International Journal for Pharmaceutical Research Scholars (IJPRS)



V-1, I-2, 2012

ISSN No: 2277-7873

REVIEW ARTICLE

Recent Developments in Diagnostic Immunology – A Comprehensive Review on Future Avenues Rao SH^{*1}, Gupta AS¹, Bidwai AN¹, Patil TS¹, Godbole RV¹

¹Department of Pharmaceutics, AISSMS College of Pharmacy, Pune, Maharashtra, India. Manuscript No: IJPRS/V1/I2/00102, Received On: 23/05/2012, Accepted On: 11/06/2012

ABSTRACT

Diagnostic Immunology is a collective term for a variety of diagnostic techniques that rely on the specificity of the bond between antibodies and antigens. There are many different tests and techniques which are used for diagnosis of different diseases. These advanced techniques help in the early detection of various chronic diseases such as cancer etc. and help in the cure of the disease immediately. These tests are serological tests and are mainly categorized into two parts: direct and indirect. There are 6 techniques which are used on a larger scale for the use of diagnostic immunology, ELISA being one of them. Diagnostic Immunology has its own advantages and disadvantages. Advantages being more, it is advisable to use these advanced techniques rather than the traditional ones for better diagnosis, because it helps in early detection of chronic diseases. There are many options of tests to choose from, the tests can be chosen suitable to our convenience, urgency, cost effectiveness, accuracy etc. All these tests are affordable and easily available everywhere. Many tests can be undergone easily even at primary levels because of the portability of the devices. As many of these tests are automated, computerized, they provide accurate and immediate results. This helps in better diagnosis again.

KEYWORDS

Diagnostic, Immunology, Antigen, Antibodies, ELISA, Cancer, Devices.

INTRODUCTION

Diagnostic immunology is a collective term for a variety of diagnostic techniques that rely on the specificity of the bond between antibodies and antigens. It is well suited for the detection of even the smallest of amounts of Bio-Chemical substances. Antibodies specific for a desired antigen can be conjugated with a radiolabel, fluorescent label, or color forming enzyme and are used as a "probe" to detect it.¹ It uses serological tests. These tests are mainly categorized in two following types:

Direct Test: Detects antigens from the patients' sample.

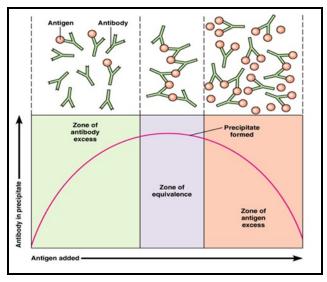
*Address for Correspondence: Shivani H Rao. Department of Pharmaceutics, AISSMS COP, Kennedy Road Near R.T.O., Pune-01, India. E-Mail Id: addi1091@gmail.com **Indirect Test:** Detects antibodies in the patients' sample.²

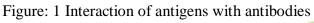
DIFFERENT TECHNIQUES USED

- 1) Precipitation Reactions
- 2) Agglutination Reactions
- 3) Complement Fixation Reaction
- 4) ELISA
- 5) Fluorescent antibody
- 6) Western blot.

1) **Precipitation Reactions**

The interaction of soluble antigens with IgG or IgM antibodies leads to precipitation reactions. Precipitation reactions depend on the formation of lattices and occur best when antigen and antibody are present in optimal proportions. Excesses of either component decrease lattice formation and subsequent precipitation.³





The precipitating ring test is performed in a small tube.

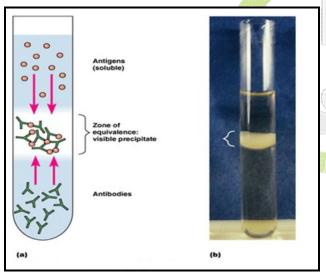


Figure: 2 Ring test

Immunodiffusion procedures are precipitation reactions carried out in an agar gel medium. Antibody and antigen are loaded in different wells and diffuse through the medium. When the optimal antigen-antibody ratio is reached a visible band appears in the gel. Immunoelectrophoresis combines electrophoresis with immunodiffusion for the analysis of serum proteins⁴

2) Agglutination Reactions

The interaction of particulate antigens (cells that carry antigens) with antibodies leads to agglutination reactions.⁵

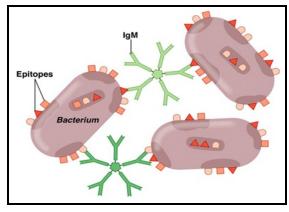


Figure: 3 Agglutination reactions

Diseases may be diagnosed by combining the patient's serum with a known antigen. Diseases can be diagnosed by a rising titer (antibody concentration in serum) or seroconversion (from no antibodies to the presence of antibodies).⁶

Direct agglutination

Direct agglutination reactions test patient serum against large, cellular antigens to screen for the presence of antibodies.⁷ Direct agglutination reactions can be used to determine antibody titer.

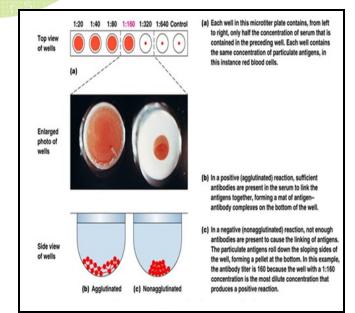


Figure: 4 Direct agglutination

Indirect agglutination

To test patients' serum for the presence of antibodies against soluble antigens, serum is mixed with latex spheres with the soluble antigens attached.⁸ Antibodies will then cause visible agglutination of the latex spheres with the soluble antigens attached.

Alternatively, antibodies may be attached to the latex spheres to test for the presence of soluble antigens in patient serum.⁹

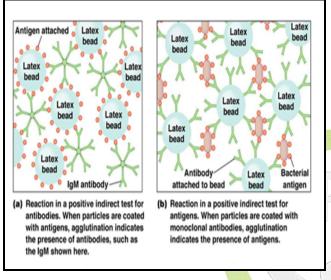


Figure: 5 Indirect agglutination

Hemagglutination reactions involve agglutination reactions using red blood cells. Hemagglutination reactions are used in blood typing, the diagnosis of certain diseases, and the identification of viruses. Viral hemagglutination occurs when spikes on the virus cause agglutination of red blood cells - there is no antigen-antibody interaction.¹⁰

3) Complement Fixation Reaction

Complement-fixation reactions are serological tests based on the depletion of a fixed amount of complement in the presence of an antigenantibody reaction.¹¹ Good for detecting very small amounts of antibody, when the amount of antibody is too low to cause a precipitation or agglutination reaction. Complement fixation was once the basis of the Wasserman test, a test to diagnose syphilis. It is still used to diagnose some viral, fungal, and rickettsial diseases. There are two steps, the complement fixation step and the indicator step.¹²

Complement Fixation Step

Add antigen and complement to serum. If the serum contains antibodies against the antigen they will bind to the antigen and fix the complement. This ties up all the free complement so it can't participate in the next step, the indicator step.

Indicator Step

Add sheep red blood cells and anti-sheep red blood cell antibodies to the serum. Antibodies to the sheep red blood cells bind and can fix complement, if any is available. If complement is available it will be fixed by the sheep red blood cell antigen-antibody complex and the sheep red blood cells will be lysed. This indicates that the serum did not contain antibodies against the antigen added in the complement fixation step and complement remains free.¹³

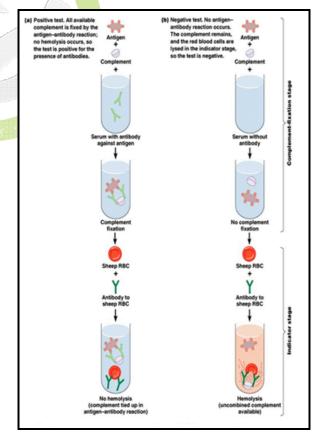


Figure: 6 Complement fixation reaction

If no complement is available the sheep red blood cells will not be lysed. This indicates there were antibodies against the antigen added in the complement fixation step and all the complement was tied up when it was fixed by the original antigen-antibody complex.¹⁴

4) ELISA

ELISA technique uses antibodies linked to an enzyme, such as horseradish peroxidase or alkaline phosphatase. Antigen – antibody reactions are detected by enzyme activity. An antibody linked to the indicator enzyme is added to the test well and is bound in the well if the antigen it is specific for is present. To determine whether or not the enzyme-linked antibody is bound in the well, substrate for the enzyme is added. If the enzyme linked antibody is present, the substrate is converted to a product that causes a color change.¹⁵

Direct

The direct ELISA is used to detect specific antigens bound in a test well. Say you want to know if a patient's serum contains a specific antigen. First coat the bottom of the test well with an antibody against that antigen. Then add patients' serum. If the antigen you're looking for is present in the patient's serum it will stick to the antibody that we coated the bottom of the well with. So let the serum sit in the well for a few minutes to give the antigen a chance to stick and then wash the excess serum away.¹⁶ Next you add your enzyme-linked antibody, which is also specific for the antigen - it is the same antibody that you coated the bottom of the well with except you linked and enzyme to it. If the antigen is present you'll end up with a sort of antibody-antigen-enzyme-linked antibody sandwich. We have to wash again to get rid of any enzyme-linked antibody that didn't bind to antigen.

Now add substrate. If there is any enzymelinked antibody present (and it should only be there if it is bound to antigen) a product will be formed that causes the color change.¹⁷

Indirect

The indirect ELISA is used to detect antibodies against an antigen bound in a test well. In this case we want to know if a patients' serum contains an antibody against a specific antigen (the opposite situation from the one in the direct ELISA test)¹⁸. First coat the bottom of the well with the antigen that the antibody would be specific for. Next add patient serum and allow it to sit in the well for a few minutes to allow any antibody a chance to bind to the antigen. Now we have to wash away the serum and any unbound antibodies. Add enzyme-linked antibody. This time the enzyme-linked antibody is specific for human immunoglobulins (it's an anti-human antibody). Let it sit for a few minutes and wash the excess away. If the patient's serum had antibodies against the antigen coated the well with you'll end up with an antigen-antibody-enzyme-linked antiantibody antibody sandwich. Now add substrate and look for the color change just like in the direct/ELISA.19

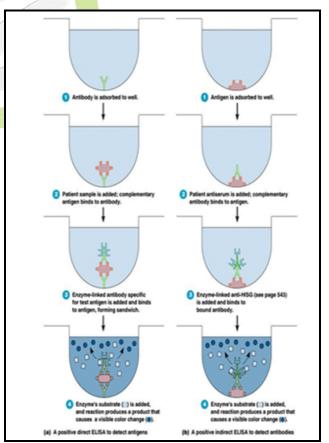


Figure: 7 Elisa testing

ELISA kits are available for both clinical diagnostics and home use. These tests are used for everything from screening blood for anti-HIV antibodies to home pregnancy tests. We can use fluorescent dyes instead of enzymes on our test antibodies but we will need a fluorescence microscope or scanner to read the results. Fluorescence is much easier and more sensitive for lab work but not practical for use outside of a well equipped lab.²⁰

5) Fluorescent antibody

Fluorescent-antibody techniques use antibodies labeled with fluorescent dyes.

Direct fluorescent-antibody tests are used to identify specific microorganisms.²¹

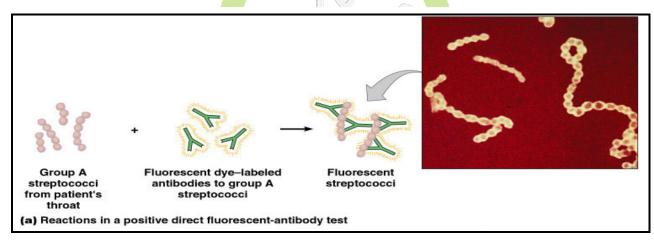
Indirect fluorescent-antibody tests are used to demonstrate the presence of antibody in serum. A fluorescence-activated cell sorter can be used to detect and count cells labeled with fluorescent antibodies.

Method

A mixture of cells is treated to label cells that have certain antigens with fluorescent antibody markers. Cell mixture leaves nozzle in droplets. Laser beam strikes each droplet. Fluorescence detector identifies fluorescent cells by fluorescent light emitted by cell. Electrode gives positive charge to identified cells. As cells drop between positively charged plates, the cells with a positive charge move closer to the negative plate. The separated cells fall into different collection tubes.²²

6) Western blot

The western blot (sometimes called the protein immunoblot) is a widely used analytical technique used to detect specific proteins in the given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide.



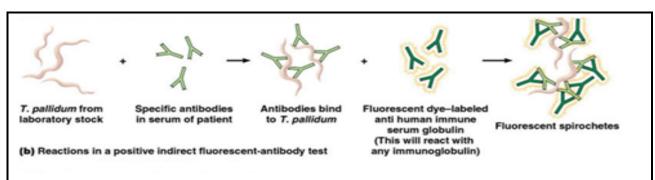


Figure: 8 Direct fluorescent

Figure: 9 Indirect fluorescent

The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein.²³

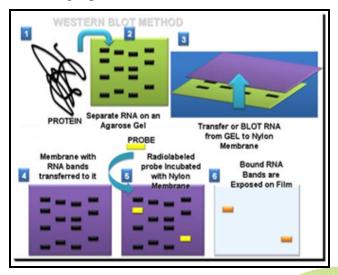
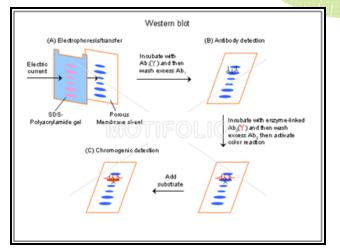
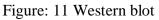


Figure: 10 Western blot test

There are now many reagent companies that providing specialize in antibodies (both monoclonal and polyclonal antibodies) against tens of thousands of different proteins. Commercial antibodies can be expensive, although the unbound antibody can be reused between experiments. This method is fields of molecular used in the biology, biochemistry, immunogenetics and





other molecular biology disciplines.²⁴ Other related techniques include using antibodies to detect proteins in tissues and cells by immunostaining and enzyme-linked immunosorbent assay (ELISA). The method originated in the laboratory of George Stark at Stanford. The name Western blot was given to the technique by W. Neal Burnette and Sushant Bhat and is a play on the name Southern blot. technique a for DNA detection developed earlier by Edwin Southern. Detection of RNA is termed Northern blot.²⁵

Advanced Applications of Diagnostic Immunology

Diagnostic immunology is used in the detection of various chronic diseases using various kinds of suitable tests. These tests are usually done on blood and serum samples of the patients. These samples are checked for particular antigens using the different kinds of antibodies. This has helped us in the detection of cancerous cells in the body of a patient, which can help us to detect various diseases like- cancer, genetic disorders which need to be detected in their early stages for better cure. Advanced research and study of immunology has proved this possible. Now we will see some tests which are useful to detect such diseases.²⁶

CANCER

Cancer is the uncontrolled growth of abnormal cells in the body. Cancerous cells are also called malignant cells.

Causes, incidence, and risk factors

Cells are the building blocks of living things. Cancer grows out of normal cells in the body. Normal cells multiply when the body needs them, and die when the body doesn't need them. Cancer appears to occur when the growth of cells in the body is out of control and cells divide too quickly. It can also occur when cells forget how to die. There are many different kinds of cancers. Cancer can develop in almost any organ or tissue, such as the lung, colon, breast, skin, bones, or nerve tissue. However, the cause of many cancers remains unknown. The most common cause of cancer-related death is lung cancer. These following cancers are mostly seen²⁷

In women these three most common cancers are seen in the world $^{\rm 28}$

- Breast cancer
- Colon cancer
- Lung cancer

Some other types of cancers include:

- Brain cancer
- Cervical cancer
- Hodgkin's lymphoma
- Kidney cancer
- Leukemia
- Liver cancer
- Non-Hodgkin's lymphoma
- Ovarian cancer
- Skin cancer
- Testicular cancer
- Thyroid cancer
- Uterine cancer
- Tongue cancer

The signs of cancer vary based on the type and location of the tumor.

Common tests include the following²⁹

- Biopsy of the tumor
- Blood tests (which look for chemicals such as tumor markers)
- Bone marrow biopsy (for lymphoma or leukemia)
- Chest x-ray
- Complete blood count (CBC)
- CT scan
- MRI scan

Most cancers are diagnosed by biopsy. Depending on the location of the tumor, the biopsy may be a simple procedure or a serious operation. Most patients with cancer have CT

scans to determine the exact location and size of the tumor or tumors. A cancer diagnosis is difficult to cope with. It is important, however, that you discuss the type, size, and location of the cancer with your doctor when you are diagnosed. You also will want to ask about treatment options, along with their benefits and risks. These tests are commonly used to detect cancer. But these are useful in detection only in the later stages of the disease where it is hard to cure it. The new advanced study of diagnostic immunology helps in such situations for early detection of cancer. So we are listing here the tests that are considered alternative or less toxic than the above written standard conventional tests. Some of these tests are not used by conventional physicians. So we need to spread awareness about these advanced technologies for betterment of the treatment.³⁰

The following tests are very advanced ones used to detect cancer.

1) Methods using immunology

In these types of tests immunological substances are used to detect the various types of cancer such as antibodies, blood sample with the help of these we can detect the cancer.

Examples

In these methods various types of tests are present as following

- 1) AMAS-Anti-malignan antibody screen test
- 2) Cancer Marker Tests
- 3) CBC Blood Test
- 4) DR-70
- 5) Lymphocytes Size Analysis
- 6) T/Tn Antigen Test³¹

1) AMAS-Anti-malignan antibody screen test

This is very extremely important test for detection of cancer. Cancer cell (including breast cancer) trigger our body to produce a chemical (peptide) called malignan. When our body detects malignan, our immune system goes on the attack by producing antimalignan antibodies. A simple blood test, looking for the presence of the antimalignan antibody is a sound and logical first step in detection of cancer.³² No antimalignan antibody present means no malignan, means no cancer. This test is extremely sensitive. Blood levels of this antibody, rise early in the course of the vast majority of cancers of all types, regardless of location in the body. The test is especially useful when cancer is suspected but has not been confirmed by biopsy.³³

Principle of the test

This blood test measures an antibody in the blood called antimalignan. Antimalignan antibody levels are higher in people with early stage cancer. In this blood test, levels of the antimalignan antibodies are checked. Antimalignan antibodies in serum (AMAS) is a naturally occurring antibody present in the serum of all people even children. AMAS is our natural immune system against cancer.³⁴

Advantages of the test

This AMAS test is over 95% reliable in the first test and over 99% reliable when a repeat test is performed. This test is valid for all types of cancer. This test is also useful for monitoring the treatment of malignancies. As compared to marker test, this test has an excellent way of screening for the detection of cancer and recoveries. It is an inexpensive test and hence is an affordable option for patients. It is effective in finding cancer cells in the early stages of the disease.³⁵

2) Cancer marker test

These are immunological methods - cancer markers that are produced as cancer grows and are detectable even before it reaches a size big enough for detection by other methods. This early detection system is vital for early medical intervention that significantly improves the chances of recovery.³⁶

These markers include the following

Alpha fetoprotein (AFP) levels are often elevated in liver cancers (hepatocellular) and testicular cancers (non-seminomatous). Raised levels are also present during pregnancy or some gastrointestinal cancers.

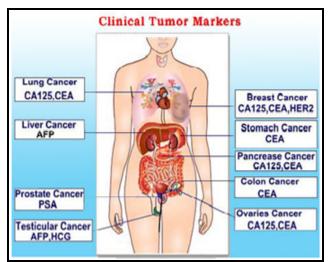


Figure: 12 Cancer marker test

CA 15.3 values are often elevated in patients with breast cancers. When there is a history of cancer among family members, patients may be advised to also do a breast mammogram. Besides breast cancer, other non-malignant conditions (eg. cirrhosis, benign diseases of ovaries & breast) have also been known to cause elevated CA 15.3 levels.³⁷ A number of tumour markers are currently being used for a wide range of cancer types. Tumor markers that are currently in common use included in table 1.

CA 19.9 - for gastric/pancreatic or stomach cancer - It is a diagnostic tool for those with stomach trouble such as symptoms of gastritis, abdominal pains, or gas. Its level is best evaluated along with CEA marker test.³⁸ CA125 has become a widely used tumor marker which is measured most often in women with cancers of the reproductive system including the uterus, fallopian tubes and ovaries. Other cancers that may cause abnormal CA125 levels include cancer of the pancreas, lungs, breast and colon. However, CA125/CA125-II can be elevated during menstruation, pregnancy or in individuals with ovarian cysts, pericarditis, hepatitis, cirrhosis of the liver or peritonitis, an infection of the lining of the abdomen, and even in 1-2% of healthy individuals. Once a cancer is diagnosed, CA125/CA125-II levels may prove to be an effective indicator of the effectiveness

of cancer treatment. A declining CA125/CA125-II value may indicate a good response to treatment and a favorable prognosis. Persistently rising CA125/CA125-II levels may be associated with a growing tumor, presence of tumor on the peritoneum that lines the abdomen or a recurrence of a previously treated tumor. Additional evaluation is necessary to make such determinations. CA 125-II, an improved version of the original CA 125 assay, is now commercially available.³⁹

Carcinoembryonic antigen (CEA) is a cancer marker to screen for colorectal cancer - it is associated with digestive tract cancers (eg of the colon) as well as other malignant and nonmalignant disorders. It is recommended for those with frequent constipation, diarrhea, or bleeding piles for an initial diagnostic tool. It can also help detect Medullary thyroid cancer (MTC).

Tumor Marker	Cancer Type	Tissue Analyzed	How it is detected
ALK gene rearrangements	Non-small cell lung cancer; anaplastic large cell lymphoma	Tumor tissue	With the help of Fluorescence In Situ Hybridization.
Alpha-fetoprotein (AFP)	Liver cancer; germ cell tumors	Blood	With the help of monoclonal antibody.
Beta-2-microglobulin (B2M)	Multiple myeloma; chronic lymphocytic leukemia; some lymphomas	Blood, urine, or cerebrospinal fluid	Western Blot Technique.
Beta-human chorionic gonadotropin (Beta-hCG)	<u>Choriocarcinoma;</u> testicular cancer	Urine or blood	Flurimetric Immuno Assay.
BCR-ABL	Chronic myeloid leukemia	Blood and/or bone marrow	Fluorescence In Situ Hybridization, Real Time PCR.
BRAF mutation V600E	Cutaneous melanoma; colorectal cancer	Tumor tissue	Polymerase Chain Reaction (PCR).
CA15-3/CA27.29	Breast cancer	Blood	ELISA, Monoclonal antibody.
CA19-9	Pancreatic cancer; gallbladder cancer; bile duct cancer; gastric cancer	Blood	Monoclonal Antibody.
CA-125	Ovarian cancer	Blood	Monoclonal Antibody.
Calcitonin	Medullary thyroid cancer	Blood	Monoclonal Antibody.
Carcinoembryonic antigen (CEA)	Colorectal cancer; breast cancer	Blood	Monoclonal Antibody Arcitumo mAb.
CD20	Non-Hodgkin lymphoma	Blood	Monoclonal Antibody, mAb RituximAb.
Chromogranin A (CgA)	Neuroendocrine tumors	Blood	Two-side Sandwitch Immunodiametric Assay Involving Monoclonal Antibodies.

Table 1: various tumor markers currently in use

EVP - Cancer marker to screen for nasopharyngeal cancer. Epstein Bar virus (EBV) has been shown to have a direct relationship with NPC where it can be detected in NPC tumors and patients with NPC tend to have higher titres of EBV specific antibodies than the general population.⁴⁰

3) CBC Blood test

This is nothing but the Complete Blood Test also called as Full Blood Test.

A variety of blood tests are used to check the levels of substances in the blood that indicate how healthy the body is and whether infection is present. For example, blood tests revealing elevated levels of waste products, such as creatinine or blood urea nitrogen (BUN), indicate that the kidneys are not working efficiently to filter those substances out. Other tests check the presence of electrolytes – chemical compounds such as sodium and potassium that are critical to the body's healthy functioning. Coagulation studies determine how quickly the blood clots.

CBC Blood test lists the amounts detected of about 44 substances normally found in the blood (and compares the blood status with known indicators of diseases.⁴¹

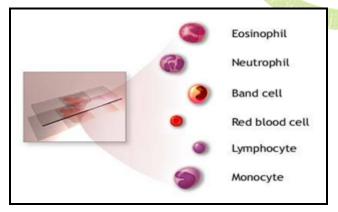


Figure: 13 Blood cells

A complete blood count (CBC) measures the size, number, and maturity of the different blood cells in a specific volume of blood. This is one of the most common tests performed. Red blood cells are important for carrying oxygen and fighting anemia and fatigue; the haemoglobin portion of the CBC measures the oxygen carrying capacity of the red blood cells while the haemocrit measures the percentage of red blood cells in the blood. White blood cells fight infection. Increased number of white blood cells, therefore, may indicate the presence of an infection. Platelets prevent the body from being bruised and bleeding easily. This helps us in the detection of presence of cancerous cells if any in our blood.⁴²

4) DR-70

DR-70 is a simple blood test that screens for 13 different cancers at the same time. It is highly specific and catches cancer long before suspecting anything was amiss.

Cancers that can be detected by the test are of the following types:

- -lung cancer,
- -colon cancer,
- -breast cancer,
- -stomach cancer,
- –liver cancer,
- -rectum cancer,
- -ovary cancer,
- -cervix cancer,
- -esophagus cancer,
- -thyroid cancer, and pancreas cancer, and trophoblast and malignant lymphoma.⁴³

The Cancer test known as DR-70 test is an exciting new development that allows us to diagnose and monitor cancer. This test gives us the ability to:

1. Diagnose early cancer development – that is the ability to diagnose cancer in those with little or no symptoms or signs;

2. Confirm the diagnosis of cancer in those with uncertain results of conventional tests such as blood tests or X-rays;

3. Monitor the effect of treatment, including conventional treatment such as surgery,

chemotherapy or radiotherapy, or complementary and alternative treatments such as nutritional approaches or herbal medicine; and

4. Detect early recurrence in those who have had cancer in the past but are now in remission.⁴⁴

It would be particularly valuable for

1. Those who have a strong family history of cancer, but are fit and well;

2. Those who have had suspicious test results from their doctors, but do not know whether they have cancer or not;

3. Those who have had cancer diagnosed and are about to embark upon a course of treatment; and those who have had cancer in the past, and want to make sure they are clear.

4. For fit and healthy people, over the age of 40, it could be argued that they should have the test every 2 years or so.

5. For fit and healthy people over the age of 55 -60 it could be argued that they should have the test every 12 months.⁴⁵

6. For people with cancer, or a history of cancer, or a family history of cancer, it could be argued that the test should be carried out more frequently.

Procedure

The DR-70 test is carried out on a fasting blood sample – that is a blood sample taken after fasting (not eating) for a period of 12 hours or longer. Patients are asked to give a blood sample in the morning having had nothing to eat after dinner the night before. It is analyzed in the laboratory as Enzyme Linked Immuno Sorbant Assay (ELISA) Based Serological Test.⁴⁶

If the result of DR-70 level is as follows:

- Low: there is no cancer in the body.
- Medium: the presence of early cancer.
- **High:** invasive cancer.

If the DR-70 levels are low or normal, then the presence of cancer is highly unlikely. A follow up test should be carried out in 12 - 24 months.⁴⁷

If the DR-70 levels are moderately raised then the test should be repeated, possibly with other tests looking for other signs of cancer. If the DR-70 levels are high, then further tests should be carried out, looking for signs of cancer. Further tests should include physical / clinical examination, blood tests, and possibly X-rays or other imaging tests. Further tests may be indicated according to individual requirements. In the presence of high, or moderately raised, DR-70 tests, seek further advice from doctor.

Advantages

Currently available and previous tests for cancer have been vague and of limited accuracy or limited usefulness. For example, the PSA test for prostate cancer is accurate, but only for prostate cancer, X-rays are useful in a large number of cancers but may miss many cancers, and is not specific enough to confirm the diagnosis.⁴⁸

5) Lymphocyte Size Analysis

It was developed by Valentin Govallo, MD, a Russian immunologist. The test measures the diameters of lymphocytes and counts the numbers of swollen versus normal cells in a sample of a patient's blood.

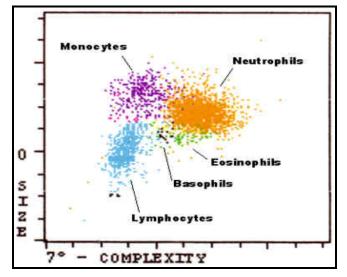


Figure: 14 Lymphocyte size analysis

If the number of swollen lymphocytes is excessive or when the ratio of swollen to normal lymphocytes is out of balance, then cancer will most likely develop. (Note: A lymphocyte is a form of white blood cell whose numbers increase during infection.)⁴⁹

6) T/Tn Antigen Test

T/Tn Antigen Test developed by Dr. Georg Springer can detect the majority of cancers before any biopsy can pick up the presence of cancer. The T and Tn antigens are proteins on the surface of blood and skin cells and can be identified by the immune system antibodies T/Tn antigen is detected by serological and immunohistochemical methods. Also with the help of anti-T antibody immunoassay (SPIA-T). we can detect the 85% of cancer patients.⁵⁰ The concentration of these antigens vary depending on the cancer type and stage. A skin prick can predict or indicate the likely development of cancers, even 6-10 years in advance of other tests. The test appears to successfully diagnosis about 94% of lung cancers and 80% of breast cancers.⁵¹

ADVANTAGES OF DIAGNOSTIC IMMUNOLOGY

Nowadays Diagnostic immunology has reached new heights in the scientific research world. It can also be used for detection of cancer. This has helped in early diagnosis of cancer and its treatment. Adults living an unhealthy lifestyle are more prone to such diseases. Smoking, consumption of alcohol, and many such things result in lung cancer, pancreatic cancer, cancer of the bladder, esophageal cancer, and distal esophagitis. In these diseases, innate immune cells play a vital role, recognizing stressed cells or undigested cellular contents when cells undergo non-apoptotic or non-autophagic death. This helps detection or diagnosis of cancer using Diagnostic Immunology. As these are the advanced methods, cancer is detected in early stage. For cancer diagnosis early stage detection is main factor.⁵²

DISADVANTAGES OF DIAGNOSTIC IMMUNOLOGY

main disadvantage of Diagnostic The Immunology is that it is not completely and fully successful in the diagnosis, detection or treatment of many more dangerous diseases like cancer, or other genetic disorders like leukoderma. After these tests are performed, another tests such as biopsy, whole body CT scan, X Rays, are prescribed by the physicians for the confirmation of the disease.⁵³ As these are very new techniques, many physicians are not even aware of them. So there is a great need to promote these tests. Many of these tests do not provide the accurate result and are hence preferentially not prescribed by the physician. It involves many tests such as serum tests, blood tests and hence is a very costly and complicated procedure for a common man to undergo. These tests make use of advanced devices and hence are not available equipment and everywhere easily. The technicians using such equipment need to be trained well for the appropriate use of these devices. These tests are available in the developed countries and hence are spread in a limited area.⁵⁴

CONCLUSION

An industrial revolution is taking place in clinical and diagnostic immunology. We are already benefiting from these new technologies, but we must keep fully abreast of these rapid changes and participate in the development of these technologies to take full advantage of new methods and to provide the leadership necessary to advance the field of clinical and diagnostic immunology. So here in the above review we have seen the different traditional techniques and advanced tests used in diagnostic immunology. We have also seen how the advanced tests are more accurate and easy than the traditional ones. The advanced technology used helps in reducing human errors and getting the results in a shorter period of time.

Many chronic diseases such as cancer, genetic disorders, can be detected in their early stages and hence help in early cure of these diseases. If these diseases are diagnosed in the early stage, it

is beneficial to the patient as the treatment will be effective only then. Such diseases if not detected early can be hazardous to life.

REFERENCES

- 1. "Diagnostic immunology prospects," Aug 2005, en.wikipedia.org/wiki/diagnostic-_immunology, Accessed: 15/08/2011.
- 2. "Immunological tests," Feb 1999, www.aacc.org, Accessed: 20/08/2011.
- 3. Kokare CR, A Text Book of Pharmaceutical Microbiology & Principles, 4th Edition, Nirali Prakshan, Pune, 2007, 23.2-23.3.
- 4. "Immunodiffusion precipitation tests," Nov 1998,classes.midlandstech.edu/carterp/cours es/bio255/.../lecture2htm, Accessed: 28/08/2011.
- Kokare CR, A Text Book of Pharmaceutical Microbiology & Principles, 4th Edition, Nirali Prakshan, Pune, 2007, 23.7.
- 6. "Immunodiffusion precipitation tests," Nov 1998, classes.midlandstech.edu/carterp/cours es/bio255/.../chapter18/lecture3htm, Accessed: 28/08/2011.
- 7. "Direct agglutination tests," Sept 2001, pathmicro.med.sc.edu, Accessed: 28/08/2011.
- 8. "Soluble antigens precipitation", June 2004, www.scribd.com, Accessed: 29/08/2011.
- "Diagnostic precipitation tests," Oct 1998, www.immuneweb.xxmu.edu, Accessed: 30/08/2011.
- 10. "Viral hemagluttination," Aug 2007, www.bartonccc.net, Accessed: 03/092011.
- Satyanaryana U, Chakrapani U, A Text Of Biochemistry, 3rd Edition, Books And Allied pvt .ltd, New Delhi, 2006, 734.
- 12. "Compliment fixation reactions" Sept 2008, www.cancerrequest.org, Accessed: 10/09/2011.
- 13. "Indicators of antigen-antibody reactions," Jan 2008,

www.ncbi.nlm.nih.gov/pmc/articles/pdf/can cer, Accessed: 20/09/2011.

- 14. "Antigen antibody complex" Jume 2007, www.onlinelibrary.willey.com, Accessed: 20/09/2011.
- Satyanaryana U, Chakrapani U, A Text Of Biochemistry, 3rd Edition, Books And Allied pvt .ltd, New Delhi, 2006, 729.
- 16. "Direct ELISA" Aug 2005, en.wikipedia.org/wiki/ELISA, Accessed: 30/09/2011.
- 17. "ELISA detection" Nov 2004, www.elisa.com, Accessed: 30/09/2011.
- 18. Wang G, Ding J, S Hu, X Yang. Res Vet Sci, 2012, 2(1), 34-37.
- 19. "Immunodiffusion precipitation tests," Nov 1998, classes.midlandstech.edu/carterp/cours es/bio255/.../chapter18/lecture5htm, Accessed: 10/10/2011.
- 20. Tanaka F, Nishi K, Arisne K, Hayashi C, Miyani K, Clinical Chemistry, 1980, 26,1647-51.
- 21. 'Immunodiffusion precipitation tests," Nov 1998, classes.midlandstech.edu/carterp/cours es/bio255/.../chapter18/lecture13htm, Accessed: 10/10/2011.
- 22. "Antibody fluorescence tests," Aug 2007, en.wikipedia.org/wiki_direct_fluroscent_ant ibody Accessed: 12/10/2011.
- 23. Satyanaryana U, Chakrapani U, A Text of Biotechnology, 3rd Edition, Books And Allied pvt .ltd, New Delhi, 2006, 100.
- Chang YD, Sinzinger MD, P Bovce-Gurts, Krause M, Dinkala S, Joosten I, Koopman WJ, Adjobo-Hermes MJ, Brock R, Biophys, 2011, 101(11), 2807-15.
- 25. "Northern Blot" Oct 2010, www.cdc.gov, Accessed: 15/10/2011.
- 26. "Applications of diagnostic immunology" Feb 2003, www.science.org, Accessed: 15/10/2011.

- 27. "Cancer-causes, incidence and risk factors" June 1999, www.cancer.org, Accessed: 25/10/2011.
- 28. Bareschino MA, Shettino C, Rossi A, Maione P, Sacco PC, Zeppa R, J Thorac Dis, 2011, 3(2), 122-33.
- 29. "Common tests for cancer" March 2001, www.cancure.gov/cancertopics/details, Accessed: 30/10/2011.
- 30. "Tests for cancer detection" Jan 2007, www.acro.org/cancer, Accessed: 30/10/20111.
- 31. "Method for immunology," March 2003, www.cancure.org/methods, Accessed: 20/11/2011.
- 32. Harman SM, Gucciardo F, Heward CB, Jr Cancer Epidemol Biomarkers Prev, 2005, 14, 2310-15.
- 33. "Antibody screen tests," Aug 2006, www.kroninstitute.org>research>completed, Accessed: 25/11/2011.
- 34. "Natural immune system," Oct 1997, www.oncolabinc.com, 25/11/2011.
- 35. "AMAS test" Jan 1997, cancerhelp.cancerresearchuk.org/aboutcance r/cancerquestions/amas, Accessed: 25/11/2011.
- 36. "Cancer marker test;" Aug 2004, www.cancer.gov/cancertopics/factsheet/dete ction/tumormarkers, Accessed: 30/11/2011.
- 37. "Markers for cancer" Oct 2006, www.tccancer.com/tumormarkers.html. Accessed: 30/11/2011
- 38. "Markers" June 2000, www.cancure.org/cancermarkertest, Accessed: 25/11/2011.
- 39. Andriole G, Crawford E, Grubb R, Journal of Medicine, 2009, 360(13), 1310-19.
- 40. Schroder FH, Huggson J, Robo MJ, New England Journal of Medicine, 2009, 360(13), 1320-28.

- 41. Buys SS, Patridge E, Black A, JAMA, 2011, 305(22), 2295-2303.
- 42. "Blood cancer" Aug 2009, www.cancure.org.completebloodtest, Accessed: 10/01/2012.
- 43. "DR-70 test, June 2004, www.medicinenet.com>home>CBC, Accessed: 10/01/2012.
- 44. "Cancer detectors", Jan 2003, www.uses.globalnet.co.uk/bhealth/cancertes t.html, Accessed: 10/01/2012.
- 45. "Cancer immunology" Aug 2006, www.cancure.org/test-a-detect_cancer, Accessed: 12/01/2012.
- 46. "Serological tests" Oct 2001, www.ncbi.nlm.nih/gov/pubmed/8243206, Accessed: 15/01/2012.
- 47. Kerber A, Trojan J, Herrlinger K, Zgouras D, Caspary WF, Aliment Pharmacol Ther, 2004, 20, 983-87.
- 48. "Advantages od DR-70," Jan 2006, www.radient-pharma.com/clinicalstudythe newDR70pdf, Accessed: 16/01/2012.
- 49. "Lymphocyte size analysis" Feb 2003, www.healingcancernaturally.com, Accessed: 16/01/2012.
- 50. "T/T_n antigen test" Aug 1995, www.cancure.org/lymphocytesizeanalysis, Accessed: 16/01/2012.
- 51. Springer GF, Desai PR, Ghazizadeh M, Tegtmever H, Cancer Detect Prev, 1995, 19(2), 173-82.
- 52. "Advantages of diagnostic immunology" Nov 2000, www.books.google.co.in/books, Accessed: 17/01/2012.
- 53. "Disadvantages of diagnostic immunology" Feb 2004, www.ncbi.nlm.nih.gov/pubmed/2244391, Accessed: 18/01/2012.
- 54. "Diagnostic immunology overview," Jan 2004,www.books.google.co.in/books/immu nology, Accessed: 18/01/2012.