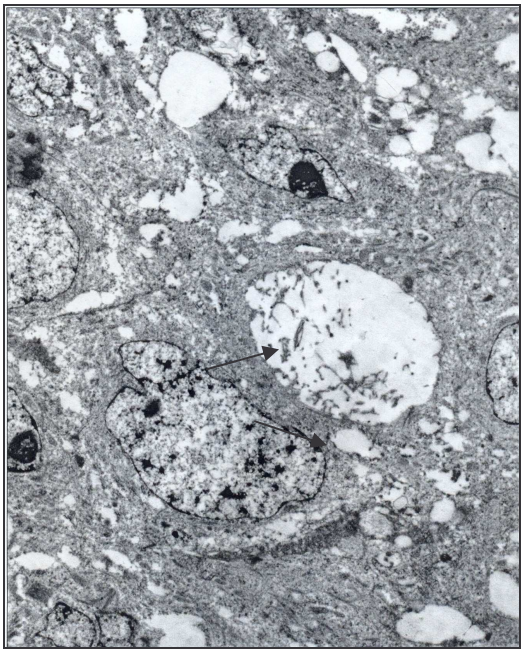

Figure 4.11: Extracellular material in tissue treated with xylene overnight (X3700)

4.3 QUESTIONNAIRE RESULTS

Of the 85 questionnaires distributed, 65 (81.25%) were completed and used in the analysis. Continuous variables were described by the mean, standard deviation (std), minimum (min) and maximum (max). Categorical variables were described by frequencies and percentages with 95% Confidence Interval (CI) for the percentage having the correct knowledge or incorrect knowledge. The number of respondents who answered the questions is indicated by ***n*** in the tables where necessary.

4.3.1 RESPONDENTS' DEMOGRAPHIC INFORMATION

The questions were:

1. Date questionnaire completed
2. What is your gender?
3. What is your age?
4. What is your highest qualification?
5. How long have you been working in a laboratory?

The respondents' demographic results are summarised in the table 4.5.

Table 4.5: Respondents' demographic results (questions 1-5)

Question	Parameters	Frequency	Percentage (%)
1. Date answered	1 May 2006 to 25 Sep 2006	65 of 80	81.25
2. Gender	Male	19	30
	Female	46	70
3. Age (years)	≤30	8	12.30
	31-40	27	41.53
	41-50	23	34.38
	>50	7	10.76
4. Qualification	Matric	6	9.23
	National diploma	43	66.15
	Degree	16	24.62
5. Period worked in the laboratory (years)	≤ 8	7	10.77
	8-15	20	30.77
	16-20	18	27.70
	20-25	8	12.31
	25-30	8	12.31
	30-42	4	6.15

4.3.2 RESPONDENTS' KNOWLEDGE REGARDING XYLENE TOXICITY AND RELATED SYMPTOMS

The questions and correct answers regarding xylene toxicity and resulting symptoms are given below. Questions and the correct answers given in bold were:

6. Can the toxicity of reagents be organized into more than one classification?
   Answers: **yes,** no, don't know
7. Which of the following statements is correct? (Choose one)
   Answers: very highly toxic, **highly toxic,** moderately toxic, slightly toxic.
8. Which of the following statements is correct? (Choose one)
   Regarding carcinogenicity.
   Answers: a known carcinogen, a probable carcinogen, a possible carcinogen, unclassifiable, **unclassifiable as to carcinogenity in humans**
9. Which of the following statements is correct? (You can choose more than one)
   Answers: **an allergen, an asthma trigger, a neurotoxin,** a reproductive toxicant, **a developmental toxicant,** nature friendly, biodegradable, non of the above, don't know
10. Are the toxicity levels of xylene in laboratories regulated by International Workers Exposure Levels?
    Answers: **yes,** no, don't know
11. The maximum level of xylene toxicity permitted is? (Choose one)
    Answers: **100ppm [part of gas vapour per million (ppm) of contaminated air volume at 25°C and 760mmHg pressure] time weighted a ten hours period,** 250ppm, don't know, no any level of toxicity is permitted

12. How long does it take for xylene to be cleared from the body? (Choose one)
    Answers: 12 hours, 24 hours, 72 hours, **more then 72 hours,** don't know
13. Is xylene biodegradable?
    Answers: yes, **no**, don't know
22. The symptoms following the inhalation of vapours of xylene may be: (You can choose more than one)
    Answers: **headache, euphoria, muscular weakness, red skin, dizziness, nausea, drowsiness, eye irritation**, blindness, **vomiting, loss consciousness, coma,** others, don't know, all of them
28. Which of the organs mentioned below are affected by xylene toxicity? (You may mark more than one)
    Answers: **eye, skin, respiratory system, central nervous system, gastrointestinal tract,** blood, **liver, kidney, muscles, foetus,** I don't know, others, all of them

The respondents' answers regarding xylene toxicity and symptoms with one correct answer are given in Table 4.6.

Table 4.6: Respondents' answers regarding xylene toxicity and symptoms (questions 6, 7, 8, 10, 11, 12, 13) with only one correct answer indicated in bold

Question	Parameters	Frequency	Percentage	95% CI
6 (n=65)	**Yes**	**55**	**84.62**	**75.9; 90.6**
	No	**2**	3.08	
	Don't know	**8**	12.31	
7 (n=65)	Very highly toxic	25	38.46	
	Highly toxic	**26**	**40.00**	**30.6; 50.2**
	Moderately toxic	8	12.31	
	Slightly toxic	1	1.54	
	Don't know	5	7.69	
8 (n=65)	A known human carcinogen	39	60.00	
	A probable human carcinogen	7	10.77	
	Possibly a human carcinogen	9	13.85	
	Unclassifiable as to carcinogenity in humans	**5**	**69**	**3.8; 15**
	Not likely to be a human carcinogen	0	0	
	Don't know	5	7.69	
10 (n=65)	**Yes**	**31**	**47.69**	**37.8; 57.8**
	No	14	21.54	
	Don't know	20	30.77	
11 (n=65)	**100ppm**	**14**	**21.54**	**14.4; 31.0**
	250ppm	3	4.62	
	Don't know	44	67.69	
	Not any level is permitted	4	6.15	
12 (n=64)	12 hours	2	3.13	
	24 hours	3	4.69	
	72 hours	4	6.25	
	More then 72 hours	**11**	**17.19**	**10.8; 26.3**
	Don't know	44	68.75	
13 (n=65)	Yes	6	9.23	**64; 81.8**
	No	**48**	**73.85**	
	Don't know	11	16.92	

The respondents' answers regarding xylene toxicity and symptoms with more than one correct answer are given in Table 4.7. Only 57 and 42 respondents answered questions 22 and 28, respectively.

Table 4.7: Respondents' answers regarding xylene toxicity and symptoms (questions 9, 22, 28) with more than one correct answer indicated in bold

Question	Parameters	'No' answer n (%)	"Yes" answer n (%)	95% CI
9 (n=65)	**Allergen**	**20 (30.77)**	**45 (69.23)**	**0.4; 5.2**
	Asthma activator	**22 (33.85)**	**43 (66.15)**	
	Neurotoxin	**39 (60.00)**	**26 (40.00)**	
	Reproductive toxicant	55 (84.62)	10 (15.38)	
	Developmental toxicant of foetus	**52 (80.00)**	**13 (20.00)**	
	Nature friendly	65 (100)	0 (0)	
	Biodegradable	63 (96.00)	2 (3.08)	
	None of the above	64 (93.46)	1 (1.54)	
	Don't know	61 (93.85)	4 (6.15)	**0.3; 6.6**
22 (n=57)	**Headache**	**13 (22.81)**	**44 (77.19)**	
	Euphoria	**40 (70.18)**	**17 (29.82)**	
	Muscular weakness	**53 (92.98)**	**4 (7.02)**	
	Red skin	**36 (63.16)**	**21 (36.84)**	
	Dizziness	**33 (57.89)**	**24 (42.11)**	
	Nausea	**22 (38.60)**	**35 (61.40)**	
	Drowsiness	**47 (82.46)**	**10 (17.54)**	
	Eye irritation	**38 (66.67)**	**19 (33.33)**	
	Blindness	57 (100)	0 (0)	
	Vomiting	**46(80.70)**	**11 (19.30)**	
	Lose consciousness	**54 (94.74)**	**3 (5.26)**	
	Coma	**57 (100)**	**0 (0)**	
	Other	57 (100)	0 (0)	
	Don't know	53 (92.98)	4 (7.02)	
	All of them	50 (87.72)	7 (12.28)	
28 (n=42)	**Eyes**	**12 (28.57)**	**30 (71.43)**	**26.8; 50.8**
	Skin	**13 (30.95)**	**29 (69.05)**	
	Respiratory system	**19 (45.24)**	**23 (54.76)**	
	Central nervous system	**32 (76.19)**	**10 (23.81)**	
	Gastro intestinal tract	**38 (90.48)**	**4 (9.52)**	
	Blood	39 (92.86)	3 (7.14)	
	Liver	**34 (80.95)**	**8 (19.05)**	
	Kidney	**35 (83.33)**	**7 (16.67)**	
	Bones	40 (95.24)	2 (4.76)	
	Muscle	**40 (95.24)**	**2 (4.76)**	
	Foetus	**39 (92.86)**	**3 (7.14)**	
	I don't know	40 (95.24)	2 (4.76)	
	Other	42 (100)	0 (0)	
	All of them	35 (83.33)	7 (16.67)	

4.3.3 RESPONDENTS' KNOWLEDGE REGARDING XYLENE SAFETY REGULATIONS, HANDLING AND DISPOSAL

The questions regarding xylene safety regulations, exposure and handling are given below with the correct answer highlighted in bold.

14. **How should xylene be discarded? (Choose more than one)**
    Answers: *via* the drain, stored in special drums and discarded by specialised services, discarded in a special drainage system that is approved by the fire brigade, don't know, there is no regulation about it
15. **Is xylene inflammable?**
    Answers: yes, no, don't know
16. **How should xylene be stored? (Choose one)**
    Answers: In fire resistent rooms with heavy metal fire-proof doors, in metal fire-proof cabinets, in well ventilated store rooms, don't know, in ordinary cupboards, no special precautions are necessary
17. **How should alcohol, acetone and xylene required for daily use be stored in the laboratory? (Choose one)**
    Answers: in fire resistent rooms with heavy metal fire-proof doors, in metal fire-proof cabinets, in well ventilated store rooms, don't know, in ordinary cupboards, no special precautions are necessary
18. **Which of the following reagents cannot be stored in the same area as xylene?**
    Answers: acetone, formalin, alcohol, nitric acid, acetic acid, not sure, all of them
19. **Specify the type of hazards which can occur with xylene. (You can mark more than one)**
    Answers: fire, explosion, exposure
20. **Which of the following hazardous prevention rules are followed in your laboratory? (You can mark more than one)**

Answers: **fire - prevention (no open flames, no sparks, no smoking), explosion - prevention (no storage with nitric acid or oxidant agents, no spillage), exposure (inhalation, skin, eyes, ingestion) (work under extractor, strict hygiene),** exposure - wear exposure tag, corrosive – equipment protection, radioactive – radiation tag, other, not sure, don't know

21. How often do you obey hazardous prevention rules?
    Answers: **always,** sometime, never

The results to the questions regarding respondents' knowledge regarding xylene safety regulations, handling and disposal where there is one correct answer, is given in Table 4.8.

Table 4.8: Respondents' knowledge regarding xylene safety regulations, handling and disposal (questions 15, 16, 17, 21) where only one answer was correct, indicated in bold

Question	Parameters	Frequency	Percentage	95% CI
15 (n=65)	**Yes**	**59**	**90.77**	**83.15; 95.2**
	No	4	6.15	
	Don't know	2	3.08	
16 (n=65)	**Special rooms**	**51**	**78.46**	**65.6; 96.0**
	Special cabinets	7	10.77	
	Well ventilated rooms	4	6.15	
	Ordinary cupboard	1	1.54	
	Don't know	2	3.08	
	No special precautions	0	0	
17 (n=64)	Special rooms	30	46.88	
	Special cabinets	**19**	**29.69**	**21.3; 39.8**
	Well ventilated rooms	8	12.50	
	Don't know	3	4.69	
	Ordinary cupboard	4	6.26	
	No special precautions	0	0	
18 (n=63)	Acetone	5	7.94	
	Formalin	3	4.76	
	Alcohol	4	6.35	
	Nitric acid	**20**	**31.75**	**21.6; 44.0**
	Acetic Acid	8	12.70	
	Not sure	34	53.97	
	All of them	12	19.05	
21 (n=57)	**Always**	**40**	**70.18**	
	Sometimes	16	28.07	
	Never	1	1.75	

Respondents' knowledge regarding xylene safety regulations, handling and disposal where there is more than one correct answer is given in Table 4.9.

Table 4.9: Respondents' knowledge regarding xylene safety regulations, handling and disposal (questions 14, 18 19, 20) with more than one correct answer (indicated in bold)

Question	Parameters	'No' answer n (%)	"Yes" answer n (%)	95% CI[#]
14 (n=65)	*Via* the drain	63 (96.92)	2 (3.08)	
	Stored in drums	**8 (12.31)**	**57 (87.69)**	
	Special drainage	**50 (76.92)**	**15 (23.08)**	**14.4; 31.0**
	Don't know	59 (90.77)	6 (9.23)	
	No regulations	60 (92.31)	5 (7.69)	
19 (n=58)	**Fire**	**8 (13.79)**	**50 (86.21)**	
	Explosion	**21 (36.21)**	**37 (63.79)**	
	Exposure	**17 (29.31)**	**41 (70.69)**	**28.2; 48.8**
	Corrosion	47 (81.03)	11 (18.97)	
	Radiation	58 (100)	0 (0)	
	Not sure	56 (96.55)	2 (3.45)	
	All of them	56 (96.55)	2 (3.45)	
20 (n=58)	**Fire**	**5 (8.62)**	**53 (91.38)**	
	Explosion	**23 (39.66)**	**35 (60.34)**	
	Exposure	**14 (24.14)**	**44 (75.86)**	
	Exposure-tag	**54 (93.10)**	**4 (6.90)**	**34.5; 55.6**
	Corrosive	54 (93.10)	4 (6.90)	
	Radioactive	56 (96.55)	2 (3.45)	
	Other	58 (100)	0 (0)	
	Not sure	56 (96.55)	2 (3.45)	
	Don't know	57 (98.28)	1 (1.72)	

[#]Regarding questions 14, 19 and 20, the 95% CI for having respectively two, three and four correct answers is given.

4.3.4 RESPONDENTS' EXPERIENCE REGARDING XYLENE EXPOSURE AND HANDLING

The questions on xylene exposure and handling experience are given below. For these questions there were no correct or incorrect answers.

23. **Have you ever experienced one or more of the following? (You may choose more than one)**
    Answers: loss of consciousness, euphoria, muscular weakness, coma, vomiting, nausea, drowsiness, eyes irritation, blindness, dizziness, headaches, red skin, others, have never experienced one of them.
25. **How often do you wear a xylene toxicity tag?**
    Answers: always, sometimes, never.
26. **How often do you use protective equipment during handling of xylene?**
    Answers: always, sometimes, never.
27. **Specify the type of protective equipment you would use. (You may choose more than one)**
    Answers: gloves, safety spectacles, breathing protection, fume extractors, safety shields, all of them, none.
29. **More companies are producing new and less harmful reagents. Which of the new reagents mentioned below have you heard of?**
    Answers: I have not heard about them, Tissueclear®, Slide-Brite Clearant, Micro-Clear, Pro-Par Clearant, Shandon Xylene Substitute.
31. **Which of the following problems have you experienced? (You may choose more than one)**
    Answer: dissolved plastic containers with specimens, dissolved pencils and pens, dissolved gloves, dissolved spectacles, dissolved window shield, dissolved plastic pipettes, detached the stickers

from containers with patient's information, dissolved the ink used to print the patient's details on the stickers, other (specify).

Respondents' answers regarding xylene exposure and handling experience with one correct answer are given in Table 4.10 and questions with more than one correct answer, in Table 4.11.

Table 4.10: Respondents' answers regarding xylene exposure and handling (questions 25, 26), with one correct answer (n=46)

Question	Parameters	Frequency	Percentage
25(n=46)	1.Always	1	2.17
	2.Sometimes	0	0
	3.Never	45	97.83
26(n=46)	1.Aways	19	41.31
	2.Sometimes	16	34.78
	3.Never	11	23.91

Table 4.11: Respondents' answers regarding xylene exposure and handling (questions 23, 27, 29, 31) with more than one correct answer

Question	Parameter	'No' answer n (%)	"Yes" answer n (%)
23 (n=51)	Loss of consciousness	50 (98.04)	1 (1.96)
	Euphoria	42 (82.35)	9 (17.65)
	Muscular weakness	50 (98.04)	1 (1.96)
	Coma	51 (100)	0 (0)
	Vomiting	45 (88.24)	6 (11.76)
	Nausea	30 (58.31)	21 (15.69)
	Drowsiness	43 (84.31)	8 (15.69)
	Eye irritation	29 (56.86)	22 (43.14)
	Blindness	51 (100)	0 (0)
	Dizziness	35 (68.63)	16 (31.37)
	Headache	13 (25.49)	38 (74.51)
	Red skin	35 (68.63)	16 (31.37)
	Others	50 (98.04)	1 (1.96)
	Never experienced	47 (92.16)	4 (7.84)
27 (n=44)	Gloves	6 (13.64)	38 (86.36)
	Safety spectacles	40 (90.91)	4 (9.09)
	Breathing protection	40 (90.91)	4 (9.09)
	Fume extractors	21 (47.73)	23 (52.27)
	Safety shields	42 (95.45)	2 (4.55)
	All of them	42 (95.45)	2 (4.55)
	None	40 (90.91)	4 (9.09)
	Others	44 (100)	0 (0)
29 (n=40)	Not heard about	22 (55.00)	18 (54.00)
	Tissueclear	19 (47.50)	21 (52.50)
	Slide-Brite	39 (97.50)	1 (2.50)
	Micro-Clear	40 (100)	0 (0)
	Pro-Par Clearant	40 (100)	0 (0)
	Shandon Xylene substitute	35 (87.50)	5 (12.50)
	Others	40 (100)	0 (0)
31 (n=33)	Dissolves plastic containers	8 (24.24)	25 (75.76)
	Dissolves pencils, pen	8 (24.24)	25 (75.76)
	Dissolves gloves	14 (42.42)	19 (57.58)
	Dissolves spectacles	26 (78.79)	7 (21.21)
	Dissolves shields	29 (87.88)	4 (12.12)
	Dissolves pipettes	18 (54.55)	15 (45.45)
	Detaches stickers	17 (51.52)	16 (48.48)
	Dissolves ink	20 (60.61)	13 (39.39)

4.3.5 USEFULNESS OF THE QUESTIONNAIRE

The results concerning the usefulness of the questionnaire are given below. Here too there were no correct answers.

24. Specify the most information about xylene that was most beneficial to you. (You may choose more than one)
    Answers: hazards, storage, symptoms, protection equipment, all of them, others.
32. Do you consider this survey as meaningful to laboratory workers?
    Answers: yes, no, not sure.
33. Do you think that more can be done in your laboratory about prevention and protection of workers' health and safety?
    Answers: yes, no, not sure.
34. If yes. Will you be willing to initiate a process of replacing xylene with a friendlier product?
    Answers: yes, no.

Respondents' opinion regarding the usefulness of the questionnaire is given in Table 4.12.

Table 4.12: Respondents' opinion regarding the usefulness of the questionnaire

Question	Parameters	'No' answer n (%)	"Yes" answer n (%)
24 (n=48)	Hazards	32 (66.67)	16 (33.33)
	Storage	41 (85.42)	7 (14.58)
	Symptoms	37 (77.08)	11 (22.92)
	Protection	39 (81.25)	9 (18.75)
	Others	47 (97.92)	1 (2.08)
	All	15 (31.25)	33 (68.75)
32 (n=31)	Yes	0	28 (90.37)
	No	0 (0)	0
	Not sure	0	3 (9.68)
33 (n=31)	Yes	0	30 (96.77)
	No.	0 (0)	0
	Not sure	0	1 (3.23)
34 (n=31)	Yes	0	29 (93.55)
	No	2 (6.45)	0

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

The aim of this study was to compare the morphological effects of Tissueclear® with xylene on tissue to be examined by EM. The reason for this was to attempt to replace one of the toxic reagents used in the EM laboratory with a less toxic one. A secondary aim was to see if Tissueclear® was less damaging than xylene to tissue exposed to the reagents for prolonged periods, thus enabling tissue processing to begin immediately. This meant that the tissue could be left in the reagent overnight and the method be continued the following morning. In this way the processing time could be shortened by a day. A peripheral issue was to assess the awareness among laboratory staff of the toxic effects of xylene and provide them with feedback information that they might not have known, and to make them more careful when working with chemicals in general.

This project raised several important issues regarding the processing method and safety awareness in the laboratory. These will now be discussed.

5.2 WAX REMOVAL FROM SPECIMENS FOR ELECTRON MICROSCOPY

The cases examined in this study were all problematic with regards to diagnosis because all that remained of the specimens to be examined was tissue that had been embedded in paraffin wax, which was suboptimal for ultrastructural examination. It was therefore very important that the tissue be retrieved as carefully as possible to

preserve the integrity of the ultrastructural organelles. If these were not visible, the examination would not contribute to a diagnosis. The traditional preparation method for wax embedded specimens involved using the clearing agent xylene to remove the paraffin wax before the tissue was processed for EM. Good morphological results were usually obtained provided the original tissue was not too degenerated. However, xylene is extremely toxic and needed to be handled with care.

5.3 SPECIMEN PREPARATION AND EVALUATION

5.3.1 SPECIMEN PREPARATION

Preparation of EM specimens is a difficult process for the following reasons. The specimens are very small and can easily become lost during handling. The cutting process is time consuming and stressful. The preparation of the glass knife requires patience because the glass bar has to be broken slowly if one is to obtain a good knife. On occasions, an entire glass bar is used in the preparation of a single good knife (Pease, 1964: 38-43). Care must be taken when trimming the small specimens that the tissue or area of interest is not trimmed, for example the glomeruli in a kidney biopsy. Ultra-thin sections are cut with a diamond knife, which is a very expensive instrument costing in the region of R50 000.00, and can be easily damaged. For this reason it must be handled with great care. The ultramicrotome is very sensitive to movement and air currents and technologists need to be patient when attempting to cut thin sections. Staining also requires patience and care. The grids are very small and are easy to squash or bend. The staining solution of lead citrate has to be used in special carbon dioxide free conditions otherwise black precipitations will form on the tissue, which makes examination and photography impossible.

5.3.2 FREQUENCY OF SPECIMENS REMOVED FROM WAX IN THE ELECTRON MICROSCOPY LABORATORY

The number of specimens from which wax was removed in our laboratory represented 43% of the total amount of work submitted for EM between 1 January 2004 to 1 June 2005, which clearly illustrates the need for the method be as optimal as possible. These were cases which provided diagnostic difficulty and where there was no other tissue available.

5.3.3 TYPES OF SPECIMENS

In EM, different tissues and tumours have different consistencies. Some tissues and tumours are easier to cut and have better preserved structures than others. For this reason we studied specimens obtained from most of the tissues of the body. The specimens removed from wax were also harder then those fixed in glutaraldehyde (the specific fixative for EM), due to the previous fixation and processing, which made sectioning more difficult.

5.3.4 SPECIMEN CONSISTENCY UNDER DISSECTING MICROSCOPE

All of the specimens that were treated with xylene for the usual period and overnight were dry, hard and brittle when examined under the dissecting microscope. When cutting these specimens they were also dry, hard and brittle making it impossible to obtain a fragment with a square shape. Usually a fragment of tissue was obtained that measured either more or less than the required 0.5 x 0.5mm. A very important and stressful part of the preparation procedure is to keep the EM specimens wet to ensure good ultrastructural preservation. With xylene this was difficult to achieve because it evaporates instantly. When cutting, the specimens were firstly evaluated under the dissecting microscope to dissect unwanted blood clot, collagen and necrotic areas, and then one area compact and suitable for examination was chosen. This process

usually takes between 5 to 7 minutes. This is too long a period of time to keep an EM specimen dry. The specimens treated with Tissueclear® for the usual period of time and overnight were all soft, wet and not brittle and this did not change as the technologist evaluated and sectioned the specimen. If the specimen becomes dry it is also harder to cut. Subsequently more glass knives have to be made and used for trimming. When cutting the ultra-thin specimens this sometimes damages the diamond knife.

5.3.5 RESIN COMPACTION AND CRACKS UNDER LIGHT AND ELECTRON MICROSCOPES

When xylene evaporates air bubbles penetrate the tissue at microscopic level and remain there even after dehydration, impregnation and embedding the specimens. This results in cracks and holes in the specimen either during cutting, drying, developing in the resin, or during the cutting and staining procedures. These can be observed under the light and electron microscopes. Cracks and holes produce tears in sections that impede evaluation and photographic recording. This may force the evaluator to search for better areas and may even require further cutting and staining. In our study all of the specimens treated with xylene for the usual period of time and overnight had cracks and holes in the resin (see Figure 4.1). This effect has been reported as air bubble artefacts in the literature (Kayton & Aktas, 1998) and has been ascribed to Spurr's epoxy resin sections reacting with xylene. For this reason in our EM laboratory, specimens could not be left overnight or over a weekend in xylene. All of the specimens treated with Tissueclear® for both periods were, however, without cracks and holes in the resin (Figure 4.2).

5.3.6 ULTRASTRUCTURAL PRESERVATION

In specimens examined by EM it is very important to have the ultrastructure well preserved so that the organelles are easy to identify

and in this way contribute to making a diagnosis (Henderson & Papadimitriou, 1982: 324-389). In our project the structures were evaluated on a scale 1-poor, 2-partial, 3-good preservation. We examined cell membrane preservation, cytoplasmic content and extracellular material.

5.3.6.1 Cell membrane preservation

The cell membrane in EM is a very important structure and in some tumours the presence of basal lamina and intercellular junctions provides evidence of a specific type of differentiation (Erlandson, 1994: 207-212).

Our results indicated that cell membrane preservation was good in 72.14% of specimens and partial in 7.86% of specimens treated with Tissueclear® over the usual period of time as well as overnight. Identical results were also obtained with xylene after the usual time. Treatment with xylene overnight produced poorer results, with 72.14% showing partial preservation and 0% good preservation (Table 4.4).

In the pictures (Figures 4.4 and 4.5, respectively) examples of cell membrane and cell junction preservation after Tissueclear® and xylene were shown. In Figure 4.4, tissue that was treated with Tissueclear® overnight had a clear cell membrane and a cell junction very similar to the classical image illustrated in the Ultrastructural Atlas of Brain Tumours (Tung, Asao & Zimmerman, 1971: 68-80). Figure 4.5 showed the same tissue that was treated with xylene overnight, with poorly preserved cell membrane and cell junction. In this case, the image was taken at higher magnification to make sure that the structure was the one needed for diagnosis. These images clearly showed a difference in ultrastructure preservation.

5.3.6.2 Cytoplasmic content

Assessment of cytoplasmic content (Figure 4.6) showed good preservation in 71.43% and partial preservation in 27.86% of specimens treated with Tissueclear® over the usual period of time and overnight, and with xylene over the usual period of time. For the specimens treated with xylene overnight, the results showed partial preservation in 80% and none with good preservation (0%).

In the pictures (Figures 4.7 and 4.8, respectively) examples of cytoplasmic content preservation after treatment with Tissueclear® and xylene are shown. In this case Figure 4.7 showed cytoplasmic content with muscle differentiation clearly (arrows), appearing identical to the image in Ghadially's tumour atlas (Ghadially, 1985: 364) and Rosai and Ackerman's Surgical Pathology (Rosai, 2004: 568). Figure 4.8 showed the same tissue with cytoplasmic content partially preserved with the muscle differentiation more difficult to identify (arrows).

5.3.6.3 Extracellular material

Specimens treated with Tissueclear® over the usual period of time and overnight both showed partial preservation in 27.87% and good preservation in 71.43% of extracellular material. Specimens treated with xylene over the usual period also showed partial preservation in 27.87% and good preservation in 71.43% of extracellular material. Specimens treated with xylene overnight, however, showed poor preservation in 20%, partial preservation in 80% and no good preservation (0%) of extracellular material (Figure 4.4).

Examples of extracellular preservation were illustrated in the pictures (Figures 4.10 and 4.11) with the Tissueclear® treated specimen (Figure 4.10) showing collagen fibers outside the cells (arrows) similar to that illustrated in Ghadially's tumour atlas (Ghadially, 1985: 429). The xylene

treated specimen (Figure 4.11) showed collagen partially preserved as seen by small pieces, broken fibers and a large empty area (arrows).

5.4 TOXICITY IN ELECTRON MICROSCOPY LABORATORY

From the literature review it became apparent that there are a multitude of toxic side effects ascribed to many of the chemical reagents used in the EM laboratory of which there has been poor awareness in the past. These include xylene, osmium tetroxide, uranyl acetate, lead citrate and resin (Bancroft & Stevens, 1982: 618). The results of the questionnaire indicated that many respondents have in the past experienced symptoms, which could be regarded as toxic side effects of xylene although they were unaware that this reagent could have caused them. There was a surprising lack of knowledge regarding some aspects of handling and disposal of the reagent and it seems that not much attention is paid to protective measures. With this project not only did the researcher herself become more aware of the danger of toxic reagents and the need for handling them with care but was able to communicate some of this knowledge to colleagues in other laboratories. This has also encouraged personnel to consider the use of alternative less toxic reagents in the future.

5.5 QUESTIONNAIRE

The questionnaire on xylene included a personnel profile, their knowledge regarding the toxic side effects and safety measures necessary when handling the reagent, the problems that they had personally encountered and their opinion on the usefulness of taking part in the survey.

5.5.1 RESPONDENTS' DEMOGRAPHICS INFORMATION

Out of the 85 (100%) questionnaires handed to personnel, 65 (81.25%) were answered and returned. Most (70%) were female with a mean age

of 40 years (range 20 to 60 years). Most respondents had a national diploma (66.15%), 9.23% had a matriculation certificate and 24.62% had a degree. On average (+/-std) they had worked for 18 (8.8%) years in the laboratory (range 3 months to 42 years). This profile reflected that typical person working in the laboratory at the NHLS in Universitas Hospital is a middle-aged female with a number of years experience.

5.5.2 RESPONDENTS' KNOWLEDGE REGARDING XYLENE TOXICITY AND SYMPTOMS

Most (84.62%) of the respondents were aware that there is more than one classification regarding toxicity while 12.31% were not aware of this. Many knew (40%) that xylene is highly toxic with 38.46% answering that xylene may be regarded as very highly toxic and 7.69% did not know in which category regarding toxicity to place xylene. Few respondents (7.69%) knew that xylene is not likely to be a human carcinogen and most (60%) thought it was a human carcinogen. Approximately half (47.69%) the respondents knew that xylene toxicity is internationally regulated and 21.54% knew the level of toxicity permitted by international law. Only 17.19% respondents answered correctly that it takes more then 72 hours for xylene to be cleared from the body and 73.85% knew that xylene is not biodegradable. Regarding xylene exposure symptoms only one person had all the correct answers. The highest percentage (30.8%) had only 2 correct answers (allergen and asthma trigger). Regarding xylene vapour inhalation and organs affected by xylene, no one had all the correct answers. The impression from this section was that there is a meaningful lack of knowledge regarding the toxicity of xylene.

5.5.3 RESPONDENTS' KNOWLEDGE REGARDING XYLENE SAFETY REGULATIONS, HANDLING AND DISPOSAL

Most respondents (90.77%) knew xylene is inflammable and 78.46% knew how to store xylene correctly. Only 29.69% knew how to store

xylene for daily use (in metal fire-proof cabinets). Most respondents (70.18%) said that they always obeyed the safety regulations, 28.07% said only some of the time, while 1.75% said they never obeyed the rules. Respondents (96.92%) knew that xylene should not be discarded *via* the drain and 87.69% answered that it be stored in special drums, which is the practice in our laboratory. Only 23.08% knew about special drainage. A third of respondents (31.75%) knew which reagents should not be stored with xylene. Regarding hazards occurring with xylene, respondents marked fire (86.21%), explosion (63.79%), and exposure (70.69%). The impression on this section was that there was greater awareness on the handling and disposal of xylene than there had been regarding the toxic side effects.

5.5.4 RESPONDENTS EXPERIENCE REGARDING XYLENE EXPOSURE AND HANDLING

Most respondents (97.83%) never wear a toxicity tag for xylene and 41.83% used protective equipment. A third (n=51, 31.4%) of respondents had experienced 3 of the adverse symptoms mentioned. The 3 symptoms were considered significant, as less than 3 could have been co-incidental. The most commonly used protective equipment were gloves (86.36%), and fume extractors (52.47%). Half (50%) of the respondents had heard of Tissueclear®. Only 12.1% (n=33) had experienced the melting of plastic containers, pencils and gloves and 77.42% had experienced apparatus damage.

5.5.5 USEFULNESS OF THE QUESTIONNAIRE

Although 17 (26.1%) respondents did not answer question 24 the remaining respondents (68.75%) considered all the information about xylene protection and hazards useful. Regarding the meaningfulness of the survey, only 31 (47.6%) respondents answered. Of these, 90.32% considered the questionnaire meaningful. Respondents (n=30, 93.55%)

considered that more needed to be done with regards to protection and prevention and 93.55% were willing to initiate a process to replace xylene in their laboratory. This section of the questionnaire was poorly answered but those who did submit answers seemed to be positive.

5.5.6 VALUE OF THE QUESTIONNAIRE

The questionnaire had a good response (81.25%) and showed that many of the personnel were affected by chemicals. Most of them had experienced mild symptoms (headaches, dizziness, nausea) and were aware of the toxicity of xylene. What was not addressed in this study was whether the toxic effects that they experienced could have been attributed to other toxic reagents. Most of the respondents did not know of all the negative effects of xylene and specifically the effect on foetal development.

Many respondents were under the impression that xylene is a known carcinogen. The questionnaire did, however, enrich their knowledge about xylene toxicity and it is my impression that it has resulted in a greater openness to the use of new and less toxic reagents on the market.

CHAPTER 6

CONCLUSIONS

6.1 THE ACCEPTABILITY OF TISSUECLEAR

Comparing the effects of xylene and Tissueclear® on specimens removed from wax submitted for EM was done with the intention of finding a better alternative to xylene and in this way to reduce the number of toxic reagents used in the EM laboratory

6.2 CONCLUSION ON MORPHOLOGICAL EXAMINATION

No difference in ultrastructural preservation was seen for Tissueclear® and xylene treatment over the usual period of time. Tissue treated with Tissueclear® was easier to handle, resulting in better sections for EM examination than that treated with xylene. The ultrastructural preservation was better with Tissueclear® after processing overnight, which means that this reagent could be used to improve laboratory turn around time. This can be accomplished because as soon as the specimen is submitted, processing can begin and the fragments can be left in the Tissueclear® overnight. With xylene processing could only begin early in the morning so a specimen, which arrives later or in the afternoon, has to stand till the next day. The conclusion is that Tissueclear® is a superior clearing agent to xylene and a better reagent to use in the EM laboratory.

6.3 AWARENESS OF XYLENE TOXICITY IN THE LABORATORY

The questionnaire submitted to personnel in the laboratory was intended to make them more aware of the dangers of xylene toxicity and to encourage them to use new and less dangerous reagents in the future.

In this regard the questionnaire appeared to be successful in increasing awareness regarding the toxic effects of xylene and hopefully this will encourage greater care in the handling of all chemical reagents and the substitution of less toxic reagents in the future. However, there does appear to be a need for more emphasis to be placed on safety measures in the laboratory.

6.4 FINANCIAL BENEFITS

The use of Tissueclear® has definite positive financial implications. The method using Tissueclear® is less expensive because there is no need for special storage and disposal. With non toxic Tissueclear® accidents in the working place are not a problem saving on medical expenses. Finally the turn around time is shortened and the patients receive the results more rapidly. This may shorten the period in hospital reducing medical expenses.

6.5 REFLECTION ON WORK DONE

The researcher wanted to prove that the medical technologists are able to achieve other goals apart from routine work at the bench.

The work on the project was hard and time consuming but enriched the experience in the research field, which will be useful in the future.

There is the satisfaction that the study showed that Tissueclear® is an acceptable non toxic alternative to xylene in the EM laboratory and also created greater awareness of safety aspects among laboratory workers.

The immediate replacement of xylene in some laboratories as a result of this project brought great satisfaction to the researcher and encouraged her to carry on research in the medical technology field.

Respondents to the questionnaire became more aware of the negative effects of xylene.

6.6 OUTCOMES

The outcomes of this project that have been achieved are;
Laboratory staff appear to be more aware of the potential dangers of the chemical reagents with which they come into contact.

Xylene was replaced completely in the cytology, EM and genetics laboratories and partially in the histology laboratory since the completion of this study.

The turn around time in our EM laboratory on specimens submitted in wax has been reduced from 3 days to 2 days and it has implications on patient financial situation.

The use of Tissueclear is more cost effective then xylene, due to the fact it is no necessary any special storage and disposal, or safety measures for the workers handling the reagent.

In May 2003 at the Congress of Medical Technology in Bloemfontein the researcher presented "Comparative study on the use of Xylene versus Tissueclear" which the company (Sakura-Bayer) placed on their website in the Scientific Literature Department.

In March 2005, at the Mini-Congress of the SMLTSA Free State Branch in Bloemfontein, the researcher did the presentation "Toxicological Profile of Xylene", and received the award "The best presented presentation".

In August 2005 at the Free State University Faculty of Health Sciences Forum she presented a poster "Toxicological Profile of Xylene".

In March 2006 she did a presentation, "A Xylene Free Laboratory?" at the Histology Symposium at Shumba Valley Lodge, Johannesburg sponsored by Bayer Health Care.

Monthly journal discussions regarding toxic reagents used in laboratories have been instituted.
It is hoped that this work will be published in a technology journal.

A follow up project may emanate from this study.

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APPENDIX A

STATISTICAL DATA SHEETS OF THE TISSUES EVALUATION

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
1	8	11511	1	NX	1	1	1	2	1	2	1	3	3	3	1
2	10	11516	1	NX	1	1	1	2	1	2	1	3	3	3	2
3	8	11520	1	NX	1	1	1	2	1	2	1	3	3	3	3
4	8	11523	1	NX	1	1	1	2	1	2	1	3	3	3	3
5	7	11524	1	NX	1	1	1	2	1	2	1	3	3	3	2
6	6	11525	1	NX	1	1	1	2	1	2	1	3	3	3	3
7	8	11526	1	NX	1	1	1	2	1	2	1	3	3	3	3
8	6	11527	2	NX	1	1	1	2	1	2	1	1	2	2	1
9	6	11531	1	NX	1	1	1	2	1	2	1	3	2	2	2
10	8	11533	1	NX	1	1	1	2	1	2	1	3	3	3	3
11	4	11535	2	NX	1	1	1	2	1	2	1	1	2	2	1
12	8	11538	1	NX	1	1	1	2	1	2	1	3	3	3	2
13	3	11546	1	NX	1	1	1	2	1	2	1	3	3	3	3
14	4	11548	1	NX	1	1	1	2	1	2	1	3	3	3	1
15	8	11558	2	NX	1	1	1	2	1	2	1	1	2	2	1
16	6	11559	1	NX	1	1	1	2	1	2	1	3	3	3	3
17	9	11563	1	NX	1	1	1	2	1	2	1	3	3	3	3
18	10	11564	2	NX	1	1	1	2	1	2	1	1	2	2	1
19	8	11565	1	NX	1	1	1	2	1	2	1	3	3	3	3
20	8	11566	1	NX	1	1	1	2	1	2	1	3	3	3	3
21	1	11567	2	NX	1	1	1	2	1	2	1	1	2	2	1
22	9	11569	2	NX	1	1	1	2	1	2	1	2	2	2	3
23	8	11570	1	NX	1	1	1	2	1	2	1	3	3	3	3
24	10	11576	2	NX	1	1	1	2	1	2	1	1	2	2	1
25	8	11583	1	NX	1	1	1	2	1	2	1	3	3	3	1
26	8	11586	1	NX	1	1	1	2	1	2	1	3	3	3	3
27	8	11588	1	NX	1	1	1	2	1	2	1	3	3	3	3
28	8	11589	1	NX	1	1	1	2	1	2	1	3	3	3	3
29	1	11590	2	NX	1	1	1	2	1	2	1	1	2	2	1
30	7	11591	1	NX	1	1	1	2	1	2	1	3	3	3	1
31	1	11598	2	NX	1	1	1	2	1	2	1	1	2	2	1
32	8	11619	1	NX	1	1	1	2	1	2	1	3	3	3	1
33	4	11622	1	NX	1	1	1	2	1	2	1	3	3	3	1
34	4	11628	1	NX	1	1	1	2	1	2	1	3	3	3	2
35	3	11535	1	NX	1	1	1	2	1	2	1	3	3	3	3
36	8	11636	1	NX	1	1	1	2	1	2	1	3	3	3	1
37	8	11636	1	NX	1	1	1	2	1	2	1	3	3	3	3
38	8	11639	1	NX	1	1	1	2	1	2	1	3	3	3	1
39	3	11646	1	NX	1	1	1	2	1	2	1	3	3	3	1
40	8	11650	1	NX	1	1	1	2	1	2	1	3	3	3	3
41	8	11644	2	NX	1	1	1	2	1	2	1	1	2	2	1

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
42	8	11652	2	NX	1	1	1	2	1	2	1	1	2	2	1
43	8	11658	2	NX	1	1	1	2	1	2	1	1	2	2	1
44	9	11660	1	NX	1	1	1	2	1	2	1	3	3	3	1
45	6	11661	1	NX	1	1	1	2	1	2	1	3	3	3	3
46	8	11663	1	NX	1	1	1	2	1	2	1	3	3	3	1
47	8	11668	1	NX	1	1	1	2	1	2	1	3	3	3	3
48	6	11669	2	NX	1	1	1	2	1	2	1	2	2	2	3
49	6	11670	2	NX	1	1	1	2	1	2	1	1	2	2	1
50	8	11673	2	NX	1	1	1	2	1	2	1	1	2	2	1
51	6	11674	1	NX	1	1	1	2	1	2	1	3	3	3	3
52	8	11675	1	NX	1	1	1	2	1	2	1	3	3	3	3
53	8	11680	1	NX	1	1	1	2	1	2	1	3	3	3	1
54	6	11685	2	NX	1	1	1	2	1	2	1	1	2	2	1
55	8	11686	1	NX	1	1	1	2	1	2	1	3	3	3	3
56	7	11687	1	NX	1	1	1	2	1	2	1	3	3	3	3
57	8	11694	2	NX	1	1	1	2	1	2	1	1	2	2	1
58	8	11699	1	NX	1	1	1	2	1	2	1	3	3	3	3
59	9	11703	1	NX	1	1	1	2	1	2	1	3	3	3	3
60	8	11708	1	NX	1	1	1	2	1	2	1	3	3	3	3
61	9	11718	1	NX	1	1	1	2	1	2	1	3	3	3	3
62	3	11719	1	NX	1	1	1	2	1	2	1	3	3	3	3
63	6	11730	1	NX	1	1	1	2	1	2	1	3	3	3	3
64	8	11738	1	NX	1	1	1	2	1	2	1	3	3	3	3
65	5	11753	2	NX	1	1	1	2	1	2	1	1	2	2	1
66	9	11755	1	NX	1	1	1	2	1	2	1	1	1	1	2
67	8	11760	1	NX	1	1	1	2	1	2	1	3	3	3	3
68	6	11762	1	NX	1	1	1	2	1	2	1	3	3	3	3
69	3	11759	1	NX	1	1	1	2	1	2	1	3	3	3	3
70	8	11773	2	NX	1	1	1	2	1	2	1	2	2	2	3
71	3	11775	1	NX	1	1	1	2	1	2	1	3	3	3	2
72	1	11776	1	NX	1	1	1	2	1	2	1	3	3	3	3
73	9	11778	1	NX	1	1	1	2	1	2	1	3	3	3	2
74	3	11782	1	NX	1	1	1	2	1	2	1	3	3	3	2
75	8	11786	2	NX	1	1	1	2	1	2	1	1	2	2	1
76	7	11793	1	NX	1	1	1	2	1	2	1	3	3	3	3
77	9	11801	1	NX	1	1	1	2	1	2	1	3	3	3	1
78	2	11802	1	NX	1	1	1	2	1	2	1	3	3	3	3
79	8	11808	1	NX	1	1	1	2	1	2	1	3	3	3	3
80	8	11814	1	NX	1	1	1	2	1	2	1	3	3	3	1
81	8	11815	2	NX	1	1	1	2	1	2	1	1	2	2	1
82	8	11816	1	NX	1	1	1	2	1	2	1	3	3	3	3

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
83	8	11822	1	NX	1	1	1	2	1	2	1	3	3	3	3
84	8	11840	1	NX	1	1	1	2	1	2	1	3	3	3	1
85	2	11841	1	NX	1	1	1	2	1	2	1	3	3	3	3
86	8	11880	1	NX	1	1	1	2	1	2	1	3	3	3	1
87	8	11884	1	NX	1	1	1	2	1	2	1	3	3	3	3
88	3	11887	2	NX	1	1	1	2	1	2	1	1	2	2	1
89	8	11890	1	NX	1	1	1	2	1	2	1	3	3	3	3
90	8	11892	2	NX	1	1	1	2	1	2	1	1	2	2	1
91	1	11899	1	NX	1	1	1	2	1	2	1	3	3	3	3
92	3	11897	1	NX	1	1	1	2	1	2	1	3	3	3	3
93	1	11899	1	NX	1	1	1	2	1	2	1	3	3	3	2
94	8	11902	1	NX	1	1	1	2	1	2	1	3	3	3	3
95	3	11904	1	NX	1	1	1	2	1	2	1	3	3	3	3
96	6	11906	1	NX	1	1	1	2	1	2	1	3	3	3	3
97	7	11907	2	NX	1	1	1	2	1	2	1	1	2	2	1
98	6	11908	1	NX	1	1	1	2	1	2	1	3	3	3	3
99	8	11932	1	NX	1	1	1	2	1	2	1	3	3	3	1
100	8	11933	1	NX	1	1	1	2	1	2	1	3	3	3	3
101	8	11939	2	NX	1	1	1	2	1	2	1	1	2	2	1
102	7	11948	1	NX	1	1	1	2	1	2	1	3	3	3	3
103	3	11951	1	NX	1	1	1	2	1	2	1	3	3	3	3
104	1	11956	1	NX	1	1	1	2	1	2	1	3	3	3	3
105	8	11957	2	NX	1	1	1	2	1	2	1	1	2	2	1
106	8	11962	1	NX	1	1	1	2	1	2	1	3	3	3	1
107	6	11964	1	NX	1	1	1	2	1	2	1	3	3	3	3
108	8	11974	2	NX	1	1	1	2	1	2	1	2	2	2	3
109	8	11975	1	NX	1	1	1	2	1	2	1	3	3	3	2
110	3	11981	1	NX	1	1	1	2	1	2	1	3	3	3	3
111	3	11983	1	NX	1	1	1	2	1	2	1	3	3	3	3
112	8	11984	2	NX	1	1	1	2	1	2	1	1	2	2	1
113	8	11986	2	NX	1	1	1	2	1	2	1	2	2	2	3
114	3	11990	1	NX	1	1	1	2	1	2	1	3	3	3	2
115	7	11995	1	NX	1	1	1	2	1	2	1	3	3	3	2
116	3	12003	1	NX	1	1	1	2	1	2	1	3	3	3	3
117	1	12010	2	NX	1	1	1	2	1	2	1	2	2	2	3
118	4	12011	1	NX	1	1	1	2	1	2	1	3	3	3	1
119	1	12013	1	NX	1	1	1	2	1	2	1	3	3	3	2
120	6	12015	1	NX	1	1	1	2	1	2	1	3	3	3	1
121	7	12016	2	NX	1	1	1	2	1	2	1	1	2	2	1
122	8	12019	2	NX	1	1	1	2	1	2	1	2	2	2	2
123	5	12024	1	NX	1	1	1	2	1	2	1	3	3	3	1

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
124	8	12025	1	NX	1	1	1	2	1	2	1	3	3	3	1
125	8	12031	2	NX	1	1	1	2	1	2	1	2	2	2	3
126	3	12045	1	NX	1	1	1	2	1	2	1	3	3	3	3
127	10	12049	1	NX	1	1	1	2	1	2	1	3	3	3	1
128	7	12066	2	NX	1	1	1	2	1	2	1	2	2	2	3
129	9	12067	1	NX	1	1	1	2	1	2	1	3	3	3	1
130	7	12077	2	NX	1	1	1	2	1	2	1	2	2	2	2
131	8	12078	2	NX	1	1	1	2	1	2	1	2	2	2	1
132	8	12085	1	NX	1	1	1	2	1	2	1	3	3	3	2
133	8	12087	1	NX	1	1	1	2	1	2	1	3	3	3	3
134	4	12088	1	NX	1	1	1	2	1	2	1	3	3	3	3
135	10	12089	2	NX	1	1	1	2	1	2	1	1	2	2	1
136	8	12094	2	NX	1	1	1	2	1	2	1	1	2	2	1
137	6	12099	1	NX	1	1	1	2	1	2	1	3	3	3	1
138	6	12100	1	NX	1	1	1	2	1	2	1	3	3	3	3
139	8	12101	1	NX	1	1	1	2	1	2	1	3	3	3	3
140	3	12110	1	NX	1	1	1	2	1	2	1	3	3	3	3
1	8	11511	1	NTS	2	2	2	1	2	1	2	3	3	3	3
2	10	11516	1	NTS	2	2	2	1	2	1	2	3	3	3	2
3	8	11520	1	NTS	2	2	2	1	2	1	2	3	3	3	3
4	8	11523	1	NTS	2	2	2	1	2	1	2	3	3	3	3
5	7	11524	1	NTS	2	2	2	1	2	1	2	3	3	3	2
6	6	11525	1	NTS	2	2	2	1	2	1	2	3	3	3	3
7	8	11526	1	NTS	2	2	2	1	2	1	2	3	3	3	3
8	6	11527	2	NTS	2	2	2	1	2	1	2	1	2	2	1
9	6	11531	1	NTS	2	2	2	1	2	1	2	3	2	2	2
10	8	11533	1	NTS	2	2	2	1	2	1	2	3	3	3	3
11	4	11535	2	NTS	2	2	2	1	2	1	2	1	2	2	1
12	8	11538	1	NTS	2	2	2	1	2	1	2	3	3	3	2
13	3	11546	1	NTS	2	2	2	1	2	1	2	3	3	3	3
14	4	11548	1	NTS	2	2	2	1	2	1	2	3	3	3	1
15	8	11558	2	NTS	2	2	2	1	2	1	2	1	2	2	1
16	6	11559	1	NTS	2	2	2	1	2	1	2	3	3	3	3
17	9	11563	1	NTS	2	2	2	1	2	1	2	3	3	3	3
18	10	11564	2	NTS	2	2	2	1	2	1	2	1	2	2	1
19	8	11565	1	NTS	2	2	2	1	2	1	2	3	3	3	3
20	8	11566	1	NTS	2	2	2	1	2	1	2	3	3	3	3
21	1	11567	2	NTS	2	2	2	1	2	1	2	1	2	2	1
22	9	11569	2	NTS	2	2	2	1	2	1	2	2	2	2	3
23	8	11570	1	NTS	2	2	2	1	2	1	2	3	3	3	3
24	10	11576	2	NTS	2	2	2	1	2	1	2	1	2	2	1

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
25	8	11583	1	NTS	2	2	2	1	2	1	2	3	3	3	1
26	8	11586	1	NTS	2	2	2	1	2	1	2	3	3	3	3
27	8	11588	1	NTS	2	2	2	1	2	1	2	3	3	3	3
28	8	11589	1	NTS	2	2	2	1	2	1	2	3	3	3	3
29	1	11590	2	NTS	2	2	2	1	2	1	2	1	2	2	1
30	7	11591	1	NTS	2	2	2	1	2	1	2	3	3	3	1
31	1	11598	2	NTS	2	2	2	1	2	1	2	1	2	2	1
32	8	11619	1	NTS	2	2	2	1	2	1	2	3	3	3	1
33	4	11622	1	NTS	2	2	2	1	2	1	2	3	3	3	1
34	4	11628	1	NTS	2	2	2	1	2	1	2	3	3	3	2
35	3	11535	1	NTS	2	2	2	1	2	1	2	3	3	3	3
36	8	11636	1	NTS	2	2	2	1	2	1	2	3	3	3	1
37	8	11636	1	NTS	2	2	2	1	2	1	2	3	3	3	3
38	8	11639	1	NTS	2	2	2	1	2	1	2	3	3	3	1
39	3	11646	1	NTS	2	2	2	1	2	1	2	3	3	3	1
40	8	11650	1	NTS	2	2	2	1	2	1	2	3	3	3	3
41	8	11644	2	NTS	2	2	2	1	2	1	2	1	2	2	1
42	8	11652	2	NTS	2	2	2	1	2	1	2	1	2	2	1
43	8	11658	2	NTS	2	2	2	1	2	1	2	1	2	2	1
44	9	11660	1	NTS	2	2	2	1	2	1	2	3	3	3	1
45	6	11661	1	NTS	2	2	2	1	2	1	2	3	3	3	3
46	8	11663	1	NTS	2	2	2	1	2	1	2	3	3	3	1
47	8	11668	1	NTS	2	2	2	1	2	1	2	3	3	3	3
48	6	11669	2	NTS	2	2	2	1	2	1	2	2	2	2	3
49	6	11670	2	NTS	2	2	2	1	2	1	2	1	2	2	1
50	8	11673	2	NTS	2	2	2	1	2	1	2	1	2	2	1
51	6	11674	1	NTS	2	2	2	1	2	1	2	3	3	3	3
52	8	11675	1	NTS	2	2	2	1	2	1	2	3	3	3	3
53	8	11680	1	NTS	2	2	2	1	2	1	2	3	3	3	3
54	6	11685	2	NTS	2	2	2	1	2	1	2	1	2	2	1
55	8	11686	1	NTS	2	2	2	1	2	1	2	3	3	3	3
56	7	11687	1	NTS	2	2	2	1	2	1	2	3	3	3	3
57	8	11694	2	NTS	2	2	2	1	2	1	2	1	2	2	1
58	8	11699	1	NTS	2	2	2	1	2	1	2	3	3	3	3
59	9	11703	1	NTS	2	2	2	1	2	1	2	3	3	3	3
60	8	11708	1	NTS	2	2	2	1	2	1	2	3	3	3	3
61	9	11718	1	NTS	2	2	2	1	2	1	2	3	3	3	3
62	3	11719	1	NTS	2	2	2	1	2	1	2	3	3	3	3
63	6	11730	1	NTS	2	2	2	1	2	1	2	3	3	3	3
64	8	11738	1	NTS	2	2	2	1	2	1	2	3	3	3	3
65	5	11753	2	NTS	2	2	2	1	2	1	2	1	2	2	1

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
66	9	11755	1	NTS	2	2	2	1	2	1	2	1	1	1	1
67	8	11760	1	NTS	2	2	2	1	2	1	2	3	3	3	3
68	6	11762	1	NTS	2	2	2	1	2	1	2	3	3	3	3
69	3	11759	1	NTS	2	2	2	1	2	1	2	3	3	3	3
70	8	11773	2	NTS	2	2	2	1	2	1	2	2	2	2	3
71	3	11775	1	NTS	2	2	2	1	2	1	2	3	3	3	2
72	1	11776	1	NTS	2	2	2	1	2	1	2	3	3	3	3
73	9	11778	1	NTS	2	2	2	1	2	1	2	3	3	3	2
74	3	11782	1	NTS	2	2	2	1	2	1	2	3	3	3	2
75	8	11786	2	NTS	2	2	2	1	2	1	2	1	2	2	1
76	7	11793	1	NTS	2	2	2	1	2	1	2	3	3	3	3
77	9	11801	1	NTS	2	2	2	1	2	1	2	3	3	3	1
78	2	11802	1	NTS	2	2	2	1	2	1	2	3	3	3	3
79	8	11808	1	NTS	2	2	2	1	2	1	2	3	3	3	3
80	8	11814	1	NTS	2	2	2	1	2	1	2	3	3	3	1
81	8	11815	2	NTS	2	2	2	1	2	1	2	1	2	2	1
82	8	11816	1	NTS	2	2	2	1	2	1	2	3	3	3	3
83	8	11822	1	NTS	2	2	2	1	2	1	2	3	3	3	3
84	8	11840	1	NTS	2	2	2	1	2	1	2	3	3	3	1
85	2	11841	1	NTS	2	2	2	1	2	1	2	3	3	3	3
86	8	11880	1	NTS	2	2	2	1	2	1	2	3	3	3	1
87	8	11884	1	NTS	2	2	2	1	2	1	2	3	3	3	3
88	3	11887	2	NTS	2	2	2	1	2	1	2	1	2	2	1
89	8	11890	1	NTS	2	2	2	1	2	1	2	3	3	3	3
90	8	11892	2	NTS	2	2	2	1	2	1	2	1	2	2	1
91	1	11899	1	NTS	2	2	2	1	2	1	2	3	3	3	3
92	3	11897	1	NTS	2	2	2	1	2	1	2	3	3	3	3
93	1	11899	1	NTS	2	2	2	1	2	1	2	3	3	3	2
94	8	11902	1	NTS	2	2	2	1	2	1	2	3	3	3	3
95	3	11904	1	NTS	2	2	2	1	2	1	2	3	3	3	3
96	6	11906	1	NTS	2	2	2	1	2	1	2	3	3	3	3
97	7	11907	2	NTS	2	2	2	1	2	1	2	1	2	2	1
98	6	11908	1	NTS	2	2	2	1	2	1	2	3	3	3	3
99	8	11932	1	NTS	2	2	2	1	2	1	2	3	3	3	1
100	8	11933	1	NTS	2	2	2	1	2	1	2	3	3	3	3
101	8	11939	2	NTS	2	2	2	1	2	1	2	1	2	2	1
102	7	11948	1	NTS	2	2	2	1	2	1	2	3	3	3	3
103	3	11951	1	NTS	2	2	2	1	2	1	2	3	3	3	3
104	1	11956	1	NTS	2	2	2	1	2	1	2	3	3	3	3
105	8	11957	2	NTS	2	2	2	1	2	1	2	1	2	2	1
106	8	11962	1	NTS	2	2	2	1	2	1	2	3	3	3	1

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
107	6	11964	1	NTS	2	2	2	1	2	1	2	3	3	3	3
108	8	11974	2	NTS	2	2	2	1	2	1	2	2	2	2	3
109	8	11975	1	NTS	2	2	2	1	2	1	2	3	3	3	2
110	3	11981	1	NTS	2	2	2	1	2	1	2	3	3	3	3
111	3	11983	1	NTS	2	2	2	1	2	1	2	3	3	3	3
112	8	11984	2	NTS	2	2	2	1	2	1	2	1	2	2	1
113	8	11986	2	NTS	2	2	2	1	2	1	2	2	2	2	3
114	3	11990	1	NTS	2	2	2	1	2	1	2	3	3	3	2
115	7	11995	1	NTS	2	2	2	1	2	1	2	3	3	3	2
116	3	12003	1	NTS	2	2	2	1	2	1	2	3	3	3	3
117	1	12010	2	NTS	2	2	2	1	2	1	2	2	2	2	3
118	4	12011	1	NTS	2	2	2	1	2	1	2	3	3	3	1
119	1	12013	1	NTS	2	2	2	1	2	1	2	3	3	3	2
120	6	12015	1	NTS	2	2	2	1	2	1	2	3	3	3	1
121	7	12016	2	NTS	2	2	2	1	2	1	2	1	2	2	1
122	8	12019	2	NTS	2	2	2	1	2	1	2	2	2	2	2
123	5	12024	1	NTS	2	2	2	1	2	1	2	3	3	3	1
124	8	12025	1	NTS	2	2	2	1	2	1	2	3	3	3	1
125	8	12031	2	NTS	2	2	2	1	2	1	2	2	2	2	3
126	3	12045	1	NTS	2	2	2	1	2	1	2	3	3	3	3
127	10	12049	1	NTS	2	2	2	1	2	1	2	3	3	3	1
128	7	12066	2	NTS	2	2	2	1	2	1	2	2	2	2	3
129	9	12067	1	NTS	2	2	2	1	2	1	2	3	3	3	1
130	7	12077	2	NTS	2	2	2	1	2	1	2	2	2	2	2
131	8	12078	2	NTS	2	2	2	1	2	1	2	2	2	2	1
132	8	12085	1	NTS	2	2	2	1	2	1	2	3	3	3	2
133	8	12087	1	NTS	2	2	2	1	2	1	2	3	3	3	3
134	4	12088	1	NTS	2	2	2	1	2	1	2	3	3	3	3
135	10	12089	2	NTS	2	2	2	1	2	1	2	1	2	2	1
136	8	12094	2	NTS	2	2	2	1	2	1	2	1	2	2	1
137	6	12099	1	NTS	2	2	2	1	2	1	2	3	3	3	1
138	6	12100	1	NTS	2	2	2	1	2	1	2	3	3	3	3
139	8	12101	1	NTS	2	2	2	1	2	1	2	3	3	3	3
140	3	12110	1	NTS	2	2	2	1	2	1	2	3	3	3	3
1	8	11511	1	OX	1	1	1	2	1	2	1	1	1	1	1
2	10	11516	1	OX	1	1	1	2	1	2	1	2	2	2	2
3	8	11520	1	OX	1	1	1	2	1	2	1	2	2	2	3
4	8	11523	1	OX	1	1	1	2	1	2	1	2	2	2	3
5	7	11524	1	OX	1	1	1	2	1	2	1	2	2	2	2
6	6	11525	1	OX	1	1	1	2	1	2	1	2	2	2	3
7	8	11526	1	OX	1	1	1	2	1	2	1	2	2	2	3

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
8	6	11527	2	OX	1	1	1	2	1	2	1	1	2	1	1
9	6	11531	1	OX	1	1	1	2	1	2	1	2	2	2	2
10	8	11533	1	OX	1	1	1	2	1	2	1	2	2	2	3
11	4	11535	2	OX	1	1	1	2	1	2	1	1	1	1	1
12	8	11538	1	OX	1	1	1	2	1	2	1	2	2	2	2
13	3	11546	1	OX	1	1	1	2	1	2	1	2	2	2	3
14	4	11548	1	OX	1	1	1	2	1	2	1	2	2	2	1
15	8	11558	2	OX	1	1	1	2	1	2	1	1	1	1	1
16	6	11559	1	OX	1	1	1	2	1	2	1	2	2	2	3
17	9	11563	1	OX	1	1	1	2	1	2	1	2	2	2	3
18	10	11564	2	OX	1	1	1	2	1	2	1	1	1	1	1
19	8	11565	1	OX	1	1	1	2	1	2	1	2	2	2	3
20	8	11566	1	OX	1	1	1	2	1	2	1	2	2	2	3
21	1	11567	2	OX	1	1	1	2	1	2	1	1	1	1	1
22	9	11569	2	OX	1	1	1	2	1	2	1	1	2	2	3
23	8	11570	1	OX	1	1	1	2	1	2	1	2	2	2	3
24	10	11576	2	OX	1	1	1	2	1	2	1	1	1	1	1
25	8	11583	1	OX	1	1	1	2	1	2	1	2	2	2	1
26	8	11586	1	OX	1	1	1	2	1	2	1	2	2	2	3
27	8	11588	1	OX	1	1	1	2	1	2	1	2	2	2	3
28	8	11589	1	OX	1	1	1	2	1	2	1	2	2	2	3
29	1	11590	2	OX	1	1	1	2	1	2	1	1	1	1	1
30	7	11591	1	OX	1	1	1	2	1	2	1	2	2	2	1
31	1	11598	2	OX	1	1	1	2	1	2	1	1	1	1	1
32	8	11619	1	OX	1	1	1	2	1	2	1	2	2	2	1
33	4	11622	1	OX	1	1	1	2	1	2	1	2	2	2	1
34	4	11628	1	OX	1	1	1	2	1	2	1	2	2	2	2
35	3	11535	1	OX	1	1	1	2	1	2	1	2	2	2	3
36	8	11636	1	OX	1	1	1	2	1	2	1	2	2	2	1
37	8	11636	1	OX	1	1	1	2	1	2	1	2	2	2	3
38	8	11639	1	OX	1	1	1	2	1	2	1	2	2	2	1
39	3	11646	1	OX	1	1	1	2	1	2	1	2	2	2	1
40	8	11650	1	OX	1	1	1	2	1	2	1	2	2	2	3
41	8	11644	2	OX	1	1	1	2	1	2	1	1	1	1	1
42	8	11652	2	OX	1	1	1	2	1	2	1	1	1	1	1
43	8	11658	2	OX	1	1	1	2	1	2	1	1	1	1	1
44	9	11660	1	OX	1	1	1	2	1	2	1	2	2	2	1
45	6	11661	1	OX	1	1	1	2	1	2	1	2	2	2	3
46	8	11663	1	OX	1	1	1	2	1	2	1	2	2	2	1
47	8	11668	1	OX	1	1	1	2	1	2	1	2	2	2	3
48	6	11669	2	OX	1	1	1	2	1	2	1	1	2	2	3

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
49	6	11670	2	OX	1	1	1	2	1	2	1	1	1	1	1
50	8	11673	2	OX	1	1	1	2	1	2	1	1	1	1	1
51	6	11674	1	OX	1	1	1	2	1	2	1	2	2	2	3
52	8	11675	1	OX	1	1	1	2	1	2	1	2	2	2	3
53	8	11680	1	OX	1	1	1	2	1	2	1	2	2	2	1
54	6	11685	2	OX	1	1	1	2	1	2	1	1	1	1	1
55	8	11686	1	OX	1	1	1	2	1	2	1	2	2	2	3
56	7	11687	1	OX	1	1	1	2	1	2	1	2	2	2	3
57	8	11694	2	OX	1	1	1	2	1	2	1	1	1	1	1
58	8	11699	1	OX	1	1	1	2	1	2	1	2	2	2	3
59	9	11703	1	OX	1	1	1	2	1	2	1	2	2	2	3
60	8	11708	1	OX	1	1	1	2	1	2	1	2	2	2	3
61	9	11718	1	OX	1	1	1	2	1	2	1	2	2	2	3
62	3	11719	1	OX	1	1	1	2	1	2	1	2	2	2	3
63	6	11730	1	OX	1	1	1	2	1	2	1	2	2	2	3
64	8	11738	1	OX	1	1	1	2	1	2	1	2	2	2	3
65	5	11753	2	OX	1	1	1	2	1	2	1	1	1	1	1
66	9	11755	1	OX	1	1	1	2	1	2	1	2	2	2	2
67	8	11760	1	OX	1	1	1	2	1	2	1	2	2	2	3
68	6	11762	1	OX	1	1	1	2	1	2	1	2	2	2	3
69	3	11759	1	OX	1	1	1	2	1	2	1	2	2	2	3
70	8	11773	2	OX	1	1	1	2	1	2	1	1	2	2	3
71	3	11775	1	OX	1	1	1	2	1	2	1	2	2	2	2
72	1	11776	1	OX	1	1	1	2	1	2	1	2	2	2	3
73	9	11778	1	OX	1	1	1	2	1	2	1	2	2	2	2
74	3	11782	1	OX	1	1	1	2	1	2	1	2	2	2	2
75	8	11786	2	OX	1	1	1	2	1	2	1	1	1	1	1
76	7	11793	1	OX	1	1	1	2	1	2	1	2	2	2	3
77	9	11801	1	OX	1	1	1	2	1	2	1	2	2	2	1
78	2	11802	1	OX	1	1	1	2	1	2	1	2	2	2	3
79	8	11808	1	OX	1	1	1	2	1	2	1	2	2	2	3
80	8	11814	1	OX	1	1	1	2	1	2	1	2	2	2	1
81	8	11815	2	OX	1	1	1	2	1	2	1	1	1	1	1
82	8	11816	1	OX	1	1	1	2	1	2	1	2	2	2	3
83	8	11822	1	OX	1	1	1	2	1	2	1	2	2	2	3
84	8	11840	1	OX	1	1	1	2	1	2	1	2	2	2	1
85	2	11841	1	OX	1	1	1	2	1	2	1	2	2	2	3
86	8	11880	1	OX	1	1	1	2	1	2	1	2	2	2	1
87	8	11884	1	OX	1	1	1	2	1	2	1	2	2	2	3
88	3	11887	2	OX	1	1	1	2	1	2	1	1	1	1	1
89	8	11890	1	OX	1	1	1	2	1	2	1	2	2	2	3

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
90	8	11892	2	OX	1	1	1	2	1	2	1	1	1	1	1
91	1	11899	1	OX	1	1	1	2	1	2	1	2	2	2	3
92	3	11897	1	OX	1	1	1	2	1	2	1	2	2	2	3
93	1	11899	1	OX	1	1	1	2	1	2	1	2	2	2	2
94	8	11902	1	OX	1	1	1	2	1	2	1	2	2	2	3
95	3	11904	1	OX	1	1	1	2	1	2	1	2	2	2	3
96	6	11906	1	OX	1	1	1	2	1	2	1	2	2	2	3
97	7	11907	2	OX	1	1	1	2	1	2	1	1	1	1	1
98	6	11908	1	OX	1	1	1	2	1	2	1	2	2	2	3
99	8	11932	1	OX	1	1	1	2	1	2	1	2	2	2	1
100	8	11933	1	OX	1	1	1	2	1	2	1	2	2	2	3
101	8	11939	2	OX	1	1	1	2	1	2	1	1	1	1	1
102	7	11948	1	OX	1	1	1	2	1	2	1	2	2	2	3
103	3	11951	1	OX	1	1	1	2	1	2	1	2	2	2	3
104	1	11956	1	OX	1	1	1	2	1	2	1	2	2	2	3
105	8	11957	2	OX	1	1	1	2	1	2	1	1	1	1	1
106	8	11962	1	OX	1	1	1	2	1	2	1	2	2	2	1
107	6	11964	1	OX	1	1	1	2	1	2	1	2	2	2	3
108	8	11974	2	OX	1	1	1	2	1	2	1	1	2	2	3
109	8	11975	1	OX	1	1	1	2	1	2	1	2	2	2	2
110	3	11981	1	OX	1	1	1	2	1	2	1	2	2	2	3
111	3	11983	1	OX	1	1	1	2	1	2	1	2	2	2	3
112	8	11984	2	OX	1	1	1	2	1	2	1	1	1	1	1
113	8	11986	2	OX	1	1	1	2	1	2	1	1	2	2	3
114	3	11990	1	OX	1	1	1	2	1	2	1	2	2	2	2
115	7	11995	1	OX	1	1	1	2	1	2	1	2	2	2	2
116	3	12003	1	OX	1	1	1	2	1	2	1	2	2	2	3
117	1	12010	2	OX	1	1	1	2	1	2	1	1	2	2	3
118	4	12011	1	OX	1	1	1	2	1	2	1	2	2	2	1
119	1	12013	1	OX	1	1	1	2	1	2	1	2	2	2	2
120	6	12015	1	OX	1	1	1	2	1	2	1	2	2	2	1
121	7	12016	2	OX	1	1	1	2	1	2	1	1	1	1	1
122	8	12019	2	OX	1	1	1	2	1	2	1	1	2	2	2
123	5	12024	1	OX	1	1	1	2	1	2	1	2	2	2	1
124	8	12025	1	OX	1	1	1	2	1	2	1	2	2	2	1
125	8	12031	2	OX	1	1	1	2	1	2	1	1	2	2	3
126	3	12045	1	OX	1	1	1	2	1	2	1	2	2	2	3
127	10	12049	1	OX	1	1	1	2	1	2	1	2	2	2	1
128	7	12066	2	OX	1	1	1	2	1	2	1	1	2	2	3
129	9	12067	1	OX	1	1	1	2	1	2	1	2	2	2	1
130	7	12077	2	OX	1	1	1	2	1	2	1	1	2	2	2

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
131	8	12078	2	OX	1	1	1	2	1	2	1	1	2	2	1
132	8	12085	1	OX	1	1	1	2	1	2	1	2	2	2	2
133	8	12087	1	OX	1	1	1	2	1	2	1	2	2	2	3
134	4	12088	1	OX	1	1	1	2	1	2	1	2	2	2	3
135	10	12089	2	OX	1	1	1	2	1	2	1	1	1	1	1
136	8	12094	2	OX	1	1	1	2	1	2	1	1	1	1	1
137	6	12099	1	OX	1	1	1	2	1	2	1	2	2	2	1
138	6	12100	1	OX	1	1	1	2	1	2	1	2	2	2	3
139	8	12101	1	OX	1	1	1	2	1	2	1	2	2	2	3
140	3	12110	1	OX	1	1	1	2	1	2	1	2	2	2	3
1	8	11511	1	OTS	2	2	2	1	2	1	2	3	3	3	3
2	10	11516	1	OTS	2	2	2	1	2	1	2	3	3	3	2
3	8	11520	1	OTS	2	2	2	1	2	1	2	3	3	3	3
4	8	11523	1	OTS	2	2	2	1	2	1	2	3	3	3	3
5	7	11524	1	OTS	2	2	2	1	2	1	2	3	3	3	2
6	6	11525	1	OTS	2	2	2	1	2	1	2	3	3	3	3
7	8	11526	1	OTS	2	2	2	1	2	1	2	3	3	3	3
8	6	11527	2	OTS	2	2	2	1	2	1	2	1	2	2	1
9	6	11531	1	OTS	2	2	2	1	2	1	2	3	2	2	2
10	8	11533	1	OTS	2	2	2	1	2	1	2	3	3	3	3
11	4	11535	2	OTS	2	2	2	1	2	1	2	1	2	2	1
12	8	11538	1	OTS	2	2	2	1	2	1	2	3	3	3	2
13	3	11546	1	OTS	2	2	2	1	2	1	2	3	3	3	3
14	4	11548	1	OTS	2	2	2	1	2	1	2	3	3	3	1
15	8	11558	2	OTS	2	2	2	1	2	1	2	1	2	2	1
16	6	11559	1	OTS	2	2	2	1	2	1	2	3	3	3	3
17	9	11563	1	OTS	2	2	2	1	2	1	2	3	3	3	3
18	10	11564	2	OTS	2	2	2	1	2	1	2	1	2	2	1
19	8	11565	1	OTS	2	2	2	1	2	1	2	3	3	3	3
20	8	11566	1	OTS	2	2	2	1	2	1	2	3	3	3	3
21	1	11567	2	OTS	2	2	2	1	2	1	2	1	2	2	1
22	9	11569	2	OTS	2	2	2	1	2	1	2	2	2	2	3
23	8	11570	1	OTS	2	2	2	1	2	1	2	3	3	3	3
24	10	11576	2	OTS	2	2	2	1	2	1	2	1	2	2	1
25	8	11583	1	OTS	2	2	2	1	2	1	2	3	3	3	1
26	8	11586	1	OTS	2	2	2	1	2	1	2	3	3	3	3
27	8	11588	1	OTS	2	2	2	1	2	1	2	3	3	3	3
28	8	11589	1	OTS	2	2	2	1	2	1	2	3	3	3	3
29	1	11590	2	OTS	2	2	2	1	2	1	2	1	2	2	1
30	7	11591	1	OTS	2	2	2	1	2	1	2	3	3	3	1
31	1	11598	2	OTS	2	2	2	1	2	1	2	1	2	2	1

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
32	8	11619	1	OTS	2	2	2	1	2	1	2	3	3	3	1
33	4	11622	1	OTS	2	2	2	1	2	1	2	3	3	3	1
34	4	11628	1	OTS	2	2	2	1	2	1	2	3	3	3	2
35	3	11535	1	OTS	2	2	2	1	2	1	2	3	3	3	3
36	8	11636	1	OTS	2	2	2	1	2	1	2	3	3	3	1
37	8	11636	1	OTS	2	2	2	1	2	1	2	3	3	3	3
38	8	11639	1	OTS	2	2	2	1	2	1	2	3	3	3	1
39	3	11646	1	OTS	2	2	2	1	2	1	2	3	3	3	1
40	8	11650	1	OTS	2	2	2	1	2	1	2	3	3	3	3
41	8	11644	2	OTS	2	2	2	1	2	1	2	1	2	2	1
42	8	11652	2	OTS	2	2	2	1	2	1	2	1	2	2	1
43	8	11658	2	OTS	2	2	2	1	2	1	2	1	2	2	1
44	9	11660	1	OTS	2	2	2	1	2	1	2	3	3	3	1
45	6	11661	1	OTS	2	2	2	1	2	1	2	3	3	3	3
46	8	11663	1	OTS	2	2	2	1	2	1	2	3	3	3	1
47	8	11668	1	OTS	2	2	2	1	2	1	2	3	3	3	3
48	6	11669	2	OTS	2	2	2	1	2	1	2	2	2	2	3
49	6	11670	2	OTS	2	2	2	1	2	1	2	1	2	2	1
50	8	11673	2	OTS	2	2	2	1	2	1	2	1	2	2	1
51	6	11674	1	OTS	2	2	2	1	2	1	2	3	3	3	3
52	8	11675	1	OTS	2	2	2	1	2	1	2	3	3	3	3
53	8	11680	1	OTS	2	2	2	1	2	1	2	3	3	3	3
54	6	11685	2	OTS	2	2	2	1	2	1	2	1	2	2	1
55	8	11686	1	OTS	2	2	2	1	2	1	2	3	3	3	3
56	7	11687	1	OTS	2	2	2	1	2	1	2	3	3	3	3
57	8	11694	2	OTS	2	2	2	1	2	1	2	1	2	2	1
58	8	11699	1	OTS	2	2	2	1	2	1	2	3	3	3	3
59	9	11703	1	OTS	2	2	2	1	2	1	2	3	3	3	3
60	8	11708	1	OTS	2	2	2	1	2	1	2	3	3	3	3
61	9	11718	1	OTS	2	2	2	1	2	1	2	3	3	3	3
62	3	11719	1	OTS	2	2	2	1	2	1	2	3	3	3	3
63	6	11730	1	OTS	2	2	2	1	2	1	2	3	3	3	3
64	8	11738	1	OTS	2	2	2	1	2	1	2	3	3	3	3
65	5	11753	2	OTS	2	2	2	1	2	1	2	1	2	2	1
66	9	11755	1	OTS	2	2	2	1	2	1	2	1	1	1	1
67	8	11760	1	OTS	2	2	2	1	2	1	2	3	3	3	3
68	6	11762	1	OTS	2	2	2	1	2	1	2	3	3	3	3
69	3	11759	1	OTS	2	2	2	1	2	1	2	3	3	3	3
70	8	11773	2	OTS	2	2	2	1	2	1	2	2	2	2	3
71	3	11775	1	OTS	2	2	2	1	2	1	2	3	3	3	2
72	1	11776	1	OTS	2	2	2	1	2	1	2	3	3	3	3

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
73	9	11778	1	OTS	2	2	2	1	2	1	2	3	3	3	2
74	3	11782	1	OTS	2	2	2	1	2	1	2	3	3	3	2
75	8	11786	2	OTS	2	2	2	1	2	1	2	1	2	2	1
76	7	11793	1	OTS	2	2	2	1	2	1	2	3	3	3	3
77	9	11801	1	OTS	2	2	2	1	2	1	2	3	3	3	1
78	2	11802	1	OTS	2	2	2	1	2	1	2	3	3	3	3
79	8	11808	1	OTS	2	2	2	1	2	1	2	3	3	3	3
80	8	11814	1	OTS	2	2	2	1	2	1	2	3	3	3	1
81	8	11815	2	OTS	2	2	2	1	2	1	2	1	2	2	1
82	8	11816	1	OTS	2	2	2	1	2	1	2	3	3	3	3
83	8	11822	1	OTS	2	2	2	1	2	1	2	3	3	3	3
84	8	11840	1	OTS	2	2	2	1	2	1	2	3	3	3	1
85	2	11841	1	OTS	2	2	2	1	2	1	2	3	3	3	3
86	8	11880	1	OTS	2	2	2	1	2	1	2	3	3	3	1
87	8	11884	1	OTS	2	2	2	1	2	1	2	3	3	3	3
88	3	11887	2	OTS	2	2	2	1	2	1	2	1	2	2	1
89	8	11890	1	OTS	2	2	2	1	2	1	2	3	3	3	3
90	8	11892	2	OTS	2	2	2	1	2	1	2	1	2	2	1
91	1	11899	1	OTS	2	2	2	1	2	1	2	3	3	3	3
92	3	11897	1	OTS	2	2	2	1	2	1	2	3	3	3	3
93	1	11899	1	OTS	2	2	2	1	2	1	2	3	3	3	2
94	8	11902	1	OTS	2	2	2	1	2	1	2	3	3	3	3
95	3	11904	1	OTS	2	2	2	1	2	1	2	3	3	3	3
96	6	11906	1	OTS	2	2	2	1	2	1	2	3	3	3	3
97	7	11907	2	OTS	2	2	2	1	2	1	2	1	2	2	1
98	6	11908	1	OTS	2	2	2	1	2	1	2	3	3	3	3
99	8	11932	1	OTS	2	2	2	1	2	1	2	3	3	3	1
100	8	11933	1	OTS	2	2	2	1	2	1	2	3	3	3	3
101	8	11939	2	OTS	2	2	2	1	2	1	2	1	2	2	1
102	7	11948	1	OTS	2	2	2	1	2	1	2	3	3	3	3
103	3	11951	1	OTS	2	2	2	1	2	1	2	3	3	3	3
104	1	11956	1	OTS	2	2	2	1	2	1	2	3	3	3	3
105	8	11957	2	OTS	2	2	2	1	2	1	2	1	2	2	1
106	8	11962	1	OTS	2	2	2	1	2	1	2	3	3	3	1
107	6	11964	1	OTS	2	2	2	1	2	1	2	3	3	3	3
108	8	11974	2	OTS	2	2	2	1	2	1	2	2	2	2	3
109	8	11975	1	OTS	2	2	2	1	2	1	2	3	3	3	2
110	3	11981	1	OTS	2	2	2	1	2	1	2	3	3	3	3
111	3	11983	1	OTS	2	2	2	1	2	1	2	3	3	3	3
112	8	11984	2	OTS	2	2	2	1	2	1	2	1	2	2	1
113	8	11986	2	OTS	2	2	2	1	2	1	2	2	2	2	3

					Dissecting Microscope			Light Microscope		Electron Microscope					
Nr	Tumour	Sample	Prev	Group	Cons1	Cons2	Cons3	Compact	Cracks	Compact	Cracks	Cell_mem	Cyt_cont	EXT_mat	Diag_cont
114	3	11990	1	OTS	2	2	2	1	2	1	2	3	3	3	2
115	7	11995	1	OTS	2	2	2	1	2	1	2	3	3	3	2
116	3	12003	1	OTS	2	2	2	1	2	1	2	3	3	3	3
117	1	12010	2	OTS	2	2	2	1	2	1	2	2	2	2	3
118	4	12011	1	OTS	2	2	2	1	2	1	2	3	3	3	1
119	1	12013	1	OTS	2	2	2	1	2	1	2	3	3	3	2
120	6	12015	1	OTS	2	2	2	1	2	1	2	3	3	3	1
121	7	12016	2	OTS	2	2	2	1	2	1	2	1	2	2	1
122	8	12019	2	OTS	2	2	2	1	2	1	2	2	2	2	2
123	5	12024	1	OTS	2	2	2	1	2	1	2	3	3	3	1
124	8	12025	1	OTS	2	2	2	1	2	1	2	3	3	3	1
125	8	12031	2	OTS	2	2	2	1	2	1	2	2	2	2	3
126	3	12045	1	OTS	2	2	2	1	2	1	2	3	3	3	3
127	10	12049	1	OTS	2	2	2	1	2	1	2	3	3	3	1
128	7	12066	2	OTS	2	2	2	1	2	1	2	2	2	2	3
129	9	12067	1	OTS	2	2	2	1	2	1	2	3	3	3	1
130	7	12077	2	OTS	2	2	2	1	2	1	2	2	2	2	2
131	8	12078	2	OTS	2	2	2	1	2	1	2	2	2	2	1
132	8	12085	1	OTS	2	2	2	1	2	1	2	3	3	3	2
133	8	12087	1	OTS	2	2	2	1	2	1	2	3	3	3	3
134	4	12088	1	OTS	2	2	2	1	2	1	2	3	3	3	3
135	10	12089	2	OTS	2	2	2	1	2	1	2	1	2	2	1
136	8	12094	2	OTS	2	2	2	1	2	1	2	1	2	2	1
137	6	12099	1	OTS	2	2	2	1	2	1	2	3	3	3	1
138	6	12100	1	OTS	2	2	2	1	2	1	2	3	3	3	3
139	8	12101	1	OTS	2	2	2	1	2	1	2	3	3	3	3
140	3	12110	1	OTS	2	2	2	1	2	1	2	3	3	3	3

APPENDIX B

QUESTIONNAIRE

Questionnaire on XYLENE exposure and use

For office use only

Instructions
Mark the appropriate option in the block on the left with an X or write the answer in the space provided

1-3

1 **Date questionnaire is completed (dd/mm/yy) …./…../…….**

d d m m y y

2 **What is your gender?**

Male(1) Female(2) 10

3 **What is your age?**

……..…….year 11-12

4 **What is your highest qualification?……..…** 13-14

5 **How long have you been working in a laboratory?**

……..……years ……months 15-16

6 **Can the toxicity of reagents be organized in more than one classification?** 19

1 **Yes**
2 No
3 Don't know

7 **Which of the following statements is correct?(Choose ONE)**
Xylene is: 20

1 Very highly toxic
2 Highly toxic
3 Moderately toxic
4 Slightly toxic
5 Don't know

8 **Which of the following statements are correct?(Choose ONE)**
Xylene is: 21

1 A known human carcinogen
2 A probable human carcinogen
3 Possibly a human carcinogen
4 Unclassifiable as to carcinogenity in humans
5 Not likely to be a human carcinogen
6 Don't know

9 **Which of the following statements is correct?(You can choose more than one)**
Xylene is:

1 An allergen 22
2 An asthma activator 23
3 A neurotoxin 24
4 A reproductive toxicant 25
5 A developmental toxicant 26
6 Nature friendly 27
7 Biodegradable 28
8 None of the above 29
9 Don't know 30

10 **Are the toxicity levels of Xylene in laboratories regulated by International Workers Exposure Levels?** 31

1 Yes
2 No
3 Don't know

11 **The maximum level of xylene toxicity permitted is(Choose ONE):**

- [1] 100ppm [part of gas vapor per million (ppm) of contaminated air by volume at 25°C and 760mmHg pressure] time weighted a ten hour period? [32]
- [2] 250ppm time weighted average over a ten hour period?
- [3] Don't know
- [4] No any level of toxicity is permitted

12 **How long does it take for xylene to be cleared from the body?(Choose ONE)**

- [1] 12 hours [33]
- [2] 24 hours
- [3] 72 hours
- [4] more than 72 hours
- [5] Don't know

13 **Is Xylene biodegradable?**

- [1] Yes [34]
- [2] No
- [3] Don't know

14 **How should xylene be discarded?(Choose more than one)**

- [1] Via the drain [35]
- [2] Stored in special drums and discarded by specialised services [36]
- [3] Discarded in a special drainage system that is approved by the fire department [37]
- [4] Don't know [38]
- [5] There is no regulation about it [39]

15 **Is Xylene inflammable?**

- [1] Yes [40]
- [2] No
- [3] Don't know

16 **How should Xylene be STORED?(Choose one)**

- [1] In fire resistant rooms with heavy metal fire-proof door [41]
- [2] In metal fire-proof cabinets
- [3] In well ventilated store rooms
- [4] In ordinary cuboards
- [5] Don't know
- [6] No special precautions are necessary

17 **How should alcohol, acetone and xylene required for DAILY use be stored in the laboratory?(Choose ONE one**

- [1] In fire resistant rooms with heavy metal fire-proof door [42]
- [2] In metal fire-proof cabinets
- [3] In well ventilated store rooms
- [4] Don't know
- [5] In ordinary cuboards
- [6] No special precautions are necessary

18 **Which of the following reagents can not be stored in the same area as Xylene? (You can mark more than one)**

1	Acetone	43
2	Formalin	44
3	Alcohol	45
4	Nitric Acid	46
5	Acetic Acid	47
6	Not sure	48
7	All of them	49

19 **Specify the type of hazards which can occur with xylene (You may choose more than one)**

1	Fire	50
2	Explosion	51
3	Exposure	52
4	Corrosion	53
5	Radiation	54
6	Not sure	55
7	All of them	56

20 **Which of the following hazardous prevention rules are followed in your lab? (You may choose more than one)**

1	Fire - prevention (no open flames, no sparks, no smoking)	57
2	Explosion - prevention (no storage with nitric acid or oxidant agents, no spillage)	58
3	Exposure (inhalation, skin, eyes, ingestion) (work under extractor, strict hygiene)	59
4	Exposure - wear exposure tag	60
5	Corrosive - equipment protection	61
6	Radioactive - radiation tag	62
7	Other(Specify)……..	63
8	Not sure	64
9	Don't know	65

21 **How often do you obey hazardous prevention rules?**

1	Always	66
2	Sometimes	
3	Never	

22 **The symptoms following the inhalation of vapors of xylene can be: (You may chose more than one)**

1	Headache		1
2	Euphoria (pathologic elevation of mood)		2
3	Muscular weakness		3
4	Red skin		4
5	Dizziness		5
6	Nausea		6
7	Drowsiness		7
8	Eyes irritation		8
9	Blindness		9
10	Vomiting		10-11
11	Loss consciousness		12-13
12	Coma		14-15
13	Other(Specify)……		16-17
14	Don't know		18-19
15	All of them		20-21

23 **Have you ever experienced one or more of the following?**
(You may chose more than one)

1	Loss of consciousness	22
2	Eouphoria	23
3	Muscular weakness	24
4	Coma	25
5	Vomiting	26
6	Nausea	27
7	Drowsiness	28
8	Eyes irritation	29
9	Blindness	30
10	Dizziness	31-32
11	Headache	33-34
12	Red skin	35-36
13	Other(Specify)……	37-38
14	Have never experience one of them	39-40

24 **Specify the most beneficial information about Xylene to you.**
(You may chose more than one)
Information about:

1	Hazards	41
2	Storage	42
3	Symptoms	43
4	Protection equipment	44
5	Other information that would be useful (Specify)……	45
6	All of them	46

25 **How often do you wear a Xylene toxicity tag?**

1	Always	47
2	Sometimes	
3	Never	

26 **How often do you use protective equipment during handling of Xylene?**

1	Always	48
2	Sometimes	
3	Never	

27 **IF YOU USE THE PROTECTION**
Specify the type of protective equipment you would use
(You may choose more than one)

1	Gloves	49
2	Safety spectacles	50
3	Breathing protection	51
4	Fume extractors	52
5	Safety shields	53
6	All of them	54
7	None	55
8	Other(specify)……	56

28 **Which of the organs mentioned below are affected by xylene toxicity?**
(You may mark more than one organ)

1	Eyes	57
2	Skin	58
3	Respiratory system	59
4	Central nervous system	60
5	Gastrointestinal tract	61
6	Blood	62
7	Liver	63
8	Kidney	64
9	Bones	65
10	Muscles	66-67
11	Foetus	68-69
12	I don't know	70-71
13	Others (specify)	72-73
14	All of them	74-75

29 **More companies are producing new and less harmful reagents.**
Which ones of the new reagents mentioned below did you heard?

1	I have not heard about it	1

2	Tissue Clear	2
3	Slide-Brite Clearant	3
4	Micro-Clear	4
5	Pro-Par Clearant	5
6	Shandon Xylene Substitute	6
7	Other(Specify)……	7

30 **Do you know of any apparatus damages from the use of Xylene?**

1	Yes	8
2	No	

31 **IF YES**

Which of the following did you experience?(You may choose more than one)

1	dissolve plastic containers with specimens	9
2	dissolve pencils and pens	10
3	dissolve gloves	11
4	dissolve spectacles	12
5	dissolve window shield	13
6	dissolve plastic pipettes	14
7	detach the stickers from containers with patient's informations	15
8	dissolve the ink used to print the patient's details on the stickers	16
9	Other(Specify)……..	17
10	……	18-19
11	……	20-21

32 **Do you consider this survey as meaningful to laboratory workers?**

1	Yes	22
2	No	
3	Not sure	

33 **Do you think more can be done in your laboratory about prevention and protection of workers' health and safety?**

1	Yes	23
2	No	
3	Not sure	

34 **IF YES**

Will you be willing to initiate a process of replacing Xylene with a friendlier product?

1	Yes	24
2	No	

YOUR CO-OPERATION IS MUCH APPRECIATED

4-9

17-18

APPENDIX C

STATISTICAL DATA SHEETS OF THE ANSWERS TO THE QUESTIONNAIRE

Nr	Q1	Q3	Q4	Q5		Q6	Q7	Q8	Question 9									Q10	Q11	Q12	Q13	Question 14			
Value	dd/mm/yy	Years	Code	yy	mm	1 to 3	1 to 6	1 to 6	1	2	3	4	5	6	7	8	9	1 to 3	1 to 4	1 to 5	1 to 3	1	2	3	4
1	08/10/06	33	4	14	1	3	1	1	1	2	3	4	5					1	3	3	2		2		
2	09/04/06	25	4	1	8	2	5	6									9	3	3	5	3				4
3	31/08/06	39	4	17	2	2	3	1					5					1	4	4	2		2		
4	31/08/06	45	3	27	8	1	1	1	1	2	3							3	3	5	2	1			
5	31/08/06	52	3	27	6	1	1	1	1	2	3							1	3	5	2				4
6	31/08/06	45	4	26	7	1	1	1			3							3	3	4	2		2	3	
7	31/08/06	46	3	27	8	1	1	1	1	2	3							1	3	5	2				4
8	21/08/06	38	4	19	7	1	1	1					5					3	3	3	2	1	2		
9	09/08/06	39	3	19	2	1	1	1								8		3	3	5	2		2		4
10	09/11/06	32	3	12	1	1	2	1		2								2	3	5	3		2		
11	31/08/06	41	4	19	1	1	1	1	1	2	3							1	3	5	2				4
12	31/08006	37	3	18	8	1	2	1	1	2	3							3	4	4	2		2		
13	31/08/06	58	4	41	8	1	2	1			3							1	3	5	2		2		
14	31/08/06	60	3	38	1	1	2	4	1									3	3	4	2		2		
15	31/08/06	48	3	25	2	1	1	1	1		3							3	3	4	2		2		
16	30/08/06	43	3	15	1	1	2	1					5					2	4	2	2		2		
17	09/01/06	43	3	23	8	1	3	1	1									1	1	4	2		2		
18	09/01/06	31	3	31	2	1	1	1	1	2	3	4						2	3	5	2		2		
19	07/11/06	45	3	8	2	1	1	3		2								2	3	5	3		2		
20	20/07/06	20	2	1	1	1	2	3							7			3	3	5	1		2		
21	14/08/06	32	3	12	1	1	2	4	1	2	3		5					2	3	5	2		2		
22	13/08/06	48	3	16	2	1	3	4		2								2	3	5	3		2	3	
23	08/11/06	38	3	13	1	1	1	1	1	2	3							2	2	4	3		2		
24	08/10/06	23	4	0	3	1	1	3		2	3		5					2	1	5	2		2	3	
25	15/08/06	35	4	15	7	1	2	1	1	2	3	4	5					2	1		2		2		
26	08/11/06	51	2	33	6	1	2	1	1	2	3	4	5					2	1	5	2		2		
27	08/11/06	42	4	19	2	1	1	1	1	2	3	4	5					1	1	2	2		2		
28	08/11/06	45	4	21	8	1	2	3	1		3	4						1	1	5	2		2	3	
29	08/11/06	56	3	25	1	1	1	1	1	2	3	4	5					2	3	4	2		2		
30	08/11/06	49	3	14	6	1	1	1		2	3	4	5					2	3	4	2		2		
31	08/11/06	42	3	24	8	1	2	1	1		3		5		7			3	3	5	1		2		
32	09/05/06	44	3	13	1	1	3	4	1				5					1	1	1	2		2	3	
33	07/01/06	36	3	15	2	1	2	2	1	2								3	3	5	2		2		

Nr	Q1	Q3	Q4	Q5		Q6	Q7	Q8	Question 9									Q10	Q11	Q12	Q13	Question 14			
	dd/mm/yy	Years	Code	yy	mm	1 to 3	1 to 6	1 to 6	1	2	3	4	5	6	7	8	9	1 to 3	1 to 4	1 to 5	1 to 3	1	2	3	4
34	15/08/06	25	3	3	2	3	5	6		2								3	3	3	3		2		
35	15/06/06	23	3	1	6	1	2	2	1	2								1	3	5	2		2		
36	07/06/06	50	2	30	2	1	2	2	1	2								1	3	5	3				4
37	08/08/06	43	2	25	3	1	2	2	1	2								1	3	5	2		2		
38	15/06/06	54	3	28	7	1	2	1									9	1	1	5	2		2		
39	08/01/06	40	3	15	6	1	2	2	1	2								3	3	5	2		2		
40	15/07/06	40	3	16	7	1	3	2	1	2								1	3	5	1		2	3	
41	09/11/06	35	4	14	2	3	2	1									9	2	3	5	3		2		
42	09/12/06	37	3	16	2	3	5	6									9	2	3	5	3		2		
43	15/08/06	45	3	23	6	3	3	2	1	2								1	1	5	2		2	3	
44	18/09/06	33	3	13	1	1	3	1	1									1	1	4	2		2		
45	08/04/06	40	3	12	7	1	2	3	1	2	3							1	3	5	1		2		
46	09/02/06	32	4	15	3	1	2	3	1	2								1	3	5	2		2	3	
47	13/09/06	39	4	16	6	1	1	1			3							1	2	2	2		2		
48	20/09/06	36	3	8	1	1	4	3			3							3	3	3	2			3	
49	13/09/06	47	3	23	2	1	3	4				4						3	2	1	3				
50	14/09/06	51	3	28	5	1	2	3	1	2								3	4	5	2		2	3	
51	14/08/06	49	3	19	8	1	2	3	1	2								1	3	5	2		2		
52	09/05/06	48	3	20	6	3	1	6	1	2								3	3	5	2		2		
53	30/08/06	42	4	18	6	1	1	1	1	2								1	3	5	1		2		
54	09/10/06	39	2	19	2	3	5	6	1	2								3	3	5	2		2		
55	09/01/06	38	3	16	3	3	5	1	1									1	3	5	2		2		
56	07/01/06	26	3	4	3	1	1	1	1	2								3	3	5	2		2		
57	20/08/06	36	3	15	2	1	1	1	1	2								3	3	5	3		2		
58	15/08/06	25	2	3	2	1	1	1	1	2								1	3	5	2		2		
59	20/07/06	28	3	7	10	1	2	1	1	2	3							1	3	5	2		2	3	
60	06/10/06	49	3	30	2	1	1	1	1	2								1	3	5	2		2	3	
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63	06/02/06	39	4	16	3	1	2	1	1	2								1	1	5	2		2	3	
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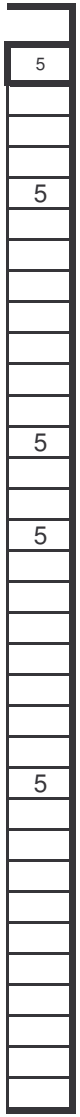
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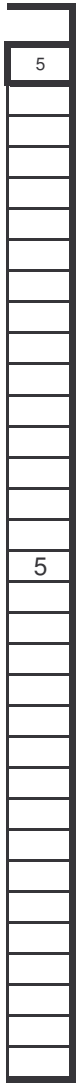
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Nr	Q15	Q16	Q17	Question 18							Question 19							Question 20								
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Nr	Q15	Q16	Q17	Question 18							Question 19							Question 20								
Value	1 to 3	1 to 5	1 to 6	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9
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Nr	Q21	Question 22															Question 23													Question 24					
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Nr	Q21	Question 22															Question 23													Question 24					
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Nr	Q25	Q26	Question 27								Question 28															Question 29							Q30
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Nr	Q25	Q26	Question 27								Question 28															Question 29							Q30
Value	1 to 3	1 to 3	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	1,2
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42		3							7													12				1							2
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Nr	Question 31											Q32	Q33	Q34
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4		2	3			6	7					1	1	1
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9	1	2	3	4	5	6	7	8				1	1	1
10	1	2	3			6	7					1	1	1
11		2	3			6	7	8				1	1	1
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18			3									1	1	1
19										10		1	1	1
20			3				7					1	1	1
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Nr	Question 31											Q32	Q33	Q34
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44	1	2										1	1	1
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46	1	2					7	8				1	1	1
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48												1	1	1
49		2										1	1	1
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