SYMPOSIUM

Biomedical techniques in translational studies: The journey so far

Imran Haruna Abdulkareem

Department of Trauma and Orthopaedics, Leeds University Teaching Hospitals, Leeds West Yorkshire, United Kingdom

ABSTRACT

Biomedical techniques have wide clinical application in many fields of medicine such as oncology, rheumatology, immunology, genomics, cardiology and diagnostics; among others. This has been made possible with the use of genetic engineering and a number of techniques like Immunohistochemistry (IHC), Fluorescent Microscopy, Cell Culture, Genetically Modified (GM) Cells, Monoclonal Antibodies (MAbs), Polymerase Chain Reaction (PCR) and Western blotting. The aim of this literature review is to explore the foundations and bases of the commonly used biomedical techniques, as well as their applications in biomedical research and clinical medicine in general. This review also aims to shed some light on more recent advances in genetic engineering, especially in relation to genetically modified cells and use of monoclonal antibodies which have found more increasing use and relevance in genomics, oncology, rheumatology, immunology, cardiology as well as diagnostics, and have revolutionised patient care, while at the same time resulting in improved standard of health care. Unfortunately, some of these new techniques are associated with unwanted side effects which may pose a risk to the people they are actually intended for. Therefore, there is need for strict regulations and guidelines to control the use and implementation of some of these novel techniques.

Key words: Biomedical techniques, cancer, cardiology, diagnostics, genetics, genetic engineering, immunology, monoclonal antibodies, rheumatology, translational studies

Address for correspondence:
Dr. Imran Haruna Abdulkareem,
Registrars' Room,
B Floor Clarendon Wing,
Leeds General Infirmary, Great George Street, Leeds LS1 3EX,
West Yorkshire, United Kingdom.
E-mail: imranharuna@yahoo.com

INTRODUCTION

Translational biomedical studies have become increasingly common and very useful in evidence-based practice of medicine. Commonly used techniques include immunohistochemistry (IHC), fluorescent microscopy, cell culture, genetically modified (GM) cells, monoclonal antibodies (MAbs), polymerase chain reaction (PCR) and western blotting among others. Many of these biomedical techniques are a result of genetic engineering, and have become increasingly more relevant in translational biomedical studies especially in the field of cancer, genomics and immunology (Fujita, 2013).[1] Other fields of medicine like cardiology have also seen a recent surge of interest in genetic engineering, especially MAbs, with a view to creating new treatments for life threatening conditions that have defied conventional treatments (Foltz *et al*., 2013).[2] These techniques have a broad spectrum of indications which include disease prevention (vaccination), diagnosis, treatment and toxicology studies, among others (Frauches *et al*., 2013).[3]

Access this article online	
Quick Response Code:	Website: www.nigeriamedj.com
	DOI: 10.4103/0300-1652.129634

IMMUNOHISTOCHEMISTRY (IHC)

This is the application of immunological techniques to the study of cells and tissues. The technique was conceptualised by Coons and consisted of labelling with a fluorescent probe an antibody that was raised in rabbit. He then searched for the antibody and the antigen to which it was bonded in tissue sections with a fluorescent microscope after incubation. This later became a very common technique in histopathology laboratories because of the subsequent improvements made to it and the obvious advantages associated with its use (Rosai, 2004).[4]

The two commonest methods used are the peroxidase-antiperoxidase immune complex method and the avidin-biotin immunoenzymatic method. The latter procedure makes use of the high affinity of avidin for biotin to couple the peroxidise label to the primary antibody. Different methods of enhancing the sensitivity of the technique have

been proposed and aim to expose the antigenic epitopes (binding sites), which may otherwise remain covered or masked- the so-called antigen-unmasking techniques. These include digestion with various proteolytic enzymes, microwave treatment of the tissue, or the use of pressure cooker which provides both heat and pressure (Rosai, 2004).[4]

The advantages include significant sensitivity and specificity, wide applicability and the ease of accurate correlation with the traditional physical parameters (Bassotti *et al*., 2007).[5] It has also been found to be compatible with the fixatives commonly used and can be applied in decalcified material or in tissue sections that were previously stained (Bender *et al*., 2007).[6] In addition, it can be applied to cytological preparations, electron microscopy and conventional techniques like silver staining in the same tissue section (Drakos *et al*., 2007).[7] It has, therefore, superseded many of the conventional special stains, as well as several electron microscopic applications and has good application in mixed-up specimens in the histopathology department (Chalmers *et al*., 2006).[8]

However, it has some limitations which may be observer dependent and need to be acknowledged and avoided by careful technique, regular checking of the antibody activity, and the appropriate employment of positive and negative controls. In this regard, a new fixation and paraffin-embedded method called the Amex method has been suggested, and helps to preserve many of the antigens that are usually destroyed by the traditional techniques (Rosai, 2004).[4] Also, a quick labelling method, which takes only 7 minutes, has been introduced for its possible indications in intra-operative situations. This can help the surgeon come to a quick and accurate decision regarding the type of procedure to carry out on the patient. Furthermore, automated devices are available and contribute to the value of this technique. Also, the sausage tissue block method of antibody testing, which was devised by Battifora, allows assessment of 100 different tissue samples at the same time on a single slide using one drop of antibody and has been subsequently improved by other workers (Torres *et al*., 2006).[9]

False negative results can occur when there is use of inappropriate, denatured or wrong concentration of the antibody, or when there is loss of antigen due to cell destruction and/or diffusion. This is more common with some antigens like factor VIII-related antigen and actin. (Taylor *et al*., 2006)[10] In addition majority of the antigens continue diffusing out following fixation, and so it is advisable to stain the tissue using the original paraffin block than old paraffin-fixed tissues. False negative results can also be seen when there is very low level of antigen to be detected due to either very low antigen production or excessive release out of the tissue. Therefore, an apparently negative immunohistochemical result should not be considered absolute in spite of a positive built-in control, especially if the clinical signs and symptoms strongly suggest the diagnosis (Rosai, 2004).[4]

On the other hand, false positive results are even more dangerous because they can lead to over-diagnosis and over-treatment of the patient with disastrous consequences. These can be as a result of the following antibody cross-reactivity with different antigens from the one being sought, non-specific binding of the antibody to the tissue in question, presence of endogenous peroxidise in some elements of the tissue or cells, or avidity of the avidin-biotin complex by these same elements, the presence of normal tissue in tumour cells. This is commonly seen as entrapment of skeletal muscles by soft tissue tumours, entrapped follicular epithelium in thyroid lymphoma or carcinoma or keratin-positive entrapped epithelial cells of the thymus in thymoma (Chalmers *et al*., 2006).[8]

Other causes of false positive results include release of soluble proteins from the normal cell cytoplasm invaded by tumour with later involvement of the interstitium and non-specific absorption and possibly uptake by the tumor cells, ectopic antigen expression by unrecognised cross reactions or sharing of some markers or antigens by different tissues or neoplasms. These antigens include neuron-specific enolase, alpha-1 anti-trypsin, S-100 protein, Vimentin and epithelial membrane antigen.[4]

Antigens detected by immunohistochemistry (IHC)

The number of antigens which have been detected with this technique has been increasing. (Rosai, 2004)[4] In principle, any antigenic substance, whose antigenicity is partially retained in tissue sections, can be evaluated using this method. (Drakos *et al*., 2007)[7] This has been made easier by the monoclonal technology. The most important diagnostic applications of IHC are related to detection of various antigens which include actin, alkaline phosphatase, alpha-actin, alpha-1 antrichymotrypsin, alpha-1 antitrypsin, alpha-fetoprotein, alpha lactalbumin, angiotensin converting enzyme, basement membrane, blood group antigens, bone gamma-carboxyglutamic acid (GLA) protein, CA19.9, CA 125, cadherins, calmodulin, cancer-associated carbohydrates, (Osborne *et al*., 2007)[11] carbonic anhydrase C, carcinoembryogenic antigen (CEA) and casein. Others include cluster of differentiation 31 (CD31), cluster of differentiation 34 (CD34), chromogranin, collagen, creatine kinase, cytokeratins, desmin, endorphins, enolase, enzymes, factor XIIIa, factor-VIII-related antigen, ferritin, fibrin, haemoglobin, histaminase, human leukocyte antigen (HLAs), hormone receptors, human chorionic gonadotropin (hCG), human placental lactogens, p53, Phosphatase and tensin homolog (PTEN), and immunoglobulins. This list is, however, not exhaustive, but serves to demonstrate the wide applicability of this technique (Rosai, 2004).[4]

The first option antibodies should be used by the immunopathologist as diagnostic tools in tumor detection. Various antibody panels have been devised to standardise immunohistochemical search based on the clinical suspicion of the pathology. Therefore, an adenocarcinoma versus mesothelioma panel may consist of stains for keratin, Carcinoembryonic antigen (CEA), Epithelial membrane antigen (EMA)and Lewis X (Leu-M1) antigen or known as CD 15. This is useful, but the number of permutations and combinations is very wide and so it impossible to devise a list of panels to cover all the diagnostic problems covered in the clinical setting (Donhuijsen *et al*., 2007).[12] However, a few examples of the applications of this technique (IHC) can be illustrated below.

Drakos *et al.*, (2006)[7] in their study assessed for Mix1 homebox-like (MixL1) gene expression using immunohistochemical methods in 193 lymphoid tumours made up of B-cell Non-Hodgkin's lymphoma, T-cell Non-Hodgkin's lymphoma and Hodgkin's lymphoma. MixL1 was detected predominantly in the nuclear fraction of all cell lines tested and was mostly nuclear in primary tumor specimens. High MixL1 expressions was detected more frequently in Burkitt lymphoma and diffuse large B cell lymphoma compared with other types of B cell Non-Hodgkin's lymphoma.

In the search for simple, reproducible methodology for measurement of endothelial cell apoptosis and proliferation, Chalmers *et al.,* (2006)[8] developed a colorimetric dual-label immunohistochemical technique for use on formalin-fixed paraffin-embedded (FFPE) human tissue. This was based on the use of single stranded deoxyribonucleic acid (SSDNA) and Ki-67 as markers of endothelial cell apoptosis and proliferation, respectively. They validated the immunohistochemical measurement of endothelial cell apoptosis and proliferation on human colorectal cancer liver metastases from a randomised placebo-controlled trial of the selective cyclooxygenase-2 (Cox-2) inhibitor, Rofecoxib.

Another application could be illustrated by the study of Osborne *et al.,* (2007),[11] in which they used immunohistochemical methods to examine the frequency of cells immunolabelled for Tyrosine receptor kinase B (trkB) receptors in two populations of spinal motor neurons, the hormone-sensitive sexually dimorphic motor neurons of the spinal nucleus of the bulbocavernosus (SNB) and the non-dimorphic motor neurons innervating the muscles of the quadriceps femoris. In both, the highly androgen-sensitive SNB motor neurons innervating the quadriceps femoris muscles, the frequency of motor neurons intensely immunolabelled for trkB receptors was regulated by the presence of testosterone.

Bassotti *et al.,* (2007)[5] compared three immunohistochemical methods to assess enteric neuronal apoptosis in patients with slow transit constipation. Several colonic tissue sections were obtained in 10 patients with this condition, and these were evaluated using the formamide-MAb method (anti-single stranded DNA IHC), the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) method and the caspase-3 method. They observed that the highest yield of apoptotic neurons were obtained by the formamide-MAb method, while the lowest yield was observed with caspase-3. They, therefore, concluded that the formamide-MAb method, which is able to distinguish apoptosis from necrosis, and not influenced by DNA breaks, may prove useful to assess neuronal apoptotic phenomena in the human enteric nervous system, and so it represents a relevant method to detect enteric neuronal apoptosis.

POLYMERASE CHAIN REACTION (PCR)

This is a technique, whereby minute amounts of DNA can be replicated very quickly and so amplified that it makes DNA detection easier. It is a popular method used to study the genetic basis of disease in DNA (Bravo-Villalta *et al*., 2007).[13] The technique was discovered by the American chemist Kary Mullis in 1983 (for which he got a Nobel prize in 1993), and by the mid 1980s, it was used to diagnose sickle cell anaemia which is an autosomal recessive haemoglobinopathy. The technique later became widespread in disease diagnosis and was subsequently introduced into forensic medicine (www.roche.com, 2007;[14] Lorenz, 2012).[15]

Using PCR tiny amounts of DNA can be quickly copied over and over to produce enough quantity that can be easily detected. It made possible the determination of the order of bases in DNA (sequencing) and only a single molecule of DNA is required for this purpose (Zawaira *et al*., 2012).[16] This is the basis of the extremely high sensitivity of this technique, and subsequently paved the way for the introduction of genomics into modern medicine and enabled the completion of the human genome project, as well as targets for the development of gene tests and other areas of genetic research) (Drakos *et al*., 2007).[7] The only challenge to this technique came from the recently introduced and very sensitive DNA chips, but even with them, it is a pre-requisite to copy or amplify the DNA of interest before proceeding and so for this reason the two techniques usually go simultaneously (Bender *et al*., 2007).[6]

Basic principles of polymerase chain reaction

This is quite a simple chain reaction in which one DNA molecule is used to produce two copies of DNA which become sequentially and continuously doubled. This is accomplished by DNA polymerases which bring together individual nucleotides to form long molecular strands. These nucleotides are Adenine (A), thymine (T), cytosine (C) and guanine (G). Small fragments of DNA known as

primers which attach to the nucleotides are also required for the reaction, as well as longer DNA molecules which serve as templates for synthesis of the new strands. In the presence of these three ingredients, the enzymes will produce exact copies of the templates. A similar principle operates during cell division, as well as synthesis of mRNA by RNA polymerases (Drakos *et al*., 2007).[7]

Therefore, these enzymes can be used in the PCR to reproduce any nucleic acid of interest. But in the case of RNA, it is usually first transcribed into DNA with the help of reverse transcriptase- a method known as reverse transcription PCR (RT-PCR). For the copying procedure, only a small piece of the DNA section of interest needs to be known which will serve as the template for producing the primers that will initiate the reaction. It is therefore possible to clone DNA whose sequence is unknown, and is one of the main advantages of this procedure. In addition to that, PCR is fast, easy to carry out and can be done *in vitro*. Also, known sections of long DNA molecules like chromosomes can be used to further look into unknown areas and characterise them (Lorenz, 2012).[15]

The DNA polymerase functions optimally at the body temperature (37°C), but in PCR the two strands must be separated to permit the primers join to them. This is only achievable at 95°C which permanently destroys the polymerases. This problem was circumvented with the introduction of polymerase from the *Thermus aquaticus* organism, which is heat-stable and known as Taq polymerase. This organism lives in hot springs at a temperature of up to 100ºC. Subsequently, PCR has been modified in many ways and adapted to different situations for routine diagnostic testing and molecular research (Zawaira *et al*., 2012).[16]

Applications of polymerase chain reaction (PCR)

Polymerase chain reaction made it possible to prepare large amounts of DNA within a limited amount of time, and therefore enabled the unravelling of the genomes of many organisms including humans. The cloning of DNA has remained one of the main applications of PCR and is used in genome sequencing projects, gene research, investigation of genomic changes and the search for targets. It is also applied in the area of SNP (single nucleotide polymorphisms) genotyping, and these genes with SNPs are potential targets for new drug development further enhancing the role of PCR in the area of drug research and pharmaceuticals (Ishmael *et al*., 2008).[17]

Qualitative PCR

This refers to the use of PCR for detecting a specific DNA segment and makes use of the standard protocol. This is a very sensitive technique that is able to detect a single DNA molecule in a given sample. This is used in the identification of pathological changes in tissue specimens as well as individual identification in forensic medicine and paternity disputes. It is also employed in the detection of microbial pathogens and in this case PCR is overtaking immunological techniques for antibody detection in patient's blood scale a positive (Zawaira *et al*., 2012).[16]

This is because antibodies are not detectable until many weeks following an infection, but PCR is able to detect the microbial DNA or RNA faster (Ishmael *et al*., 2008).[17] In addition to that, antibodies can be present long after the infection and can lead to diagnostic confusion. As such, PCR is commonly used to detect micro-organisms in body fluids, foodstuff, or drinking water and has clinical application in the screening of donor blood for HIV and Hepatitis C infections. It has been found to be a quick and simple test and most importantly, cheap and affordable on a large scale (Zawaira *et al*., 2012).[16]

Quantitative PCR

This indicates not just whether a specific DNA segment is present in the sample, but even the amount present. This can be applied in diagnostic testing, target searches for drugs and even basic clinical and cancer research (Jozefczuk *et al*., 2011).[18] It is possible to calculate the amount of DNA originally present in a sample directly from the amount found at the end of a PCR run, but this depends on the differential conditions present before or after the procedure (Purcell *et al*., 2011).[19]

Real-time PCR

This technique allows the determination of the number of new DNA molecules formed in the reaction after each cycle, and affords continuous observation of the reaction which is in real time (Ishmael *et al*., 2008).[17] Therefore, such tests require conjugating the new DNA copies to a dye thus making it possible to determine the quantity of the template. Real-time PCR allows for the detection of PCR amplification in the exponential growth phase of the reaction and is much more quantitative than traditional RT-PCR {Reverse transcriptase PCR} (Mo *et al*., 2012).[20]

Target research

Quantitative PCR is used to help search for and evaluate targets, which are the sites in the body at which new drugs can act. This makes use of target validation where the gene products rather than the genes themselves are considered. Proteins provide only a limited amount of information concerning gene expression; therefore, quantitative PCR measures the mRNAs which are the working copies of the corresponding genes (Purcell *et al*., 2011).[19]

STEP

One way of determining the quantity and nature of working copies of genes in different samples is by single target expression profiling (STEP). Here, attention is focused on a specific gene (target), for which an expression profile is prepared by measuring its expression in various tissues of

healthy and/or ill persons. When the results are entered into a diagram, the active or inactive areas of the gene in question can be readily visualised in the body. This is called the body map, and can reveal whether a target is really associated with disease or not. Furthermore, this method can also be used in the development of model systems and in basic research (Duggan *et al*., 1999).[21]

MAMMALIAN CELL CULTURES

This was pioneered by Margaret Murray, Arthur Purdy Stout and Luciano Ozzello at Columbia-Presbyterian hospital in New York, and enabled them to obtain histogenetic clues from the examination of primary cultures of human tumours such as thymoma, synovial sarcoma and haemangiopericytoma (Rosai, 2004).[4] Cell culture has diagnostic applications in human tumours because tumour cells can express features of differentiation outside the body which are not observed or displayed in the body (Nakajima *et al*., 2007).[22] A common example is seen in Neuroblastoma which grows neuritis within 24 hours of placement in a suitable culture medium. Also, amelanotic melanoma has been noticed to occasionally become deeply pigmented outside the body. Sometimes the maturation and differentiation of the cells is enhanced in the culture system by addition of exogenous substances such as cyclic adenosine monophosphate (AMP), AMP or 12-O-tetradecanoylphorbol-13-acetate (TPA) (Rosai, 2004).[4]

This can be exemplified by Ewing's sarcoma cells which differentiate upon addition of AMP or TPA. In view of this, the use of short-term tissue culture has been suggested recently in arriving at a differential diagnosis of small round cell tumor of infancy including Ewing's sarcoma and other childhood tumours (Rosai, 2004).[4] It is important to note that cells grown in culture can be studied with any of the modern diagnostic techniques such as immunohistochemistry, electron microscopy, and ultra-structural immunohistochemistry (Chu *et al*., 2004).[23] In spite of its great utility in medicine, at the practical, diagnostic level cell culture has limitations that make it difficult to become a routine diagnostic procedure in surgical pathology. In many areas, including the small round cell tumors of infancy, it has been overtaken by molecular techniques, though some pathologists still occasionally resort to this technique for some selected tumours (Waite *et al*., 2005).[24]

Advantages of cell culture techniques

The main advantage of using mammalian cell culture in research and studies is in replacing animal experiments. Efforts are being made to reduce the number of laboratory animals used in experiments as far as possible, not just for ethical reasons, but also for valid scientific reasons so as to reduce animal suffering (www.roche.com, 2007).[14] Hence the principle of the three Rs: Replace (replace animal experiments as far as possible by pain-insensitive systems, or compensate by using methods that do not involve laboratory animals), Reduce (develop methods so that fewer laboratory animals need to be used), and Refine (improve methods so that animals undergo less discomfort during the experiments). Also, in cell culture techniques, the use of anaesthesia is not necessary as opposed to the animal experiments which require some form of restraints to enable the study to be easily carried out (Van Gele *et al*., 2001).[25]

Furthermore, genetic modification of the cells is much easier and more likely to succeed in cell cultures than in live animals, where the modified cell may fail to survive due to immune rejection in the whole animal or may fail to produce an appreciable response at the cellular or molecular level, which may be more easily discernible on cultured cells (Flores-Delgado *et al*., 2007).[26] In addition to that, some people prefer to carry out toxicology studies on cell cultures as opposed to whole animals mostly for compassionate reasons and hoping to avoid long term effects of the toxic substances being tested which can maim, incapacitate or even kill the animal. Again, it may be argued that it is better to do genetic studies on cell cultures rather than live animals for similar compassionate reasons, because genetic modification may interfere with the immune mechanism of the animal and reduce its chances of survival even if it doesn't die immediately.

Because of the ethical considerations involved in procuring and using human tissue for laboratory studies, many researchers fall back to cell cultures which are easier to procure, cheaper and have a great advantage because they lack the medico-legal implications relating to the use of human tissues. Cell cultures are particularly important in comparative experiments for screening substances with similar sites and mechanisms of action, and can provide specific understanding which may be missed due to the complexity of the metabolic reactions in a complete organism. This is why cell cultures are commonly used in blood disorders and reproduction toxicology (Stambolic *et al*., 2001).[27]

Disadvantages of cell culture techniques

Studies on simple systems such as mammalian cells or isolated tissue samples can provide beneficial information on questions regarding toxicology or drug trials. But since cell cultures are merely accumulations of similar cells in a nutrient solution of culture medium, they only show limited metabolism (Sharrard *et al*., 2007).[28] Quantitative data on the toxic dose for the whole body are difficult to derive from such studies, and it is impossible to determine the target organs of the active drug or the clinical symptoms, as well as profile or time of a harmful effect (Chu *et al*., 2004).[23] In addition, it is difficult to simulate the effects resulting from the interactions of

various organ systems or tissues in the whole body using cell cultures. In the same vein, pain, narcotic effects, blood pressure, fever, swellings or intestinal movements cannot be accurately predicted from the cell culture studies and then extrapolated to the whole organism (human). Finally, it is a fact that physiological responses to drugs and chemicals may be modified by age, sex and total genetic make-up of the humans, which cannot be seen in cell culture studies. This can easily be understood by the differential metabolism of drugs and chemicals by different age groups of people (Delves *et al*., 2007).[29]

Genetically modified cells (GM Cells)

These cells are used to produce substances that are identical to their human equivalents, such as antibodies or insulin. This is made possible by the insertion of the appropriate human gene into the cultured cells. The resultant genetically manipulated cells, therefore, possess the enzymes required for correct folding and processing of the proteins as well as genetic instructions for the synthesis of the desired product (Xue *et al*., 2013).[30] The responsible gene is then placed under the control of a super-active DNA signal element. This results in the production of GM cells which produce large quantities of the desired product in its active form. In addition to that, the cells can be used to carry out studies or research into the genetic basis of disease as well as target search for novel drug development (Singh *et al*., 2013).[31]

But multiplying these mammalian cells is usually a difficult task, because they are living organisms and so are very sensitive to even minute changes in their environment. This relates to their nutrition, temperature, pressure and chemical gradient around the cells which can adversely affect them if not properly regulated and optimised. These factors decide the cell's lifespan as well as the yield and structure of the desired and undesired products (Ahmed *et al*., 2013).[32]

Cell lines are commonly used in research and biopharmaceuticals production because they are amenable to standardisation, and this allows global reproducibility of the results and flexibility in research. Biotechnology researchers usually insert structural and control genes into the cells so as to modify their properties and produce the desired effects necessary for genetic research (Fu *et al*., 2013).[33] These cells are then allowed to reproduce and safely stored at low temperatures in master cell banks. If the cells need to be stored for prolonged periods of time, they can be kept almost indefinitely in liquid nitrogen at –196°C. Cells can then be drawn from the banks as and when due (Zhou *et al*., 2013).[34]

Monoclonal antibodies (MAbs)

To enable the production of monoclonal antibodies in large amounts, and with a high degree of purity, short-lived antibody producing cells from the mouse spleen are used with immortal continuously dividing tumor cells to form hybridomas (www.roche.com, 2007).[14] These genetically uniform cell lines can be kept and propagated in cell cultures, each cell line producing a single type of monoclonal antibody, and this had led to a renewed interest in the use of monoclonal antibodies in biomedical research (Fujita, 2013).[1] The uniform structure and composition of these antibodies (MAbs) makes them suitable for research, diagnostic and therapeutic purposes. Humanised monoclonal antibodies can be used as highly effective drugs in the treatment of cancer (Foltz *et al*., 2013).[2]

CONCLUSION

Biomedical techniques have been very important in translational studies, and have increasingly found more relevance and clinical applications in the field of cancer, genomics, rheumatology, immunology, diagnostics, as well as toxicology studies. More techniques are constantly being evolved and tried, but there are problems of cost, side effects, as well as strict regulations which need to be carefully observed in the clinical application of some of these techniques, especially genetic engineering. This will ensure that these novel techniques do not pose any hazards, or at least minimise these hazards to patients and the general public.

REFERENCES

1. Fujita-Yamaguchi Y. Renewed interest in basic and applied research involving monoclonal antibodies against an oncofetal Tn-antigen. J Biochem 2013.
2. Foltz IN, Karow M, Wasserman SM. Evolution and emergence of therapeutic monoclonal antibodies: What cardiologists need to know. Circulation 2013;127:2222-30.
3. Frauches TS, Petretski JH, Arnholdt AC, Lasunskaia EB, de Carvalho EC, Kipnis TL, *et al*. Bothropic antivenom based on monoclonal antibodies, is it possible? Toxicon 2013;71:49-56.
4. Rosai J. Ackerman's surgical pathology. 9th ed. Amsterdam: Elsevier Health Sciences; 2004. p. 37-50.
5. Bassotti G, Villanacci V, Fisogni S, Cadei M, Galletti A, Morelli A, *et al*. Comparison of three methods to assess enteric neuronal apoptosis in patients with slow transit constipation. Apoptosis 2007;12:329-32.
6. Bender FL, Fischer M, Funk N, Orel N, Rethwilm A, Sendtner M. High-efficiency gene transfer into cultured embryonic motoneurons using recombinant lentiviruses. Histochem Cell Biol 2007;127:439-48.
7. Drakos E, Rassidakis GZ, Leventaki V, Guo W, Medeiros LJ, Nagarajan L. Differential expression of the human MIXL1 gene product in non-Hodgkin and Hodgkin lymphomas. Hum Pathol 2007;38:500-7.
8. Chalmers CR, Fenwick SW, Toogood GJ, Hull MA. Immunohistochemical measurement of endothelial cell apoptosis and proliferation in formalin-fixed, paraffin-embedded human cancer tissue. Angiogenesis 2006;9:193-200.
9. Torres EM, Meldrum A, Kirik D, Dunnett SB. An investigation of the problem of two-layered immunohistochemical staining in paraformaldehyde fixed sections. J Neurosci Methods 2006;158:64-74.
10. Taylor CR, Levenson RM. Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment II. Histopathology 2006;49:411-24.

11. Osborne MC, Verhovshek T, Sengelaub DR. Androgen regulates trkB immunolabeling in spinal motoneurons. J Neurosci Res 2007;85:303-9.
12. Donhuijsen K, Hannig H, Schroeder HG, Korinth D, Petersen I. Synchronous adenocarcinomas of intestinal type of the inner nose and the colon. Hum Pathol 2007;38:373-7.
13. Bravo-Villalta HV, Yamamoto K, Nakamura K, Bayá A, Horiuchi R. A novel intronic mutation that may affect genotyping result of CYP2C8 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is strongly associated with CYP2C8*3 in a South American population. J Hum Genet 2007;52:195-9.
14. Available from: http://www.roche.com; 2007 [Last accessed on 2013 November 16].
15. Lorenz TC. Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. J Vis Exp 2012;e3998.
16. Zawaira A, Pooran A, Barichievy S, Chopera DA. Discussion of molecular biology methods for protein engineering. Mol Biotechnol 2012;51:67-102.
17. Ishmael FT, Stellato C. Principles and applications of polymerase chain reaction: Basic science for the practicing physician. Ann Allergy Asthma Immunol 2008;101:437-43.
18. Jozefczuk J, Adjaye J. Quantitative real-time PCR-based analysis of gene expression. Methods Enzymol 2011;500:99-109.
19. Purcell MK, Getchell RG, McClure CA, Garver KA. Quantitative polymerase chain reaction (PCR) for detection of aquatic animal pathogens in a diagnostic laboratory setting. J Aquat Anim Health 2011;23:148-61.
20. Mo Y, Wan R, Zhang Q. Application of reverse transcription-PCR and real-time PCR in nanotoxicity research. Methods Mol Biol 2012;926:99-112.
21. Duggan DJ, Bittner M, Chen Y, Meltzer P, Trent JM. Expression profiling using cDNA microarrays. Nat Genet 1999;21(Suppl 1):10-4.
22. Nakajima H, Sakaguchi K, Fujiwara I, Mizuta M, Tsuruga M, Magae J, *et al*. Apoptosis and inactivation of the PI3-kinase pathway by tetrocarcin A in breast cancers. Biochem Biophys Res Commun 2007;356:260-5.
23. Chu EC, Chai J, Tarnawski AS. NSAIDs activate PTEN and other phosphatases in human colon cancer cells: Novel mechanism for chemopreventive action of NSAIDs. Biochem Biophys Res Commun 2004;320:875-9.
24. Waite KA, Sinden MR, Eng C. Phytoestrogen exposure elevates PTEN levels. Hum Mol Genet 2005;14:1457-63.
25. Van Gele M, Leonard JH, Van Roy N, Cook AL, De Paepe A, Speleman F. Frequent allelic loss at 10q23 but low incidence of PTEN mutations in Merkel cell carcinoma. Int J Cancer 2001;92:409-13.
26. Flores-Delgado G, Liu CW, Sposto R, Berndt N. A limited screen for protein interactions reveals new roles for protein phosphatase 1 in cell cycle control and apoptosis. J Proteome Res 2007;6:1165-75.
27. Stambolic V, Macpherson D, Sas D, Lin Y, Snow B, Jang Y, *et al*. Regulation of PTEN transcription by p53. Mol Cell 2001;8:317-25.
28. Sharrard RM, Maitland NJ. Regulation of protein kinase B activity by PTEN and SHIP 2 in human prostate-derived cell lines. Cell Signal 2007;19:129-38.
29. Delves PJ, Martin SJ, Burton DR, Roitt IM. Roitt's Essential Immunology. 11th ed. Oxford: Blackwell publishing; 2006.
30. Xue X, Shah YM. In vitro organoid culture of primary mouse colon tumours. J Vis Exp 2013.
31. Singh H, Figliola MJ, Dawson MJ, Olivares S, Zhang L, Yang G, *et al*. Manufacture of clinical-grade CD19-specific T Cells stably expressing chimeric antigen receptor using sleeping beauty system and artificial antigen presenting cells. PLoS One 2013;8:e64138.
32. Ahmed FE, Ahmed NC, Vos PW, Bonnerup C, Atkins JN, Casey M, *et al*. Diagnostic MicroRNA markers to screen for sporadic human colon cancer in stool: I. Proof of principle. Cancer Genomics Proteomics 2013;10:93-113.
33. Fu X, Rivera A, Tao L, Zhang X. Genetically modified T cells targeting neovasculature efficiently destroy tumor blood vessels, shrink established solid tumors, and increase nanoparticle delivery. Int J Cancer 2013.
34. Zhou X, Li J, Wang Z, Chen Z, Qiu J, Zhang Y, *et al*. Cellular immunotherapy for carcinoma using genetically modified EGFR-specific T-lymphocytes. Neoplasia 2013;15:544-53.

How to cite this article: Abdulkareem IH. Biomedical techniques in translational studies: The journey so far. Niger Med J 2014;55:99-105.

Source of Support: Nil. **Conflict of Interest:** None declared.