NUCLEAR MEDICINE APPLICATIONS

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INTRODUCTION

Nuclear applications in medicine cover many aspects of modern health care. They contribute to disease prevention, diagnosis and cure. The nuclear techniques in wide application nowadays include:

1. Medical x-rays,
2. Dental x-rays,
3. Brachytherapy,
4. Teletherapy,
5. Nuclear Medicine diagnosis,
6. Nuclear medicine therapy.

In the field of radiography, the initial visualization of bones has evolved into modern radiology in dentistry and orthopedics. X-rays now provide cheap and non invasive means to understand the pathological processes of disease and provide a guide for efficient treatment.

Nuclear imaging applications today range from the relatively cheap dental x-rays machines to dedicated sites with particle accelerators to produce isotopes for positron emission tomography (PET). Investments in hardware range from a few tens of thousands of dollars for an x-ray unit to millions of dollars for sophisticated nuclear imaging systems.

More than two billion x-rays imaging procedures are conducted every year. Seven out of every ten Americans received some form of diagnostic x-rays in 2002. PET imaging techniques have grown recently with about 375 centers worldwide in 2002 and an investment in equipment of more than a half billion dollars.

Radiotherapy is widely used for the treatment of cancer at more than 5,000 treatment centers worldwide treating millions of patients each year. Proton therapy is still expanding and is used in 11 countries at 22 treatment centers.

Radioactive tracers or tags in biomedical research are central to the progress in genomics and proteomics. Radiopharmaceuticals tagged with radioisotopes play a new role in targeting specific organs for both imaging and treatment. Of the 31.7 million patients admitted to hospitals in the year 2000, one third of them had medical radioisotopes administered to them.

The USA market for medical radioisotopes is about one hundred million dollars. The market for radio-pharmaceuticals is in the billion dollars range. The cost of all nuclear procedures in the USA is in the eight to ten billion dollars range.

NUCLEAR MEDICINE

Nuclear medicine is the medical specialty using small amounts of radioisotopes as tracers to diagnose disease, or larger amounts for therapy. Tracers are substances that are

attracted to special organs bones or tissues, like Iodine to the thyroid gland. After being injected into the body, tracers emit characteristic radiations. Special electronic instruments such as scintillation or a gamma camera, which displays these emissions into images, can detect these emissions. A gamma camera with its associated computer is shown in Fig. 1. The images such as the bone scan in Fig, 2 yield information about the anatomy and the function of the body organ being imaged. The nuclear medicine physician interprets the image to determine the cause of a given disease.

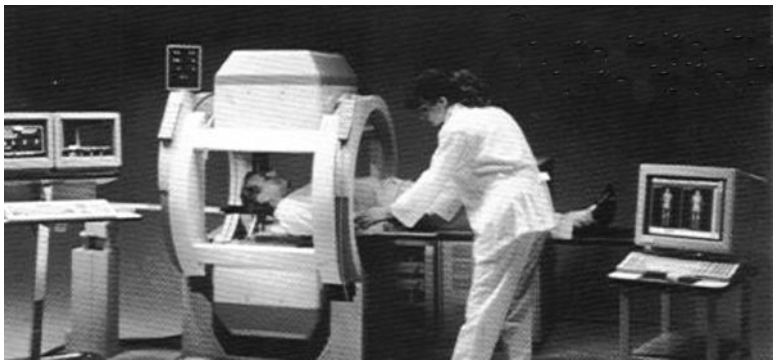

Fig. 1: Gamma Camera and its associated computer.

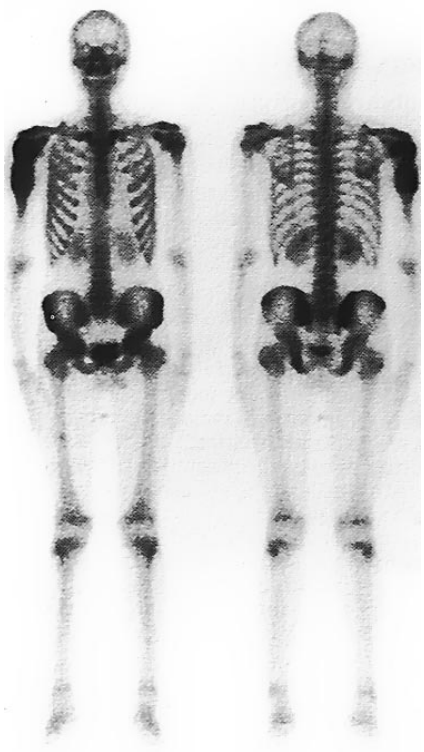

Fig. 2: Anterior and posterior views of an isotope bone scan

Nuclear medicine is also used for therapeutic purposes such as the treatment of hyperthyroidism, thyroid cancer, blood imbalances and pain relief from certain bone cancers. It is a safe and effective way of obtaining information that would otherwise be unavailable, or can only be obtained by intrusive riskier techniques such as surgery and biopsies.

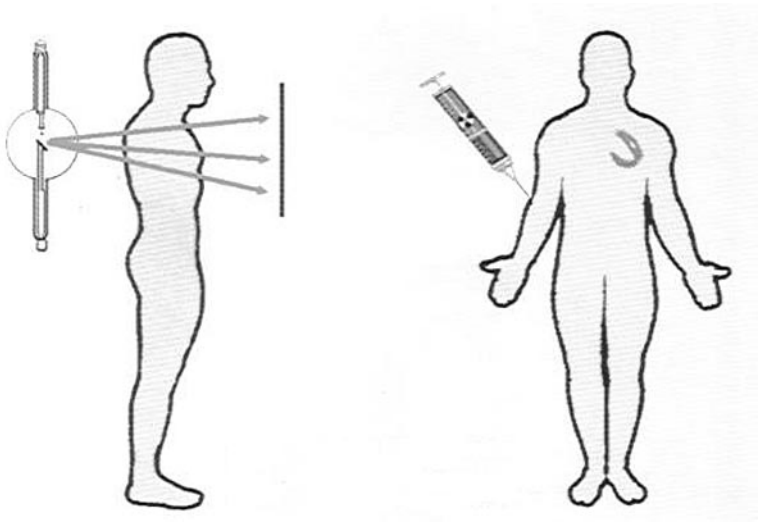

Fig. 3: Difference between x-ray and Nuclear Medicine (NM) imaging.

Nuclear medicine tests are extremely sensitive to abnormalities in body organs structure and function. Tests using nuclear medicine techniques are more sensitive and specific for disease detection than most tests because they identify abnormalities very early in the progression of a disease, long before the medical problem would be apparent with other diagnostic tests.

NUCLEAR MEDICINE IMAGING

Imaging is one of the most common nuclear techniques. Its distinctive feature is that it shows the functional state of an organ or a system in the body, rather than just the anatomical structure as do x-rays. For instance, in the case of acute infection as in acute osteomyelitis, significant destruction of the bone with 30 percent demineralization has to take place before it can be detected by such x-rays procedures as Computerized Tomography (CT) scans. A bone scintigram may detect the lesion at a very early stage of the infection process at less than 5 percent demineralization.

A distinctive feature of Nuclear Medicine (NM) imaging is that the radiation source is internal, administered directly to the patient, whereas diagnostic radiology passes a beam of radiation from an external source through the patient and onto a sensitive surface on the other side, as shown in Fig. 3.

PROCEDURES

Many different types of nuclear medicine procedure are used on a routine basis, these include:

1. Bone scans to evaluate orthopedic injuries, fractures, tumors, or unexplained bone pain. Figure 2 shows a whole body bone scan both in the anterior and posterior views.
2. Heart scans to identify normal or abnormal blood flow to the heart muscle, measure heart function or determine the existence or extent of damage to the heart tissues after a heart attack episode.
3. Thyroid Iodine scans to analyze the thyroid function and show the structure of the gland. Larger doses of radioactive iodine are used to destroy thyroid nodules in the case of Graves' syndrome.
4. Gallbladder or hepatobiliary scans to evaluate both liver as well as gallbladder function. This test can determine obstructions caused by the presence of gallstones.
5. Lung scans to evaluate the flow of blood and movements into and out of the lungs, as well as the determination of the presence of blood clots.
6. Gallium scans to evaluate infection and certain types of tumors.
7. Brain scans.
8. Gastrointestinal bleeding scans.

GAMMA CAMERA

The gamma rays emitted from the internal radiation source in NM imaging are usually detected by the key diagnostic imaging tool used in NM; the gamma camera. Its development dates back top the late 1950s.

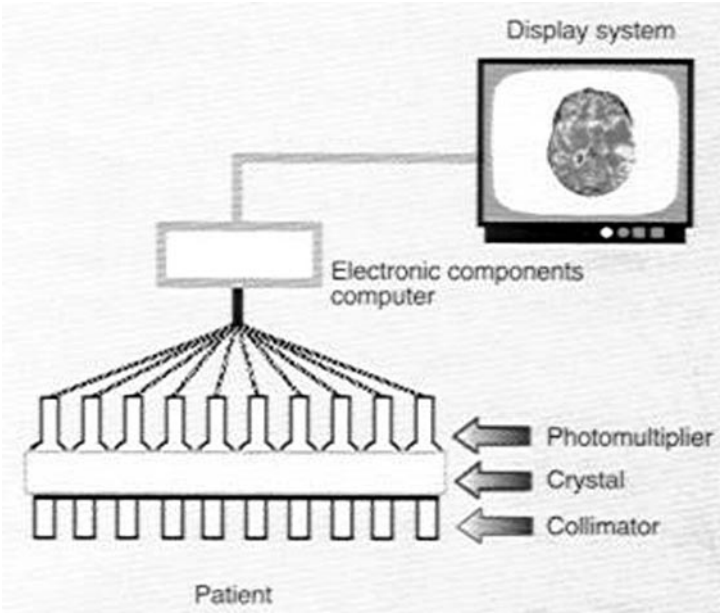

Fig. 3: Construction of Gamma Ray Camera.

In the gamma camera, gamma ray radiation is converted to light photons by a collimator and a crystal, then to an electric signal by a photomultiplier, and finally to an image by a computer, as shown in Fig. 3.

NUCLEAR TECHNIQUES

Nuclear techniques can be either in vitro or in vivo. In vitro methods make use of samples taken from the patient such as blood. In vivo methods involve the direct examination of the patient with the help of specialized equipment.

A vast array of techniques is available to help diagnose and manage disease. They make a controlled use of radiation or radioactivity. Radiation is considered in this context as a release of energy. The detection of this release of energy is the basis of the nuclear techniques used in medicine. For instance, a patient is exposed to a controlled beam of radiation and the results detected by a gamma camera. Alternatively, blood samples are tested with radioactively labeled probes that detect the presence of specific pathogens. Some of the known techniques are:

1. Radioimmunoassay and Immunoradiometric Assay,
2. Molecular methods,
3. Nuclear imaging,
4. Nonspecific organ imaging,
5. Scintigraphy.

In most cases nuclear techniques complement conventional diagnostic techniques. In some applications like in infection and infectious diseases, the nuclear techniques are unique and offer distinct advantages:

1. They provide sensitive and fast diagnosis,
2. They help monitor the spread of infectious diseases,
3. They allow the identification of drug resistant organisms, cheaper and faster than other methods,
4. They confirm whether a drug is working or not,
5. They allow the detection of organisms that are particularly virulent, being aggressive and causing more serious illness.

IN VIVO TECHNIQUES

There are two types of in vivo techniques: imaging and non-imaging techniques. Imaging is the most commonly used technique. A patient is usually given a radioactive material or radiopharmaceutical intravenously. It accumulates in the target pathological area and can thus be detected by external equipment. The detected radioactivity is the basis of the generation of images called scintigrams. A scintigram provides information about the location, extent and type of disease such as urinary tract infection.

In some cases, the radiopharmaceutical will only concentrate in the normal tissue, and not in the pathological area. Should diseased or damaged tissue interfere with the distribution of the radiopharmaceutical, the scintigram will show a defect in the otherwise normal distribution patterns of activity.

In the non-imaging in vivo technique, part of the pharmaceutical administered to the patient, usually by ingestion, is eventually measured from breath or blood samples. An example of this application is in the diagnosis of Helicobacter pylori infections. These are recognized as being responsible for most cases of peptic ulcer disease and associated with stomach cancer. This is an example of a disease that was previously

classified as non-communicable, but has been reclassified as being actually the result of an infection.

INFECTION IMAGING

Infection imaging depends on the understanding of the physiological processes associated with infection. The inflammatory reaction is the body's response to infection. It is activated by many factors including the presence and the release of specific biologically active substances from the pathogens themselves.

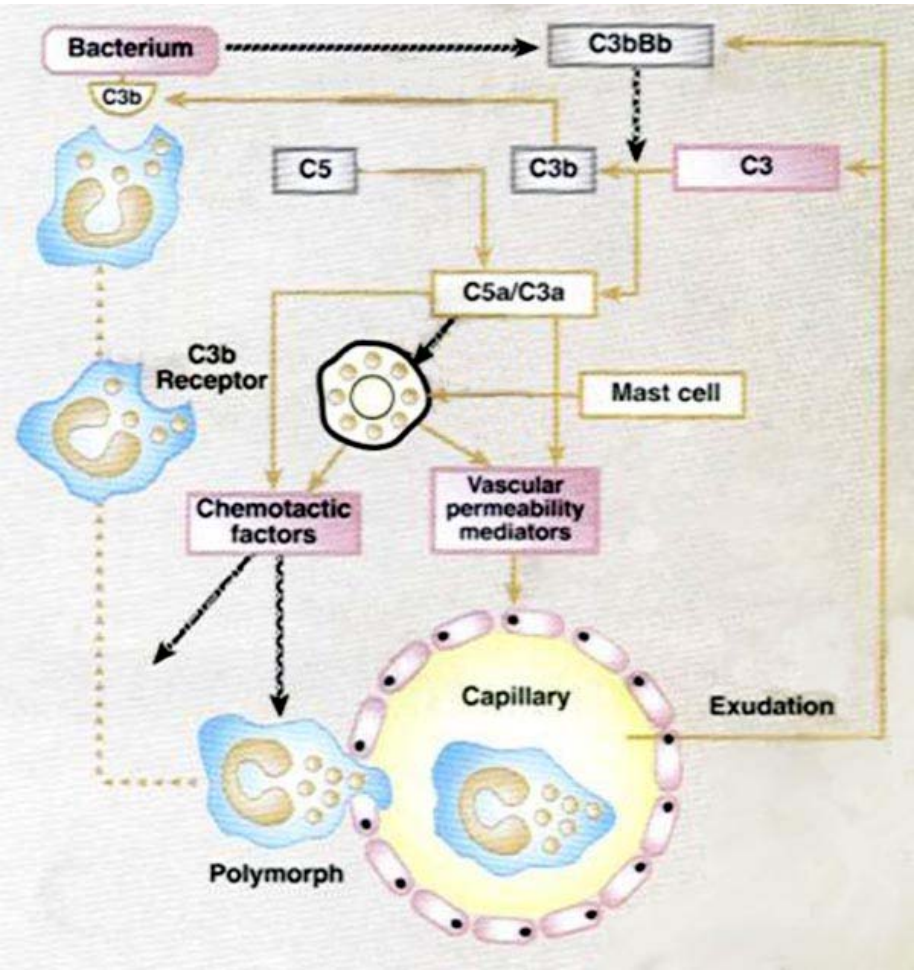

Fig. 4: The defensive strategy aspects of acute inflammation.

The presence of bacteria activates the alternate C path way shown in Fig. 4, which is a cascade of plasma proteins and factors which produces a response. The bacteria activates the enzyme C3bBbC3 convertase, which splits C3 into C3a and C3b. The bacteria binds with C3b. C3a binds to C5a, which then activates the mast cells and acts directly on the vascular permeability mediators and chemotactic factors. These cause the blood capillaries to dilate, releasing polymorph cells, which have receptors for C3b.

Once released from the capillaries, they will attack those bacteria to which C3b has bound.

The inflammatory response is associated with local hyperaemia, or increase in blood flow, oedema, or abnormal accumulation of watery fluid, swelling and pain. An increased capillary permeability causes the plasma proteins to accumulate in the extra vascular space and white blood cells to migrate out of the blood vessels into the interstitial space in and around the site of infection.

RADIOPHARMACEUTICALS

Infection scintigraphy uses different types of radiopharmaceuticals to detect infection.

1. Organ specific imaging pharmaceuticals: These are radiotracers that accumulate in normal organ tissue, and not in non-functioning pathological tissue of an organ. The presence of a functional impairment such as infection, scars, cysts, abscesses, tumor, or malformation can give rise to defects. These procedures can be positive at a very early stage, but are not specific to the kind of disease actually caused. To obtain a correct diagnosis, clinical findings, laboratory tests or other diagnostic procedure are needed. Examples are static renal scans, colloid liver scans and spleen scans.

2. Non-specific increased uptake pharmaceuticals: These tend to accumulate directly in the inflamed or infected organ or system, in contrast to the first type that accumulates in healthy tissue. The information gathered is not specific since the pathological accumulation can also be seen in conditions other than inflammation. Thus if the scintigram is positive, further investigations are warranted. Examples are bone scans and brain scans.

3. General inflammation and imaging pharmaceuticals: These provide the same information as the last group, but are more specific for the presence of inflammation or infection. The likelihood that conditions other than inflammation and infection cause accumulation is less pronounced. These cannot distinguish inflammation from infection. This property is a distinctive feature of the fourth type of radio paharmaceuticals. These pharmaceuticals include:

a) Metallic ions which bind to metalloproteins after intravenous injection, such as Gallium^{67} citrate.

b) Labelled proteins, which pass into the inflammation sites as a result of increased vascular permeability such as Tc^{99m} nanocolloids and Tc^{99m} immunoglobulins.

c) Leukocyte or white blood cells labels which trace leucocyte localization at the site of inflammation such as the Tc^{99m}/In^{111} leukocyte.

4. Infection Imaging using disease specific or organism specific radiopharmaceuticals: These tracers have the distinctive feature to differentiate if the cause of the inflammation is due to bacteria or viral pathogens. These radiopharmaceuticals include:

a) Monoclonal antibodies such as I^{123} antigranulocyte monoclonal antibodies, and monoclonal antibodies against BCG.
b) Radiolabelled peptides.
c) Radiolabelled Interleukin.
d) Radiolabelled antibiotics for bacterial infection such as Tc^{99m} labeled Ciproflaxin derivative-Infecton.

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY (SPECT)

Radiopharmaceuticals injected into the patient can be visualized using a gamma camera. The resulting images or scintigrams can be two-dimensional or they can be more complex three dimensional slices. Single Photon Emission Computed Tomography (SPECT) uses a rotating gamma camera to obtain images from multiple angles of the organ under study.

Radioisotopes administered to the patient produce a radioactive signal which can be detected by the gamma camera. Using a collimator, crystal, and photomultiplier is transformed into an image.

The SPECT technique is particularly valuable because of its unique ability to locate the precise location of an abnormality from the three dimensional image it produces.

DMSA SCINTIGRAPHY

DMSA Scintigraphy using a compound called DMSA, is the most sensitive and reliable diagnostic test available today.

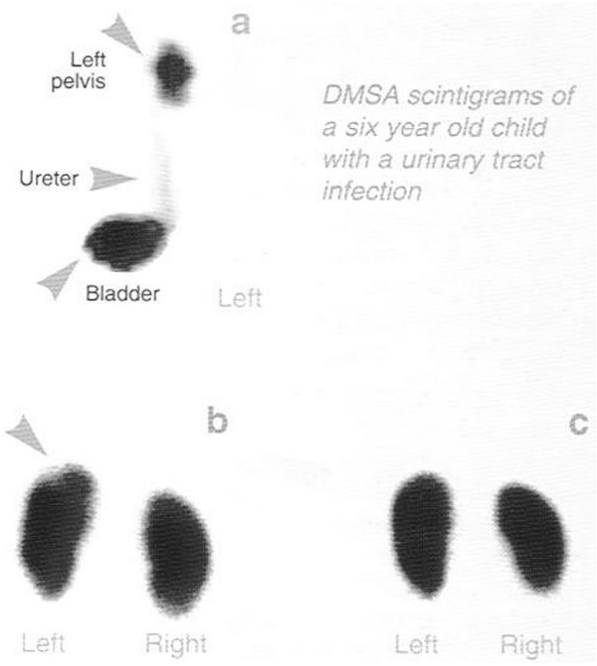

Fig. 5: Gamma camera DMSA scintigram of urinary tract infection.

Acute pyelonephritis is an infection of the kidney, specifically the parenchyma and the pelvis of the kidney. It causes morbidity or illness in children, possibly leading to a variety of ailments including high blood pressure and kidney failure. Detected early, it can be cured by antibiotic therapy. The most sensitive and reliable diagnostic test available is radionuclide DMSA scintigraphy. It detects renal scar, which is a highly characteristic finding at a very early stage.

In Fig. 5, a direct radionuclide image of the bladder through which the radiopaharmaceutical is directly infused is shown. It reveals a gross urinary back flow from the bladder to the ureter and pelvis on the left side in the anterior view (a). The posterior view DMSA scan (b) shows a defect at the left upper pole of the kidney due to renal infection. The infection resolved completely after one month as shown in (c) after the successful antibiotic treatment of the urinary tract infection.

UREA BREATH TEST

A most common human infection in the world is caused by Helicobacter pylori. The means of transmission of this bacterium is little known. It was first linked to stomach and gastric disease and ulcers as recently as 1989. In the industrialized world, the infection covers 20-40 percent of the population. In developing countries its prevalence is over 80 percent with the infection being highest among children. In the new born, H. pylori is linked to chronic malnutrition and diarrhea syndrome with failure to thrive and mental retardation. Under such circumstances, infection can be fatal, even though its diagnosis and treatment is simple.

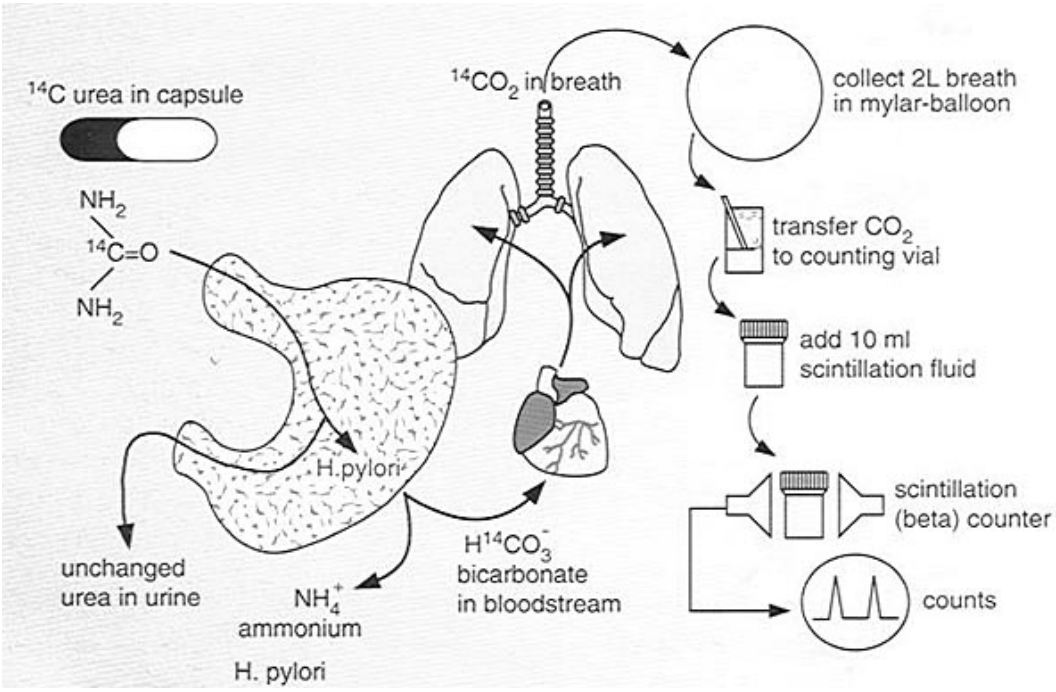

Fig. 6: Urea breath test using Carbon^{14} as a radiotracer.

An in vivo test to detect the presence of H. pylori is the urea breath test. It uses radioactively labeled urea to test for urease, a specific enzyme produced by H. pylori. The patient is given an oral preparation containing the radiolabelled urea. H. pylori naturally produces the enzyme urease which breaks the urea into two components: ammonia and bicarbonate. If the patient were infected, the urea will be broken down into ammonia and radiolabelled bicarbonate.

The radiolabelled bicarbonate will be metabolized by the body into carbon dioxide and will be expired as the patient breathes. The radioactive label can be detected in a scintillation beta counter 30 minutes after ingestion of the radiolabelled urea. The urea breath test is the preferred diagnostic test used after treatment to show that the H. Pylori has been eradicated.

TC-99M INFECTON IMAGING

The Tc99m Infecton radiopharmaceutical consists of a synthetic broad spectrum antibiotic Ciproflaxin linked to the radioisotope Tc^{99m}. The antibiotic is taken up and bound specifically by the DNA of living bacteria. It does not bind to dead bacteria. Studies in animals have shown that that the agent is not taken by sterile inflammation or abscesses, but concentrates only at sites of active infection.

A scintigram in Fig. 7 using Tc99m Infecton of the right shoulders shows intensely increased radiotracer uptake at the site of infection confirming septic arthritis. Another scintigram in Fig. 8 of the knees of a patient with a right knee prosthesis and signs of infection identified by the Tc^{99m} radiotracer uptake around the right prosthesis confirming infection.

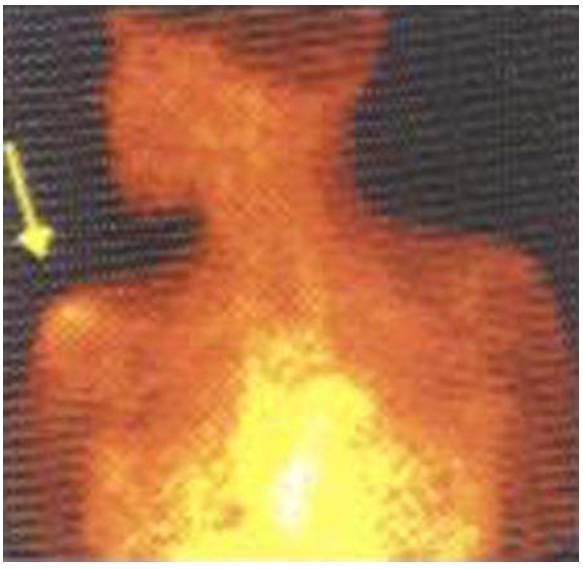

Fig. 7: Scintigram using Tc^{99m} Infecton of the right shoulders shows intensely increased radiotracer uptake at the site of infection..

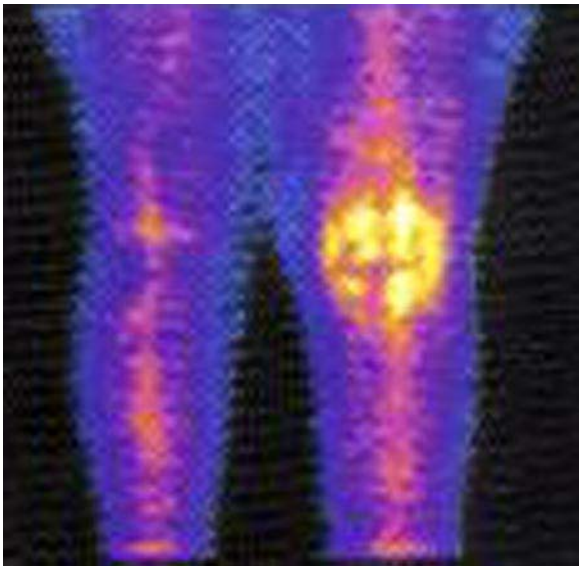

Fig. 8: Scintigram of the knees of a patient with a right knee prosthesis and signs of infection identified by the Tc^{99m} radiotracer.

IN VITRO TECHNIQUES

These normally use radioactively labeled compounds to detect pathogens or signs of infection from blood samples. Some of the techniques used to diagnose infectious disease are;

1. Radioimmunoassay (RIA),
2. Immunoradiometric assay,
3. Molecular methods.

The technique of RIA identifies the pathogen responsible for an infection by using antibodies and isotopes. The presence of a disease such as hepatitis can be detected at an early stage. Moreover, it can identify the carriers of infection, determine the infectivity of the disease, and predict response to treatment.

Radionuclide molecular methods use radioisotopes and the polymerase chain reaction (PCR) to detect the genetic material of the pathogen.

These methods have been developed for the rapid and sensitive detection of infectious agents and play an important role in the epidemiological study, management and prevention of a number of diseases such as malaria and tuberculosis.

RADIOIMMUNOASSAY (RIA) METHOD

Radioimmunoassay (RIA) is a very sensitive method to identify biomolecules such as pathogens, hormones, drugs, and cancer cells. It relies on the specific nature of the antibody-antigen interaction. In the Laboratory, mice are injected by an antigen which could be a protein from a bacteria or virus. Thje mouse's immune system recognizes the protein as an invader, and will produce antibodies that are specific to that protein in an attempt to kill it.

Its basic principle is to demonstrate when a combination has occurred between the biomolecule between the molecule to be identified and an antibody shown in Fig. 9 raised by an animal against that molecule. The demonstration is confirmed by using a radioisotope to tag one of the combining molecules.

Once radiolabelled, the antibodies can then be used to detect the presence of the same bacteria or virus in blood samples. Since the antibody-antigen interaction as at the molecular level. The RIA method is capable of detecting minute amounts of the antigen, which makes it a very sensitive method.

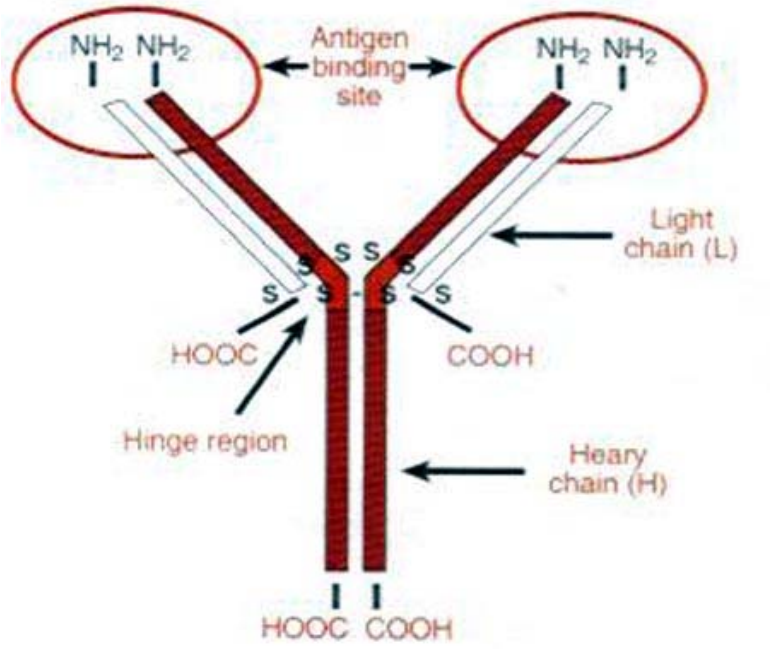

Fig. 9: Diagram of the structure of an antibody.

HYBRIDOMAS PRODUCTION OF MONOCLONAL ANTIBODIES

Antibodies are produced by white blood cells called lymphocytes. If a biomolecule is introduced into a healthy animal and is recognized by the immune system of the animal as a foreign molecule, the lymphocytres of the animal will eventually start producing antibodies against the intruding biomolecule.

In the process of producing antibodies, the lymphocytes will also divide, forming clones of cells that produce the same antibody. This is part of the immune system response to infection.

Some lymphocytes will direct themselves toward one region or epitope of the biomolecule. Other lymphocytes will target other regions or epitopes. When dividing, each lymphocyte will produce a clone of itself which is directed toward the same epitope. The antibodies produced by the different clones are not the same. They are directed against the different epitopes of the same molecule and are said to be polyclonal.

It is possible to artificially produce in the laboratory lymphocytes and produce antibodies that are monoclonal. To be able to achieve this, the cell lines of the lymphocytes should first have to acquire the ability to divide in perpetuity. Under normal conditions, this is not possible to do under laboratory conditions, because the lymphocytres die after a limited number of cell divisions. The key idea here is to fuse a clone of the lymphocyte with cancer cell lines of lymphocytes or plasmacytoma cells from the mouse. In his case they form a hybrid of both clones called a hybridoma, as shown in Fig. 10.

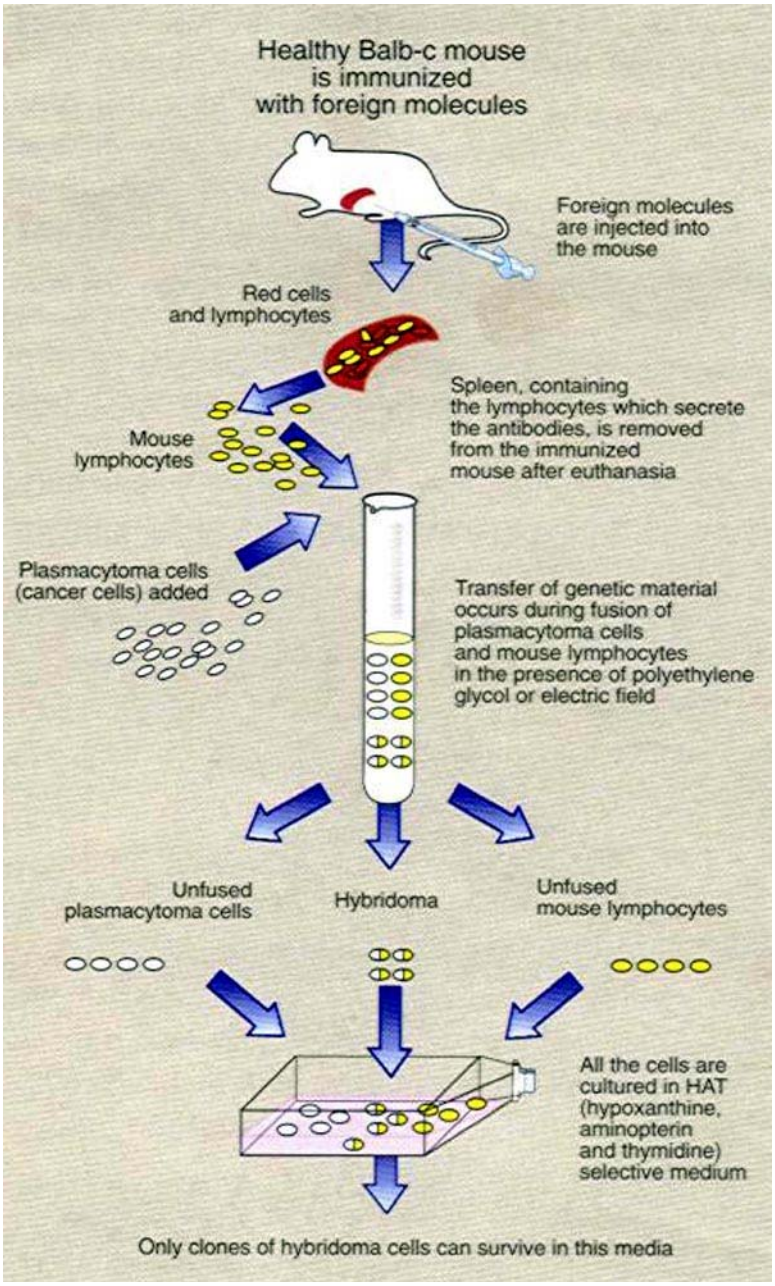

Fig. 10: Hybridomas Production of Monoclonal Antibodies.

The hybridoma cells inherit the characteristics of both parents. From the lymphocyte clone, they are able to secrete the antibody. From the cancer cell, they acquire the longevity to divide perpetually under laboratory conditions. The end result is a cell line that produces one kind of antibody or monoclonal antibody against the specific epitope of the biomolecule against which it was raised. Monoclonal antibodies can also be produced in bacteria by the recombinant DNA technique. There are attempts at manufacturing monoclonal antibodies in cow's milk and harvesting them from plants.

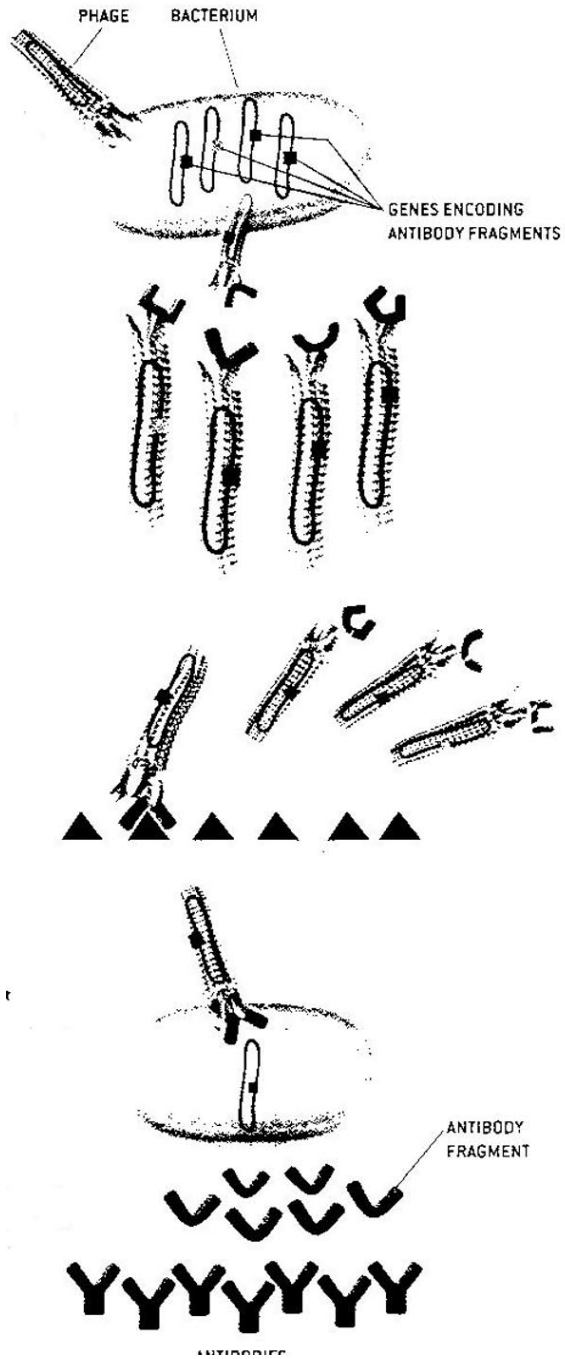

Fig. 11: Production of monoclonal antibodies from phages.

PHAGES PRODUCTION OF MONOCLONAL ANTIBODIES

Viruses called phages that can infect bacteria can also be used in the production of monoclonal antibodies. The steps in the process are as follows:

1. Insert genes encoding a variety of the antigen-binding fragments of antibodies into bacteria then infect the bacteria with phages.
2. The infected bacteria make new phages, each of which incorporating a different antibody fragment at its tip.
3. The mixture phages is screened to select only those with the antibody fragments that specifically bind to the target antigen. This process is repeated several times.
4. The genes from the selected phages are added back to the bacteria to make more of the specific antibody fragments.
5. Place the antibody fragments oto the antibody backbones to make whole antibodies.

This process is illustrated in Fig. 11.

RADIOIMMUNOASSAY REACTION

Detection systems have been devised to show the reaction between the biomolecule to be identified and the monoclonal antibody that will react with it. The detection systems try to immobilize the monoclonal antibody onto a solid phase such as a plastic bead or on the wall of a plastic tube. The immobilization process is monitored in quantitative manner by tagging a radioisotope to some component of the reaction. This process can also be visualized by using a scanning probe microscope or a scanning electron microscope. Two major types of detection systems exist:

1. The Competitive RIA system:

In this method, a limited fixed amount of monoclonal antibody is immobilized onto a solid phase such as the wall of a plastic tube as shown in Fig. 12. A patient sample which may or may not contain the biomolecule, is added together with a fixed and known amount of the radiolabelled biomolecule against which the antibody was raised.

If the biomolecule is in fact present in the sample, it will compete with the radiolabelled biomolecule for the binding sites on the antibody. The intensity of the radiation signal from the radiolabelled biomolecules through the antibody onto the solid phase will thus be inversely proportional to the concentration of the sought –after biomoleculke in the sample. The concentration of the biomolecule in the sample is eventuallt calculated from a calibration curve as shown in Fig. 13.

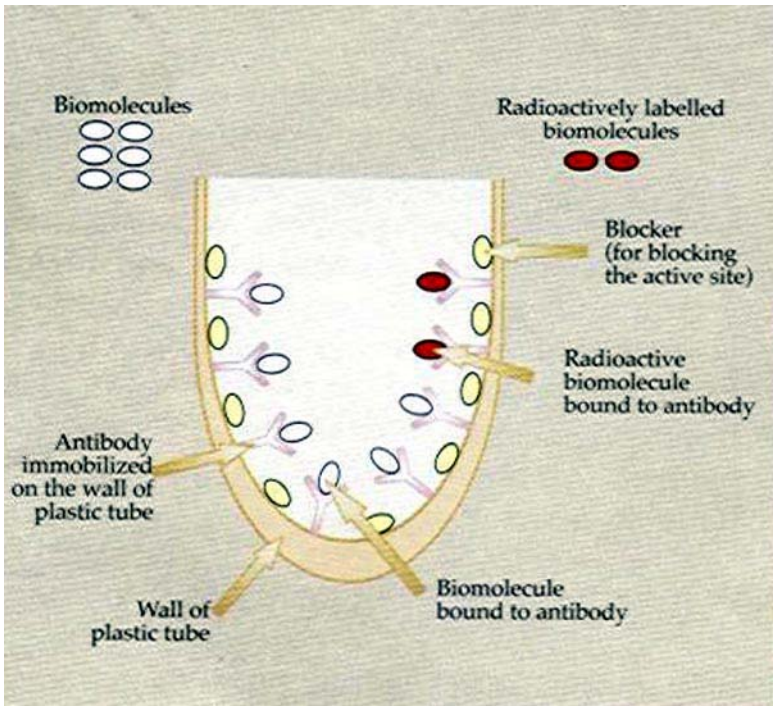

Fig. 12: The Competitive Radioimmunoassay system.

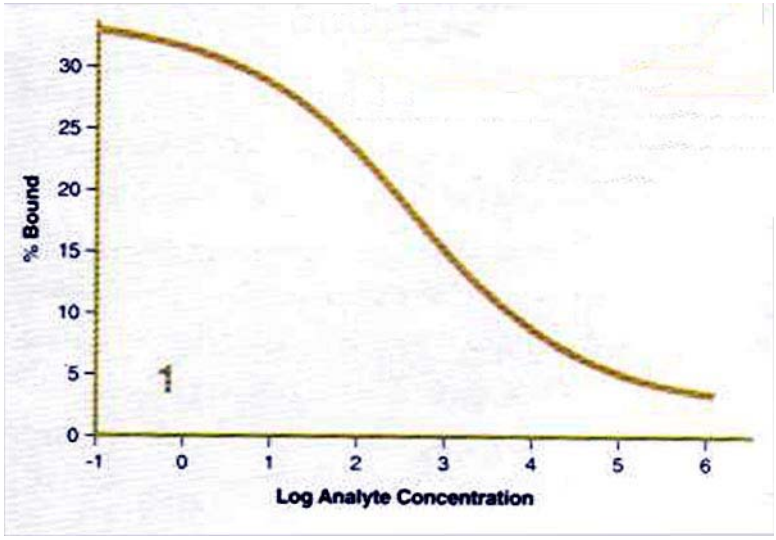

Fig. 13: Calibration curve for the Competitive Radioimmunoassay system.

2. The Sandwich Immunoradiometric Assay (IRMA)system.

This approach is specifically used to identify and quantify large biological molecules. The first step in the sandwich IRMA is to prepare an excess of immobilized monoclonal antibodies with excessive binding sites.

The next step is to add the biomolecules to be identified exposing them to the immobilized antibodies. If the biomolecule is the same as the one to which the immobilized antibodies have been raised, they will be captured by the antibodies and form the first part of a sandwich as shown in Fig. 14.

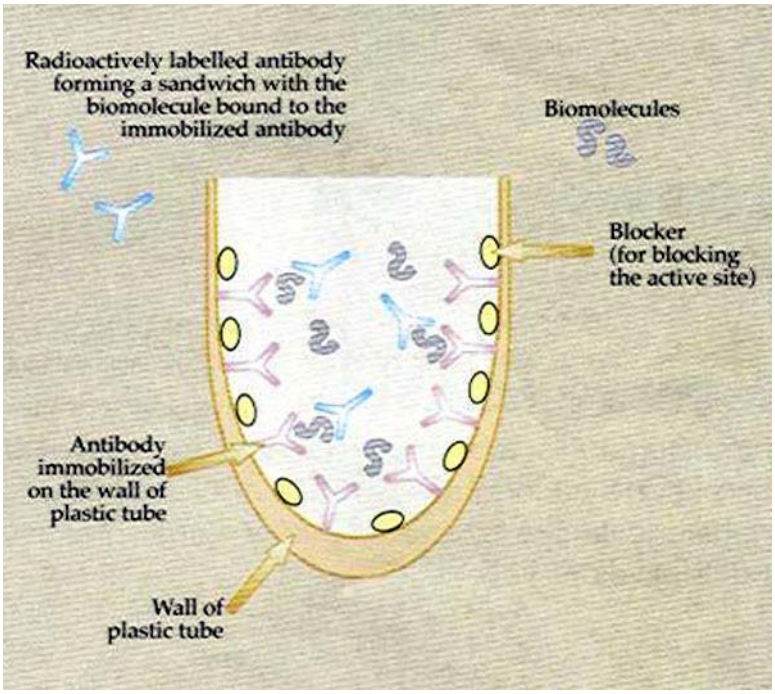

Fig. 14: The Sandwich Immunoradiometric assay system.

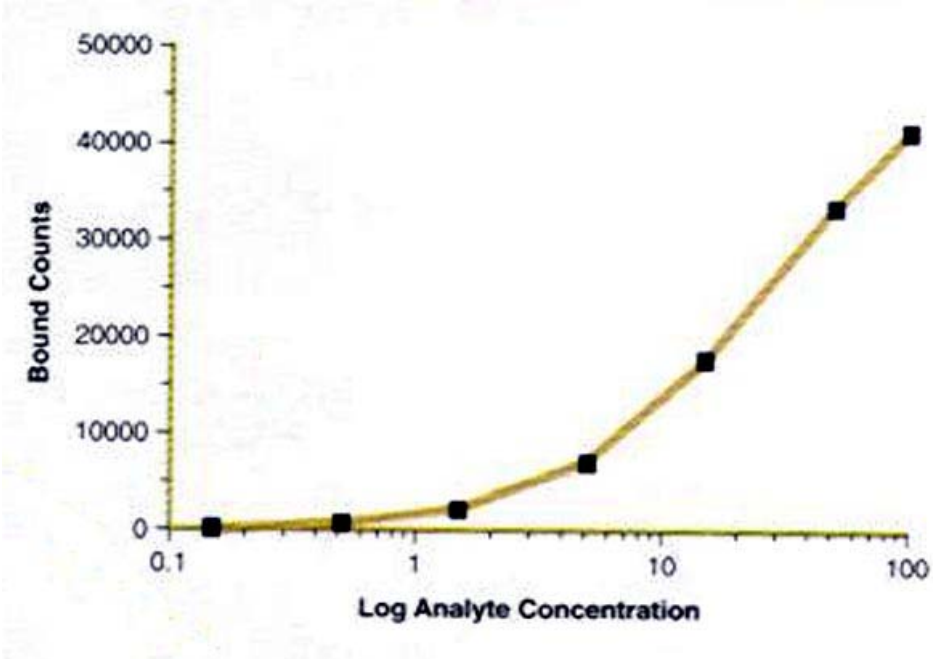

Fig. 15: Calibration Curve for the Sandwich Immunoradiometric assay system.

A second and radioactively labeled antibody is then added. This antibody has been raised against a different epitope of the same biomolecule from that of the immobilized antibody. It will bind as the top layer of the sandwich to the biomolecule bound to the immobilized first antibody. Thus the radiation signal of the second antibody will be directly proportional to the concentration of the captured biomolecule in the sample. This can be measured using a gamma counter. The amount of radioactivity measured is directly proportional to the amount of antigen in the blood sample. The exact concentrations can be inferred from the calibration curve shown in Fig. 15.

CONJUGATED MONOCLONALS

New forms of monoclonal antibodies other than the mouse ones have been developed: chimeric (66 percent human), humanized (90 percent human) and fully human.

This opens the door of achieving the dream of deploying the conjugated monoclonals, which are ones that carry radioactive chemicals or toxins directly to the tumors as a new form of cancer therapy.

Two notable monoclonals of that kind are already available and target an antigen called CD20 on the surfaces of B lymphocytes, cells that grow uncontrollably in the type of cancer called non-Hodgkin's lymphoma:

1. Zevalin developed at SanDiego by IDEC Pharmaceuticals and Shering AG. This is tagged with the radioisotope Y^{90}.
2. Bexxar, developed by Corixa in Seattle and Glaxo-Smith Kline. This is tagged with the radioisotope I^{131}.

INDUSTRIAL PRODUCTION

Just 10 large-scale anribody plants are operating worldwide. The production of monoclonals from hybridomas is carried out in large tanks called bioreactors. A giant 60,000 liters bioreactor plant would be able to accommodate four monoclonal product lines. If 100 monoclonals will be needed at least 25 new facilities will to be built. These would cost about $5 billion and would need 3-5 years to be licensed by the Food and Drug Administration (FDA).

The void needs to be filled by turning to transgenic animals and plants. These are organisms engineered to carry genes for selected antibodies. Mammals such as transgenic goats or cows that would secrete monoclonals in their milk can produce one gram of antibody at a cost of $100, which is one third the cost of the traditional method. This approach would involve the tedious step of isolating the monoclonals from the milk proteins. Genetically engineering and breeding the animals can take years. This is still much cheaper than the cost of the 10,000 liters bioreactors.

Green plants can also be the answer to the monoclonal production shortfall. Plants have the advantage of being economical and easily scalable to any level of demand, yielding metric tons of monoclonal products, even though the purification problems would still remain.

Dow and Epicyte are researching corn plants able to generate monoclonal antibodies that will be formulated as creams or ointments for mucosal surfaces such as the lips, or as orally administered drugs for gastrointestinal or respiratory infections.

Monoclonal antibodies are bound to become a major part of 21st century medicine. This is leading to a future of a new industry of "Pharming," replacing the conventional "farming."

POLYMERASE CHAIN REACTION (PCR) TECHNIQUE

The polymerase chain reaction (PCR) technique uses deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and multiplies them into easily measured amounts. The fundamental principle of applying the PCR method in diagnostics is that if a given DNA fragment is present, it will be multiplied by about a million fold through the reaction, yielding so much of the substance that it can be detected easily.

The process starts by heating the DNA fragment to separate it into strands. An enzyme which copies the DNA: DNA polymerase, is then added to create two complete copies of the original fragment. By repeating this process, millions of copies of the original DNA fragment can be made in about an hour.

Radiolabelled nucleic acid probes, which attach to DNA fragments at the molecular level, can then be used to identify the DNA fragment.

The method is very sensitive and specific, requiring only a small sample of blood. The million fold amplification permits the accurate detection of very small numbers of pathogens, such as might be present in cases of tuberculosis or Chagas disease. It is possible to detect infection much earlier than with conventional methods, thereby reducing the severity of illness and the risk of death as a result of earlier treatment.

PCR DOT BLOT METHOD

Dot blot hybridization uses DNA extracted from the pathogen to be identified and then amplifies it using the polymerase chain reaction. The amplified DNA is directly "dotted" onto a nylon membrane.

Radioactively labeled DNA probes, specific to the pathogen DNA, are added. The membrane is then exposed to an x-ray film. Spots on the x-ray film will appear only when the pathogen has first been bound to the nylon membrane and the radioactively labeled probe has been bound to the pathogen.

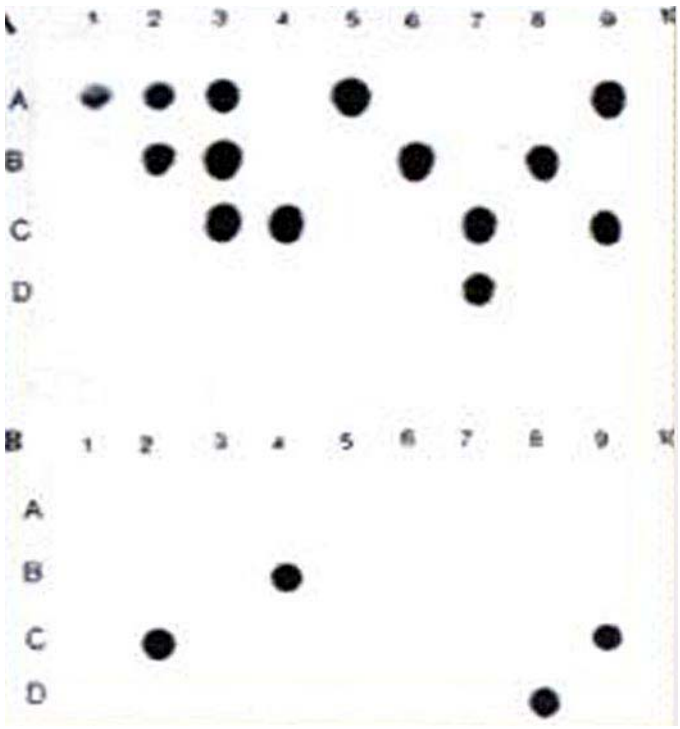

Fig. 16: PCR dot blot hybridization of codon 315 and codon 463 in the KatG gene of M. tuberculosis.

The advantage of this method is that the dots derived from numerous samples can be analyzed simultaneously. Another advantage is that the radioactive probe can be removed and a new one, to detect another mutation of the pathogen, added. Thus several mutations can be screened for sequentially. In this context, PCR dot blot can be used to detect the drug resistant Tuberculosis (TB) starins, as shown in Fig. 16.

MOLECULAR BIOLOGY METHODS

Molecular biology is the study of small biomolecules in cells. Its goal is to explain the chemical interactions occurring between these molecules and their environment.

Even though time is devoted to the study of the hereditary material of the cell, DNA, other biomolecules are also such as RNA, which translates information from the DNA into proteins, amino acids, fatty acids, and carbohydrates; all components of the human body.

Central to the analytical techniques of molecular biology is the use of restriction enzymes, which cut through DNA and RNA and the PCR technique, which can make multiple copies of the resulting DNA or RNA fragments.

Radionuclide molecular methods make use of restriction enzymes and PCR, but also use radioactively labeled probes or tags to detect specific biomolecules.

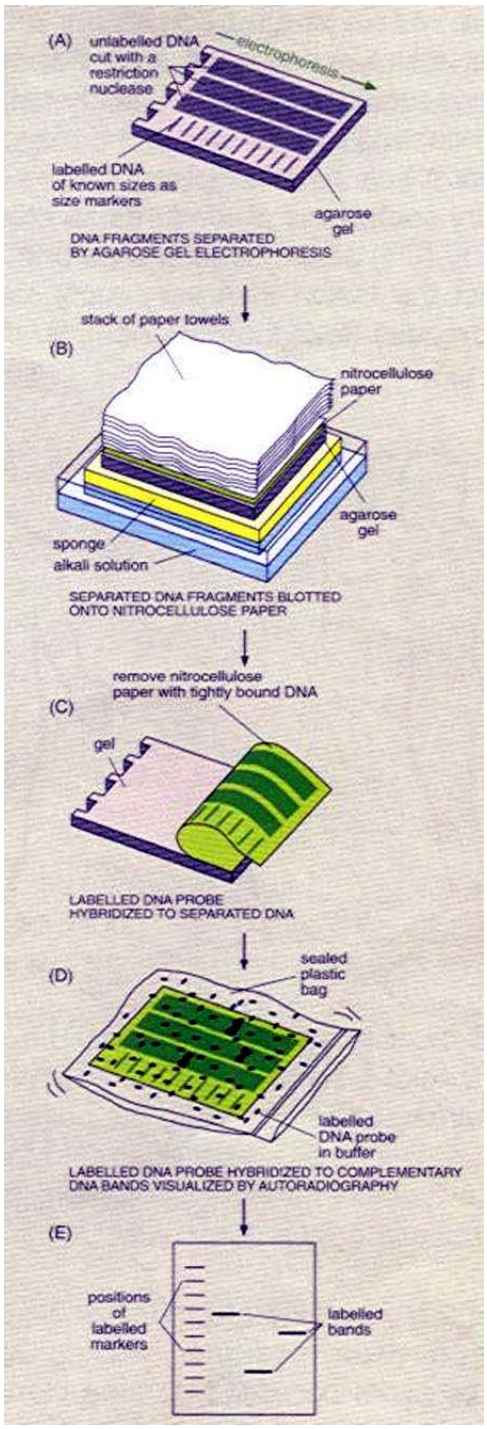

Fig. 16: DNA Fingerprinting technique.

DNA FINGERPRINTING

DNA fingerprinting is a sensitive method to identify different strains of pathogens. Double stranded DNA from the pathogen to be identified are chemically fragmented into segments using enzymes which cut the DNA. The double stranded

segments are then separated by placing them onto a slab of porous gel: agarose, and then applying an electrical current, a process called gel electrophoresis.

DNA segments have a slight negative charge and will move towards the positive electrode of the slab according to their size. Smaller segments move quickly than do larger segments, resulting in a ladder pattern of segments.

The agarose slab is then treated with chemicals to denature the DNA, a process which causes the two complementary DNA strands to separate.

The next step is to cover the treated slab with a nylon membrane covered with layers of absorbent paper, which will then draw the denatured and separated segments onto the nylon, just like drying wet ink with blotting paper.

The technique was introduced by Edwin Southern in 1975, and is known as "Southern blot."

The nylon membrane with the denatured and separated segments is then placed in a bath with radioactively labelled DNA probes. These probes are short pieces of single stranded DNA that complement and bind to a specific chain of DNA. This process is called: hybridization. When x-ray film is exposed to the nylon membrane, radioactivity will appear as dark bands on the developed film.

Thus wherever the probe matches a known DNA chain on the segment, it will show a characteristic and recognizable pattern that is unique to the specific pathogen. The steps in the DNA fingerprinting technique is shown in Fig. 16.

CONCLUSIONS

It must be noted that nucleic acids, the building blocks of genes, can be labeled with nonradioactive chemicals, radioisotopes remain the most reliable and sensitive meand of labeling nucleic acids. They retain their role as the gold standard both for reference and new technology, and for the quality control of the established techniques.

Radionuclides used with monoclonal antibodies open a new field for diagnosis and treatment of the human body replacing external radiation beams with an internal distributed source for probing and delivering energy.