COMPARISON OF UPTAKE $^{99m}Tc0_4^-$, ^{99m}Tc-DTPA AND ^{99m}Tc-MDP BY BREAST CANCER CELL LINE.

by

SITI NORAISYAH BINTI RAFZI

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Health Sciences (Medical Radiation)

JUNE 2015

CERTIFICATE

This is to certify that the dissertation entitled

"Comparison Of Uptake $^{99m}Tc0_4^-$, ^{99m}Tc-DTPA and ^{99m}Tc-MDP By Breast Cancer Cell Line"

Is the bonafide record of research work done by

Siti Noraisyah Binti Rafzi

During the period of January 2015 to June 2015

Under my supervision

Supervisor,

Dr. Wan Nordiana binti Wan Abdul Rahman

Lecturer,

School of Health Sciences,

Universiti Sains Malaysia,

Health Campus,

16150 Kubang Kerian,

Kelantan, Malaysia

Date:

Co-supervisor,

Mr. Safri bin Zainal Abidin

Staff,

School of Health Sciences,

Universiti Sains Malaysia,

Health Campus,

16150 Kubang Kerian,

Kelantan, Malaysia

Date:

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious, and the Most Merciful.

Firstly, I am grateful to the God for the good health and wellbeing to complete this final year project. Without His blessing and help, I would not be able to finish this entire work in such a short time.

I wish to express my sincere thanks to Prof. Ahmad Zakaria, Dean of School of Health Science, for his permission to get this study worked out until it is complete, and also for his numerous tips and ideas he gave us throughout this entire year. Next, I also grateful to my supervisor, Dr. Wan Nordiana Wan Abdul Rahman, and my co-supervisor, Mr. Safri Zainal Abidin for valuable guidance and encouragement supervising me throughout this research.

I take this opportunity to express gratitude to all of the Medical Radiation lecturers and staffs for their help and support until this thesis finished successfully.

I also thank my parents, my friends, my acquaintance and those who directly and indirectly support me throughout all the venture, there is no greater appreciation I can express, may God bless all of you for your kindness and patience. Thank you.

TABLE OF CONTENT

List of Tables

List of Figures

Abbreviations

μCi	microCurie
$^{99m}TcO_4^-$	technetium-99m pertechnetate
MDP	Methylene-diphosphonate
DTPA	Diethylene-triamine-pentaacetate acid
DMSA	Dimercaptosuccinic acid
MIBI	hexakis-isobutyl-isonitrile
℃	degree Celsius
DMEM	Dulbecco's Modified Eagle's Medium
FBS	Fetal Bovine Serum
Trypsin-EDTA	Trypsin-Ethylenediaminetetraacetic acid
μL	microliter
BSC	Biological safety cabinet
keV	Kilo Electron Volt

ABSTRACT

Objective: The aim of this study is to determine the percentage uptake of different tracer by the breast cancer cell line (MCF-7), hence finding the optimum condition for the cells to absorb the radiopharmaceuticals. In addition, this study is to investigate the number of cell viable for different radioactivity with different type of tracers used. In the other hand, this study also to show the reliability and applicability of well counter in measuring different activity of tracer.

Methods: 100k number of MCF-7 cell lines were prescribed with 100, 200, 300, 400, and 500 μCi of ^{99m}Tc-MDP, ^{99m}Tc-DTPA and $^{99m}TcO_4^-$ inside 6-well plate and incubated for an hour at 37℃. After the incubation finished, the cells were washed with phosphate buffer saline solution (PBS) to remove the extracellular tracers (^{99m}Tc-MDP, ^{99m}Tc-DTPA and $^{99m}TcO_4^-$) suspended in the cell media, leaving cells monolayer to detach them from the well's bottom, so that the cells can be transferred into centrifuge tubes. Trypsine was added into the cell monolayers to detach the from the well's bottom. The measurement of activity of the cells and the washed media were taken by using well-counter. The percentage uptake of ^{99m}Tc-MDP, ^{99m}Tc-DTPA and $^{99m}TcO_4^-$ into cells was calculated and analyzed.

Results and discussion: Both ^{99m}Tc-DTPA and ^{99m}Tc-MDP shows the same pattern of percent intercellular uptake. While, TcO_4^- shows different pattern of percent intercellular uptake. All of the tracers (^{99m}Tc-MDP, ^{99m}Tc-DTPA and $^{99m}TcO_4^-$) have optimum uptake in the range of 200 μCi to 400 μCi. The percentage uptake of ^{99m}Tc-MDP, ^{99m}Tc-DTPA and $^{99m}TcO_4^-$ are 66.04%, 65.91% and 44.06%,. The number of cell viable (cell count/mL) for each tracer was found to be reduced as the activity increased.

Conclusion: It was highlighted that different radioactivity used (μCi) give different intercellular uptake measured from each tracer. Different radioactivity used (μCi) also affected the number of cell viability for each tracer. In future, for study of cell uptake by using well-counter with wipe test software is not so applicable as it show high sensitivity toward low radioactivity. The acceptance counting activities is up to approximately 37 kBq.

ABSTRAK

Objectif: Penyelidikan ini bertujuan untuk mengetahui peratusan serapan radiofarmaseutikal yang berbeza oleh sel payudara (MCF-7), sekaligus mengenalpasti keadaan optimum bagi sel tersebut untuk menyerap bahan radiofarmaseutikal. Kajian ini juga adalah untuk mengenalpasti jumlah sel hidup bagi radioaktiviti dan radiofarmaseutikal yang berbeza. Di samping itu, kajian ini adalah untuk menunjukkan kesesuaian *well-counter* dalam mengukur aktiviti radiofarmaseutikal.

Kaedah: Sel MCF-7 telah diberi sebanyak 100, 200, 300, 400 dan 500 μCi ^{99m}Tc-MDP, ^{99m}Tc-DTPA dan $^{99m}TcO_4^-$ di dalam piring 6-lubang dan dieram selama sejam pada suhu 37°C. Selepas pengeraman, sel-sel telah dibasuh dengan menggunakan cecair *phosphate buffer saline (PBS)* untuk membersihkan sisa ^{99m}Tc-MDP, ^{99m}Tc-DTPA dan $^{99m}TcO_4^-$ yang melekat pada piring, supaya hanya lapisan sel sahaja yang tinggal di dalam piring. Tripsin ditambah kepada lapisan sel untuk menanggalkannya dari dasar pisring. Sel-sel telah dialih ke dalam tiub emparan. Kesemua sampel sel dan sampel sisa basuhan diempar, seterusnya diukur bacaan radioaktivitinya dengan menggunakan *well-counter*. Seterusnya peratusan serapan ^{99m}Tc-MDP, ^{99m}Tc-DTPA dan $^{99m}TcO_4^-$ dikira dan dianalisis.

Hasil kajian: Kedua-dua ^{99m}Tc-MDP dan ^{99m}Tc-DTPA menunjukkan peratusan penyerapan yang tinggi jika dibandingkan dengan $^{99m}TcO_4^-$. Kesemua radiofarmaseutikal (^{99m}Tc-MDP, ^{99m}Tc-DTPA dan $^{99m}TcO_4^-$) menunjukkan keadaan optimum untuk serapan adalah pada 200 μCi hingga 400 μCi iaitu sebanyak 66.04%, 65.91% dan 44.06%. Jumlah sel yang hidup juga menurun sekiranya radioaktiviti ditambah.

Kesimpulan: Radioaktiviti yang berbeza (μCi) akan menyebabkan penyerapan yang berbeza bagi setiap radiofarmaseutikal yang digunakan. Radioaktiviti yang berbeza juga memberi kesan kepada jumlah sel yang hidup bagi setiap radiofarmaeutikal. Bagi kajian yang melibatkan penyerapan sel pada masa akan datang, penggunaan *well-counter* dengan menggunakan perisisan *wipe test* adalah tidak sesuai kerana ianya terlalu sensitif kepada radioaktiviti yang rendah. Nilai maksimum aktiviti yang diterima untuk pengiraan oleh *well-counter* adalah 37 kBq.

CHAPTER 1

1.0 INTRODUCTION

1.1 Nuclear Medicine

The basic practice of clinical in nuclear medicine is administration of tracer amounts of compounds labelled with radioactivity (radionuclide). These radionuclide are used in order to provide diagnostic information in a wide range of disease states and to identify any abnormalities in the early stage of a disease. Some radionuclide also function in treatment for some disease, such as Iodine-131 is used for thyroid cancer patient.

By principle, in nuclear medicine study, it involves injecting a compound which is labelled with a gamma-ray-emitting radionuclide into the circulatory system of the patient's body. The radiolabel compound is called a radiopharmaceutical, or more commonly, a tracer or radiotracer. When the radionuclide decays, gamma rays are emitted from the patient's body. These gamma rays exit the patient's body because the energy of these gamma rays is that the significant number can exit the body without being scattered or attenuated. Then, the exit gamma rays from the patient's body are detected by the gamma camera to image and quantify regional physiological biochemical processes.

There are two broad classes of diagnostic imaging methods in nuclear medicine which are single photon imaging and positron emission tomography (PET) (Lopes et. al., 2004). A planar image is obtained by taking a picture of the radionuclide distribution in the patient from one particular angle. PET is a more precise and sophisticated technique using isotopes produced by the cyclotron. New procedures

combine PET with computed X-ray tomography (CT) scans to give co-registration of the two images (PET/CT), enabling 30% better diagnosis than with traditional gamma camera alone. It is a very powerful and significant tool which provides unique information on a wide variety of diseases from dementia to cardiovascular disease and cancer (oncology) (World Nuclear Association, 2015).

Nuclear medicine is able to diagnose stage of cancer by determining the presence or spread of cancer in various part of the body. Cancer cell growth is different from normal cell growth. Cancer begin when parts of the body start to grow out of control. Cancer cell often spread to the other part of the body which become a tumor and replace the normal tissues. This process is called metastases which is the cancer cell spread into bloodstream or lymph vessel of our body. The cancer can be treated by many ways such as surgery, chemotherapy, or radiation therapy.

Thus, useful information is important during diagnostic or imaging procedure to determine the stage of malignancy or as a guide for biopsy and surgery by using radioisotopes. After surgery and radiotherapy, the radionuclide is used in nuclear medicine to examine the persistence of tumor, differentiate recurrence from radiation necrosis and gliosis, also to monitor the disease.

1.2 Technetium-99m Pertechnetate

In nuclear medicine, technetium-99m pertechnetate ($^{99m}TcO_4$) is commonly used in medical diagnostic procedure. Technetium-99m pertechnetate is a metastable nuclear and it become stable state when adjusting itself by emitting energy in the form of photons which known as gamma rays with energy 140 keV that are very favorable for use with a gamma camera. This process is called as radioactive decay.

By principle, the $^{99m}TcO_4$ is produce by elution process in technetium generator. Technetium generator is a device used to extract ^{99m}Tc from decaying Molybdenum-99 (^{99}Mo). ^{99}Mo is loaded in the generator and bound with aluminium column. The daughter ^{99m}Tc activity, produced in the form of $^{99m}TcO_4$ which is not strongly bound to alumina and eluted from the column with 5 to 25 mL of normal saline (Simon et. al.2003)

After elution process of $^{99m}TcO_4$, technetium-99m activity will build up again and maximum activity is available about 24 hours later. Typically they are used for about 1 week and then discarded because of natural decay of ^{99}Mo parent. Half-life of ^{99}Mo is about 66 hours, while $^{99m}TcO_4$ has short half-life which is 6 hours and it has low energy (140 keV). These characteristic is good in nuclear medicine procedure in order to minimize the radiation dose to patient and worker.

This $^{99m}TcO_4$ can be tagging with radiopharmaceuticals such as MDP, DTPA, MAG 3 and MIBI for diagnosing and functional study of the bone, kidneys, lungs, and also tumors. Depending on the procedure, the $^{99m}TcO_4$ transport it to its specific organ and diagnosing can be made by imaging with gamma camera.

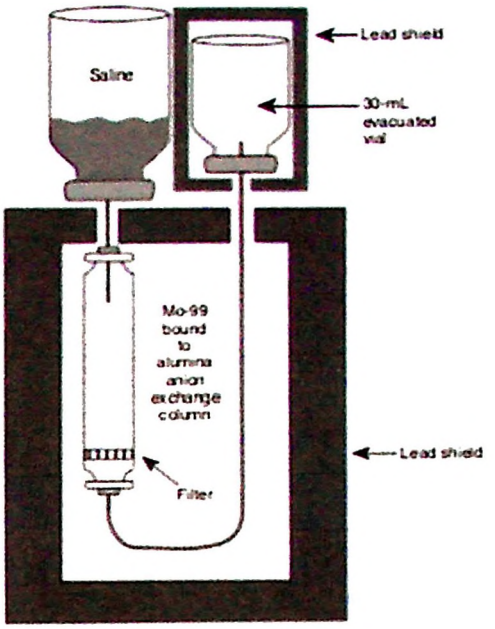


Figure 1.0.1. Molybdenum 99/Technetium-99m Radionuclide Generator. (Requisites in Nuclear Medicine, 4th edition, (2014))

1.3 Molecular imaging

The term molecular imaging can be broadly defined as the in vivo characterization and measurement of biologic processes at the cellular and molecular level. (Weissleder & Mahmood, Radiology, 2001). Molecular imaging techniques either directly or indirectly monitor and record the spatiotemporal distribution of molecular or cellular process. The process includes for biochemical, biologic, diagnostic or therapeutic applications.

The field of molecular imaging emerged in the early 1990's as scientist from multiple specification including cell biology, biomedical engineering, chemistry, mathematic, medicine, pharmacology, and genetic. Their mission is to elucidate molecular mechanism in biology and medicine. The aims of molecular imaging is to non-invasively visualize, characterized and quantify normal and pathologic processes

within the cellular and subcellular level (Cherry et. al. 2007). Besides, the extent and location of the disease can be accurately estimated, before and after the treatment. Treatment may be better selected and unnecessary procedures can be avoided.

Molecular imaging is becoming a key bridging technology to translate experimental preclinical finding into the clinical environment. It provides detailed of pictures of the processes inside the body at the molecular and cellular level.

1.4 Basic Cell Culture

Cell culture playing a major role in model system especially for research. This is because cell culture involve studying of basic cell biology, interaction between disease causing agents and cells, effect of drug on cells, process and triggering of aging and also nutritional studies. In molecular biology, cell culture is one of the most important tools for studying the normal and abnormal physiology of cells.

Cell culture is the process by which cells are grown under controlled condition, generally outside of their natural environment. Tissue from an explant is dispersed, mostly enzymatically into a cell suspension which may then cultured as a monolayer or suspension culture. Different types of cell grown in culture includes connective tissue element such as fibroblasts, skeletal tissue, cardiac, epithelial tissue (liver, breast, skin, kidney) and many different type of tumor cells. The major advantage of using cell culture is the consistency and reproducibility of results that can be obtained from using a batch of clonogenic cells.

1.4 Aim

This study is to investigate the uptake of different tracer by breast cancer cell line.

1.5 Objectives of the study

1. To determine the percentage uptake of different tracer by breast cancer cell lines.
2. To investigate the reliability and applicability of well counter in measuring different activity of tracer.

CHAPTER 2

2.0 LITERATURE REVIEW

The basic principle of radiopharmaceuticals is to bring the tagged radioactive materials with specific pharmaceutical to a targeted site in human body. There are two purposed of radiopharmaceutical either to diagnose or function as a therapeutic agent. The examples of radiopharmaceutical that function to diagnose such as technetium labeled methylene disphosphonate (^{99m}Tc-MDP), technetium labeled diethylene-triamine-pentaacetate (^{99m}Tc-DTPA), ^{99m}Tc-MIBI, ^{99m}Tc-MAG 3, and ^{99m}Tc-HIDA. While, Iodine- 131 is used for thyroid cancer therapy.

The most commonly used diagnostic radiopharmaceuticals are mainly ^{99m}Tc-based. The tracer is either used to radiolabel blood cells ex vivo before extravenous injection into the patient, or is administered directly. Technetium-99m is obtained from a Mo-99 generator. Its mode decay is by isometric transition to emit gamma rays with energy 140 keV with no tissue burden. This source can be easily shielded by using Lead pots to protect operators, and the half-life of the isotope (6 hours) is convenient during a clinical working day (Tsopelas, C., 2015).

There are several studies performed by the researchers that involve in determine the uptake of radiopharmaceuticals by breast cancer cell lines. For example, the study performed by Sergio *et al.* (1995), technetium-99m-methylene diphosphonate (MDP) uptake within breast lesions was investigate during routine pre surgical bone scintigraphy in a cohort of women at high risk for cancer who were candidates for surgery or excisional biopsy. Extraskeletal accumulation of ^{99m}Tc-MDP has been reported in some malignant tumors, including neuroblastoma, sarcoma, osteosarcoma,

and soft-tissue lymphoma. The same results have been reported for large primary breast tumours.

Uptake mechanism of ^{99m}Tc-MDP in human body could be seen after three hours intravenous administration of this ^{99m}Tc-phosphonate. It is essentially located in the blood with 1-3% bound to plasma proteins, ≤59% is excreted in urine, and ~45% remains in the skeleton with a high bone-to-soft tissue ratio. The polymeric anionic structure governs the mechanism of uptake by binding to Ca(II) ions in calcium hydroxyapatite crystals comprising bone (Tsopelas, C., 2015).

The majority of patient with advanced breast cancer evidence of skeletal metastases by the time of death (Galasko, 1981). Commonly patient with bone metastases have a protracted illness due to breast cancer itself has a variable and often long clinical course.

In the past study, breast cancer cell line is diagnose by Pentavalent ^{99m}Tc-dimercaptosuccinic acid (^{99m}Tc(V)-DMSA). ^{99m}Tc(V)-DMSA is formed from labelling of meso-2,3-dimercaptosuccinic acid (DMSA) with ^{99m}Tc under alkaline conditions, has been found to be a tumor-seeking agent (Fatma J. , 2013). ^{99m}Tc(V)-DMSA is also useful to detect or diagnosed many types of cancers such as head and neck, soft tissue tumors, breast, brain, lung, bone and particularly for metastatic and high-grade tumors.

There are several types of breast cancer cell lines which are T-47D, MCF-7, MDA-MB-231, and MDA-MB-468. Each of these breast cancer cell lines has different response towards radioactive materials. Also, they have different type of metastases in human body. For example, MDA-MB-231, and MDA-MB-468 shows high level of brain metastases compared to T-47D and MCF-7 (Lautenschlaeger, T. et. al., 2013).

The different intensity or contrast in imaging occurs due to present of different activity of cells which called radiosensitivity. The mitotic rate of the cells in the tissue and their degree of differentiation are depends on their radiosensitivity. In general, if the mitotic rate of the cells is higher, then they are more radiosensitive.

The radionuclide bone scan is based on the principle that tumor metastatic to bone generates reactive new bone formation. This new bone has a higher rate of turnover of bone mineral and osteoid than normal bone, and the bone agent 99mTc-diphosphonate accumulates preferentially. Scans detect bone metastases in many cancer types, including prostate, lung, breast, and oesophagus, and they do so earlier than skeletal radiographs can. Although highly sensitive, the bone scan has low specificity for cancer metastases (Eary, J. F., 1999).

Generally, when cells are exposed to radiation, the level of protective molecules released, such as superoxide dismutase (SOD), glutathione, and metallothionine, increases and DNA repair mechanisms are intensified. Although the protective and repair mechanisms for cells are efficient, they are not capable of blocking or rectifying all of the damage.

The study involving radiation-induced cellular damage is done by Tarnuzzer, R.W., et al. (2005), shows that MCF-7 cells showed no statistically significant protection. This could be due to several reasons such as differential uptake, differential intercellular activity, or differences in chromatin structure or free-radical targets. Tumor cells tend to have a more relaxed chromatin structure that can expose more bases as targets for free-radical attack.

There are several factors affecting the uptake of radioactive agents. The study conducted by Hayes (1976), the factors are vascularity, blood flow, interstitial fluid space, cell proliferation, presence of carrier, capillary and cell permeability, presence of inflammation and pH.

CHAPTER 3

3.1 MATERIALS

3.1.1 Cell Line

MCF-7 is a human breast adenocarcinoma cell line. The cell line was originally derived at the Michigan Cancer Foundation from a malignant pleural effusion from a postmenopausal woman with metastatic breast cancer who had been previously treated with radiation therapy. It has been used extensively in research to monitor cancer progression. The cell line is an established and supplied by the American Type Culture Collection (ATCC).

3.1.2 Cell Culture Media and Supplements

3.1.2.1 Dulbecco's Modified Eagle's Medium (DMEM) Complete Media

The media used is Dulbecco's Modification of Eagle's Medium (DMEM) was supplied by Gibco Life Technologies. Gibco media is standard cell culture media that contain a stabilized form of L-glutamine, the dipeptide L-alanyl-L-glutamine, that prevents degradation and ammonia build up in both adherent and suspension cultures. Besides, Gibco media is able to increase media stability, minimize toxic ammonia build up, and maximize cell performance even during long-term cultures.

The culture media was supplemented with 10% FBS and a 100 unit/mL penicillin-streptomycin for complete media to use in cell seeding

Figure 3.0.1. DMEM Complete Media. (Requisites in Life Technologies, n.d).

Fetal bovine serum (FBS) is the most widely used serum-supplement for the in vitro cell culture of eukaryotic cells. In cell culture, serum provides a wide variety of macromolecular proteins, low molecular weight nutrients, carrier proteins for water which is insoluble components, and other compounds necessary for in vitro growth of cells, such as hormones and attachment factors. Serum also adds buffering capacity to the medium and binds or neutralizes toxic components. FBS also maintain basal growth of the cultured cells in a medium.

Figure 3.0.2. Fetal Bovine Serum. (Requisites in Life Technologies, n.d).

Penicillin and streptomycin is used to prevent bacterial contamination of the cell cultures. Chemical Penicillin and Streptomycin also supplied by Gibco Life Technologies. Penicillin was originally purified from the fungi *Penicillium* and acts by interfering directly with the amount of the bacterial on the cell wall and indirectly by triggering the release of enzymes that will alter the cell wall. This solution contains 10,000 units/mL of penicillin and 10,000 µg/mL of streptomycin to protect cell culture from infection.

Figure 3.0.3. Penicillin and Streptomycin. (Requisites in Life Technologies, n.d).

For a 50 mL complete media, 45 mL of DMEM media was dissolved with 5 mL FBS and 500 µL Penicillin-streptomycin antibiotic. Then the complete media were stored at 4℃ until use.

3.1.2.2 Trypsin-EDTA

Trypsin-EDTA (25%) brand Gibco by Life Technologies was used for cell detachment from the surface of the culture flask. Trypsin-EDTA is commonly used about 15 minutes for any procedure to maintain live cell in the culture flask. Due to strong chemical reaction of Trypsin-EDTA, the cells might be die.

Figure 3.0.4. Trypsin-EDTA (25%). (Requisites in Life Technologies, n.d).

3.1.2.3 Phosphate Buffer Saline (PBS)

Phosphate Buffer Saline (PBS) supplied by Gibco by Life Technologies was commonly used in biological research. PBS is a water-based salt solution containing sodium phosphate, sodium chloride and, in some formulations, potassium chloride and potassium phosphate. The buffer also help to maintain a constant pH of cell culture media. Besides, PBS also act in inhibiting trypsin activity.

Figure 3.0.5. Phosphate Buffer Saline. (Requisites in Life Technologies, n.d).

3.1.3 Cell Culture Equipment

3.1.3.1 Biological Safety Cabinet

Biological safety cabinet (BSC) is designed to protect laboratory personnel and the environment from exposure to biohazards through the combined use of laminar flow and High Efficiency Particulate Air (HEPA) filters. BSC provides an aseptic working area and also contained infectious splashes or aerosols generated by many microbiological procedures. There are three classes of BSC, designated as Class I, Class II, and Class III. In this study, the BSC Class II was used for handling cell culture and radioisotopes. Besides, Class II BSC provide an effective partial barrier system for the safe manipulation of low-moderate, and high-risk microorganisms.

This BSC class II also comes with ultraviolet (UV) light that was turned on for several times to sterile the area inside BSC. Material like pipette, container, beaker and racks must be sterilized with 70% isopropanol alcohol and lay inside BSC before culturing procedure start.

3.1.3.2 Pipette and tips

The Appendorf pipette is used to transfer or measuring out small quantities of cell and media from one container to another. 500 µL and 1000 µL are commonly used in this study to transfer media and other reagent into the six well plate. The new tips is used every time involve the procedure of new cell and media to avoid contamination.

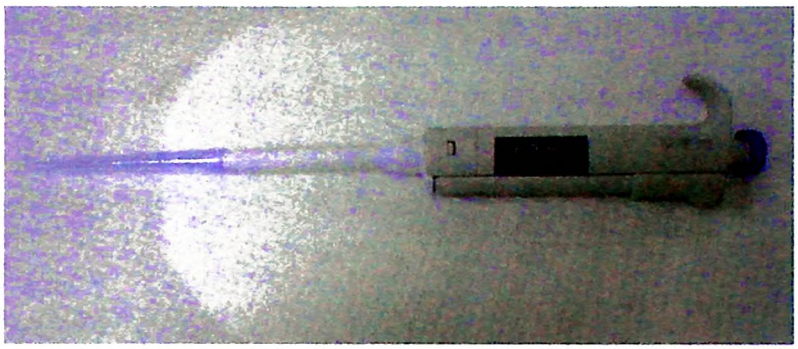


Figure 3.0.6. Appendorf pipette

3.1.4 Cell Viability Assay materials

3.1.4.1 Hemacytometer

Hemacytometer is used to count the cells. Hemacytometer is a specimen slide which is used to determined the concentration of cells in a liquid sample. The cover glass on this slide does not simply float on the liquid (sample), but is held in place at a specified height (usually 0.1 mm). Additionally, there is a grid is marked into the glass of the hemacytometer. This grid, an arrangement of squares of different sizes, allows for an easy counting of cells. This way is possible to determine the number of cells in a specified volume.

Figure 3.0.7. Hemacytometer

3.1.4.2 Trypan Blue

Trypan blue is a vital dye that has been used for selective staining of dead tissues or cells. The reactivity of trypan blue is based on the fact that the chromopore is negatively charged and it does not interact with the cells unless the membrane is damaged. Therefore, all the cells which exclude the dye are viable.

3.1.4.3 Inverted Microscope

Inverted microscope provides a large field of view which make it ideal for observing live and dead cell culture in culture plate. The cells can be observed directly after transferred the cells into hemacytometer slide and then put it on the microscope. The inverted microscope can be adjust to focus the image in order to get sharp image.

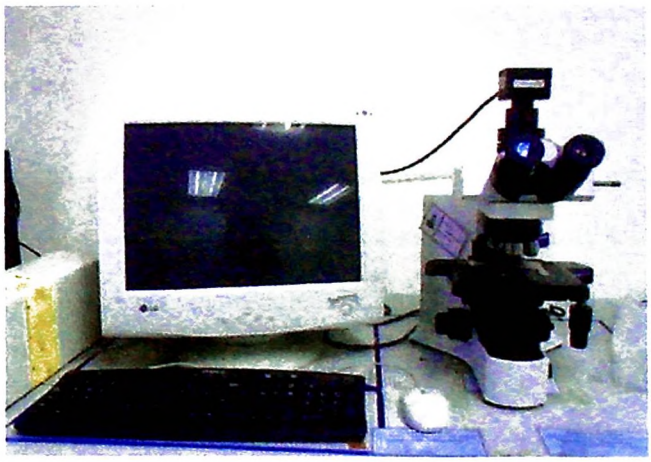


Figure 3.0.8. Inverted microscope

3.1.5 Generator $^{99}Mo/^{99m}Tc$

The $^{99}Mo/^{99m}Tc$ generator is an automatic and highly protected system which produces a sterile and pyrogen free ^{99m}Tc solution, in the form of sodium pertechnetate. The generators are "milked" by drawing a saline solution across an inner molybdenum/alumina capsule. During this milking process, any technetium that has formed will be drawn away with the saline and can be used in test.

The molybdenum/alumina sample is placed in the centre of the device, surrounded by shielding. Saline is injected through one of the tubes at the top of the device and flows into a shielded container through the outer tube. The decay process of ^{99m}Tc by the emission of gamma radiation with a mean energy of 140 keV and half life of 6 hours.

3.1.6 Dose Calibrator

The dose calibrator is used in nuclear medicine to measure activity of radionuclide. This dose calibrator consist of ionization chamber which is completely digital detector that gives a fast and reliable reading.

3.1.7 Well Counter

The well-counter is a device used to measure gamma radiation emitting from radioisotopes. Wipe test is used to measure uptake by cells and media. Well-counter with wipe test software is commonly used to measure radioactive contamination for nuclear medicine department and laboratories.

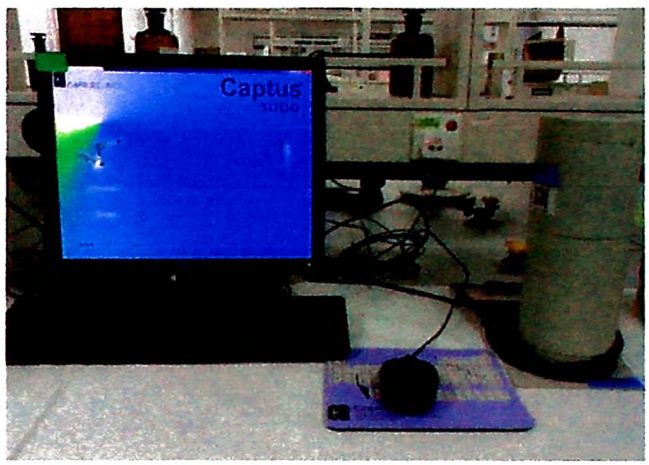


Figure 3.0.9. Well-counter Captus 3000

3.1.8 Syringes and needles

Syringes 1 mL is used to transfer ^{99m}Tc into 100 μmCi, 200 μmCi, 300 μmCi, 400 μmCi and 500 μmCi before inject into 6-well plate of cells.

3.1.9 Counting test tube

Counting test tube is used to measure uptake of cells in which the tube is filled with cells and media and then put it in the well counter. Counting test tube is reusable.

3.1.10 Lead carrier and lead syringe shield

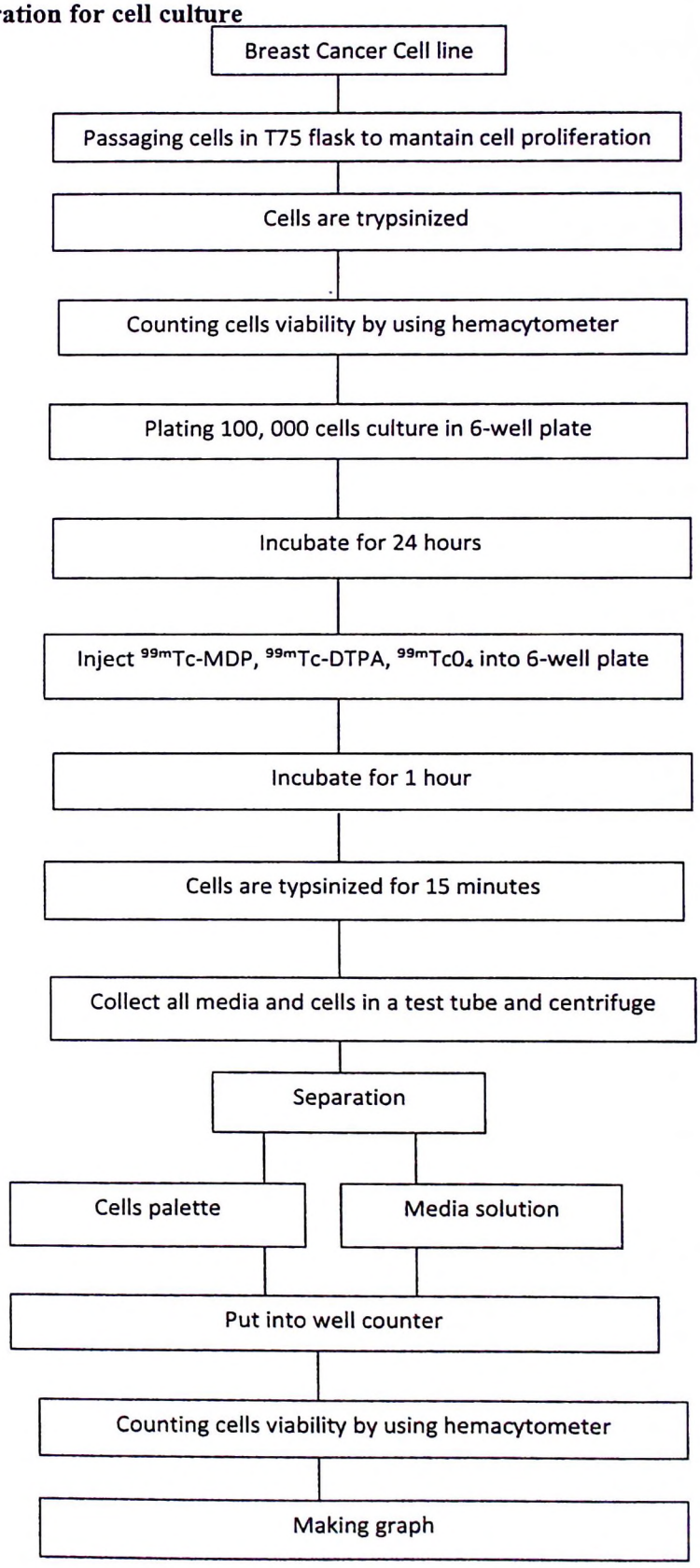
Lead shielding carrier was used for transporting the radionuclides ($^{99m}TcO_4$, ^{99m}Tc-MDP, or ^{99m}Tc-DTPA) from Nuclear Medicine Department, Hospital Universiti Sains Malaysia (HUSM) into Medical Radiation Laboratory. As a protection, all of the radionuclides is put in lead syringe shield before put it inside lead shielding carrier.

Figure 3.0.10. Lead Syringe Shield

Figure 3.0.11. Lead Carrier

3.2 Methods

3.2.1 Preparation for cell culture



3.2.1 Preparation for Cell Culture

In this study, breast cancer cell line (MCF-7) is used. The protocol for cell preparation is same with other type of cell lines. Different cell lines used different media.DMEM is used for MCF-7 to provide the nutrients, growth factor and hormones for cell growth. All solution and equipment that will be contact with cell should be sterile as a precaution step. 70% isopropyl ethanol is used to spray all the solution and equipment. In order to maintain sterility, all the preparation should be done inside a biological safety cabinet (BSC).

Before starting any procedure, the UV light is exposed to the BSC around 15 minutes before use. All of the materials including cell culture flask, media, tube racks, waste container, pipette, and 70% isopropyl ethanol spray must be prepared and properly located inside BSC.

First step in cell culture process is passaging process. Media in the flask was discarded and the cells are washed with phosphate buffer saline (PBS) about three times. After that, trypsin is added to the flask about 2 minutes in 37℃ incubator for cell detachment. The flask is gently shaken to ensure all the trypsin cover the whole cell layer. After 2 minutes trypsinized, the cell is observed under an inverted microscope for detachment. If the cells are less than 100 000 detached, the flask will be incubated a few more minutes. If more than 100 000 of the cells have detached, the flask tilts for a minimal length of time to allow the cell to drain.

Then, 2 mL of DMEM was added into the flask to stop the reaction of trypsin. Then, pipette all solutions and transfer them into the test tube to centrifuge at 5000 rpm for 15 minutes. After centrifuge, there is complete separation between cell and media. The total number of cells can be determined by count cells using hemacytometer or automated count cell machine.

3.2.2 Cell Viability Assay

The first step of cell counting is mix 0.1 mL of trypan blue with 20 µL of cell suspension to give colour to the background. The cover slip and chamber of hemacytometer was cleaned with alcohol and fixed cover slip on the chamber of hemacytometer. Then pipette 20 µl of the dyed cells to fill the chamber but not overfill. The hemacytometer was then examined under a microscope. If a cell is dead, it will take up trypan blue and they are considered non-viable. The viability is determined on life cell on hemacytometer and calculated as the number of viable cells divided by the total number of cells. To calculate the number of viable cells per ml of media, the formula below is used.

$$\text{Number of viable cells} \times 10^4 \times 1.1 = \text{cells/ml media}$$

3.2.3 Plating Cell Culture

The first step in plating cell culture is determining the cell number per ml of media. Then, 100 000 cells were pipette into each well of 6-well plate. 1 mL of DMEM is added into each well to spread all the cells uniformly. The plate need to label with the date of cell preparation, type of cell culture and the number of passaging. This is important to avoid any mistaken of the cells. The 6-well plate is sealed with parafilm to protect contamination or spillage of media.

The cell plate is returned to the incubator for 24 hours for cell attachment on the bottom of the plate. After 24 hours, the cells are ready to be injected with ^{99m}Tc-MDP, ^{99m}Tc-DTPA and $^{99m}TcO_4$.

3.2.4 Radiopharmaceuticals Preparation

Technetium-99m Pertechnetate

Technetium-99m Pertechnetate is a clear and colourless solution of sodium pertechnetate that produced through elution process. In the standard commercial ^{99m}Tc generator, the parent ^{99}Mo is chemically bound to the alumina column and this binding is strong enough to prevent molybdenum from being easily washed from the column by the isotonic saline solution used to elute the ^{99m}Tc. However, there will always be a trace of molybdenum that appears in the ^{99m}Tc product eluted from the generator. The risk of ^{99}Mo if injected into patient's body, it would cause an unnecessary radiation dose to patients.