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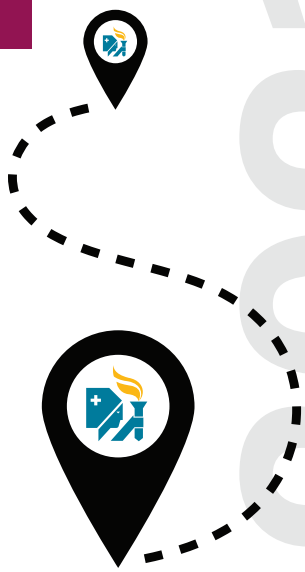
AD EXPRESS

ISSUE 2
2022



2021

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From the editor's desk -

Dear Reader
It gives us immense joy to place the second edition of AD Express in your hands Apollo Diagnostics (AD) has continued to shoulder arms along with the doyens of healthcare workers who have worked tirelessly to keep the COVID virus at bay & serve our patients in the best possible way.

The market for diagnostic services has been growing in India over the past couple of years at a rate of 15 – 20 % and is expected to grow at a CAGR of approximately 16 per cent accounting to approximately 802 billion in the financial year 2020. Within the diagnostics market, the pathology segment is estimated to contribute approximately 58 percent of total market, by revenue. The current rate of growth is expected to continue, driven by increasing awareness in the population, improving payer coverage and rising incidence of lifestyle diseases.
The COVID pandemic has also opened up avenues for 'tele-pathology' & 'tele-health' with unprecedented momentum. The market shares of these relatively esoteric branches are bound to rise in the times to come driven by the onus on preventive health & increasing consumer awareness.

In the second edition of AD express we have given special importance to advanced diagnostics & the'cutting edge' aspects of laboratory medicine. Forays of diagnostics medicine such as genomics & tele-diagnostics have garnered special attention in recent times & we have given these branches ample coverage in our second issue.

We are pleased to inform that in association with SRMC, Chennai we have completed, whole-genome shotgun sequencing of 2 bacterial strains (Enterococcus faecalis (LREF-1) & E.coli (CREC 1) to study the genes responsible for antibiotic resistance. The same is available in the NCBI registry. Given your patronage & support AD is poised to ramify across the nation & beyond keeping aloft the goal of pursuing excellence & serving our patients better than ever.

Dear Reader, We humbly request you to share your feedback on 'AD express' & we assure you that feedback from you will make each next issue ever more interesting !

Best regards,

Dr. Srivatsa. P
DGM – Technical and Advanced Diagnostics
Apollo Diagnostics

Dr. Marquess Raj
ZTC – TN & Pondicherry
Apollo Diagnostics

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WHY APOLLO DIAGNOSTICS

Case report

1. Resolving a Blood Group Discrepancy- Detection of a Para-Bombay B Phenotype.

-Dr. Prashanth R., MD (Pathology). Consultant Pathologist, RRL Apollo Diagnostics, Bengaluru

Foreword:

Blood group is one of the routine tests ordered as part of a health check-up or as a preoperative measure. It is an identity of a person and thus the laboratory testing the blood group is required to take adequate care to determine the correct one. At times, the laboratory encounters a discrepancy between forward grouping and reverse grouping, requiring a few additional tests to be performed to find the resolution. One such resolution process is detailed in this discussion.

Case details:

A 31 year old male requested blood group as part of a routine health check at RRL, Bengaluru. The test was performed on adequately collected EDTA anticoagulated sample on Immucor Galileo automated analyzer. This uses microplate agglutination technique with both forward and reverse grouping to present an optically captured blood group agglutination pattern. In this case however, a discrepancy was noted. Forward cell grouping showed no agglutination with anti-A and anti-B reagents, agreeing to "O" group, whereas reverse plasma grouping showed agglutination with known "A" cells reagent and none with known "B" or "O" cells, agreeing to "B" group. The same results were confirmed with slide agglutination method for forward grouping, and tube agglutination tests with reverse grouping. Rhesus grouping however was Rh positive.

The discrepancy had to be resolved with the help of referral blood bank with further testing. Additional testing for "H" antigen on RBCs was performed with anti-H lectin, and it was negative. This was a picture of "Bombay phenotype" with absence of H antigen on RBCs' surface. With this it is to be expected to find naturally occurring anti-A, anti-B and anti-H isohemagglutinins in patient's serum. Yet, the reverse grouping was revealing only the presence of anti-A natural antibodies, indicating to presence of "H" and "B" elsewhere in the body.

The patient was contacted and requested to provide saliva sample to determine the secretor status. Reverse grouping was performed on saliva sample, and this indicated to "B" group. Hence it was proved that the patient's tissues like salivary glands, gastrointestinal and genitourinary tissue would have "H" and "B" antigens. Antibody screening was negative indicating absence of detectable anti-H antibodies in patient serum. With the overall picture, it was concluded that the patient is an H-deficient Secretor, a rare "Para-Bombay B" phenotype.

Discussion:

The ABO blood group system is the most clinically relevant one with respect to blood transfusion, hematopoietic stem cell transplantation, and solid organ transplantation. The H antigen is the precursor of A and B antigens on red blood cells. The ABO locus determines the A and B antigens, whereas α-(1,2)-fucosyl transferase (FUT) genes FUT-1 and FUT-2 both on Chromosome 19q13.3, determine the H antigen on RBCs and extra hematologic secretory tissues.

The H antigen is ubiquitously expressed on all red cells except in case of a Bombay phenotype patient, who do not express H antigen on RBCs and also are non-secretors. Para-Bombay phenotype individuals are homozygous for a non-functional H gene (FUT1), but inherit at least one functional

secretor genes (FUT2). Thus RBCs lack serologically detectable H- antigens, but they express H, A, B antigens (depending on Para-Bombay A or B) in secretions and plasma.

Presence of anti-H antibodies in serum of individuals with Bombay phenotype due to lack of H antigens on RBCs is of a major clinical concern, as they are potent natural antibodies capable of activating complement and cause hemolysis. Hence they can only be transfused with another Bombay phenotype unit.

Individuals with Para-Bombay B phenotype can be transfused with another compatible Para-Bombay B unit, but as the availability is scarce, they can be transfused with group B or group O packed RBCs compatible by indirect anti-globulin test. The survival of RBCs with such transfusion is expected to be almost normal, even with the presence of weak anti-H antibodies, as problems with these antibodies are most often not clinically significant.

Need for an adequate systematic testing for blood group determination of individuals is being reiterated, and the table below summarizes the pattern of results in different blood groups.

	Reagent	Para-Bombay B	Bombay group	O group	B group
Cell grouping	**Anti-A**	-	-	-	-
	Anti-B	-	-	-	+
	Anti-AB	-	-	-	+
Serum grouping	**A1 cells**	+	+	+	+
	B cells	-	+	+	-
	O cells	-	+	-	-
Test for H antigen -RBCs	**Anti H Lectin**	-	-	+	+
Saliva secretor status	**A1 cells**	+	+	+	+
	B cells	-	+	+	-
	O cells	-	+	-	-

Afterword:
It is essential to perform both forward and reverse blood grouping, and with a good knowledge of principles of blood grouping to judiciously use or request for anti-H reagent testing for "H" antigen detection in certain cases like these. Otherwise, with only a forward grouping, this person would have been wrongly labeled as "O" group

2. A Case of Suddenly Detected Chronic Lymphocytic Leukemia

Dr. Zishan Akhtar, (*Consultant Pathologist, RRL Kolkata)*
Dr. Abhik Banerjee, *(Zonal Technical Chief, East Zone, RRL Kolkata)*
Dr. Debojyoti Singha Roy, *(Consultant Pathologist, RRL Kolkata)*

Abstract:
Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder of monoclonal origin. It is characterized by increased number of lymphocytes with insufficient function (1). CLL constitutes approximately 30% of all leukemia cases in the West.However incidence is less common in Asian countries. There are limited studies on CLL from the Indian subcontinent. In this report, we have discussed about a case of CLL which was initially diagnosed through examination of peripheral smear.

Introduction:
Chronic lymphocytic leukemia (CLL) is a well-known leukemia among adults, which is one of the causes of massive splenomegaly. Though CLL can affect B, T lymphocytes and even natural killer (NK) cells, most of the CLL cases are found to be of B-cell phenotype. CLL results from uncontrolled clonal growth of small B lymphocytes and affects bone marrow and peripheral blood and ultimately lymph nodes, liver, and spleen. It is assumed that CLL is associated with environmental factors such as radiation, infectious factors, exposure to toxic drugs and chemicals apart from genetic factors. The initial symptoms of CLL may include malaise, weight loss, enlarged lymph nodes, and splenomegaly. However many patients remain asymptomatic even for years together. Recurrent infections, autoimmunity, bone marrow failure are common long-term complications.

Case presentation:
We received a blood sample of a 60 years aged male for "Complete Blood Count" only. The sample was received in lab in EDTA vacutainer from a nearby patient care centre within 2 hours of collection. The result of the fully automated hematology analyzer was as follows:

- Hemoglobin: 12.5 g/dL;
- Lymphocytes: 41050/ /µL
- Total Leukocyte count: 50,000/µL
- Platelet: 160,000/µL

As the total WBC count was high & as absolute lymphocytosis was noted, a peripheral blood smear was ordered by Pathologist. Leishman stain was used to stain the slides. Under high power, the smear showed lymphocytic leukocytosis with smudge cells (Fig. 1).

Under oil immersion, the smear showed predominantly monomorphic, small mature lymphocytic population (Fig. 2) with presence of Prolymphocytes (Fig. 3).

There were approximately 12% prolymphocytes. Prolymphocytes were identified by large cell with clumped chromatin, a large prominent vesicular nucleolus and abundant cytoplasm.
The WBC differential count reported was as follows: Monomorphic, mature-appearing Lymphocytes 87%, Prolymphocytes 8%, Neutrophils 4%, Eosinophil 1%.

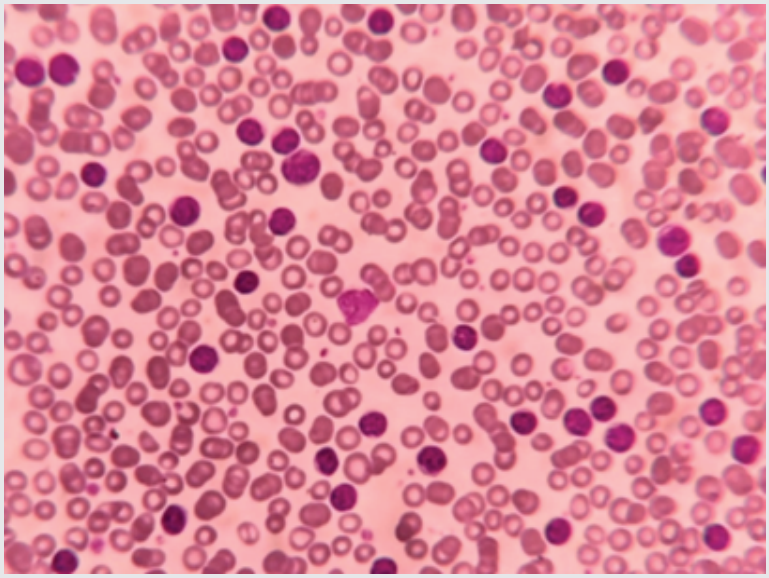

Figure 1 : High power view showing mature lymphocytes & smudge cells

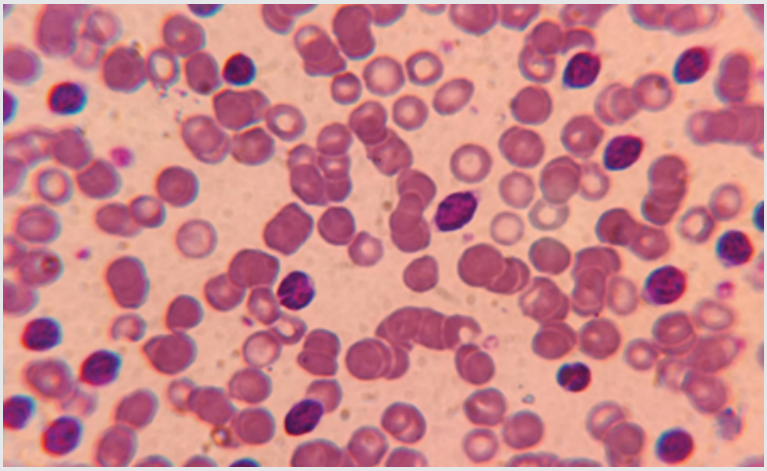

Figure 2 : Low power view showing mature lymphocytes & smudge cells

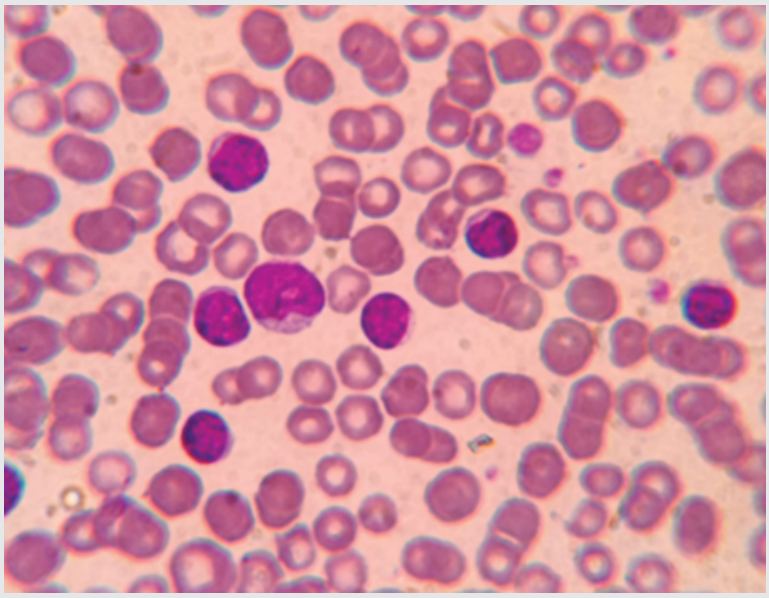

Figure 3 : Prolymphocyte (Red arrow)

The peripheral smear findings were reconfirmed by another Pathologist.
A phone call was made by Pathologist to patient as well as referring physician. However no definite clinical history was found in this case. The patient visited referring physician with a complaint of weakness only since last few weeks. Apparently there was no fever, lymphadenopathy, weight loss or any other significant clinical history. However according to patient's son, patient's brother died of some "blood related cancer" almost 4 years ago and was treated by a local "Ayurvedic practitioner". He had no idea about details of that case. The patient was not a known case of diabetes, hypertension or any chronic disease.

Though laboratory advised patient as well as the referring physician for further investigations, the patient was not interested and informed that, he will visit tertiary care centre for comprehensive care following discussion with his family physician. Hence Biochemical investigations, Flow Cytometry, Bone marrow biopsy or aspiration reports are not available with this laboratory.

Discussion:
CLL in most of the cases is asymptomatic on presentation and may be kept under observation without any need for therapeutic intervention for years. According to WHO 2004, CLL is in peripheral blood smear shows small lymphocytes with clumped chromatin and scanty cytoplasm. Prolymphocytes are usually less than 2% in typical CLL. In this instance >55% prolymphocytes were notd , fulfilling the definition of prolymphocytic leukaemia. CLL with 2-55% prolymphocytes is usually characterized by a slightly aggressive course. Hence identifying prolymhocytes in peripheral blood smear is important for therapeutic management of patients.

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Article - Vitamin vigyan

Vitamin B12 in its whole form is, elusive …being water soluble.
Active B12 – Peril, pitfall or probiotic.

Dr. Shalini Singh
ZTC - Karnataka

Dr.Marquess Raj
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Dr. Srivatsan.R
Consultant biochemist, RRL, Chennai.

First cut :
Vitamin B12,is essential for DNA synthesis,hematopoiesis,& CNS integrity.Since the absorption of B12 is complex involving several anatomical niches & cofactors, estimation of the active form of B12 is quite challenging.

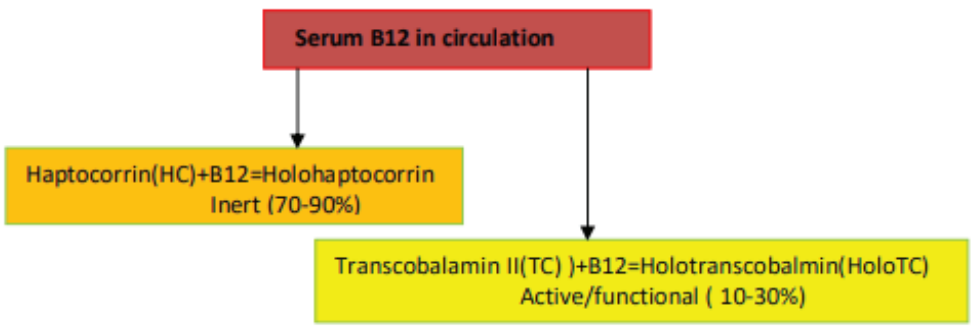

Figure 1 : Forms of B12 in circulation

Technology for B12 status estimation in humans exists within the realm of a handful of industry leaders at this point. While B12 studies are relevant & fairly common in the current clinical scenario,yet the analysis and clinical estimation of active B12 is not common. We are only grasping at straws when it comes to estimation of metabolites such as holo transcobalamine (holoTC/active B12) & methyl malonic acid (MMA). [1]

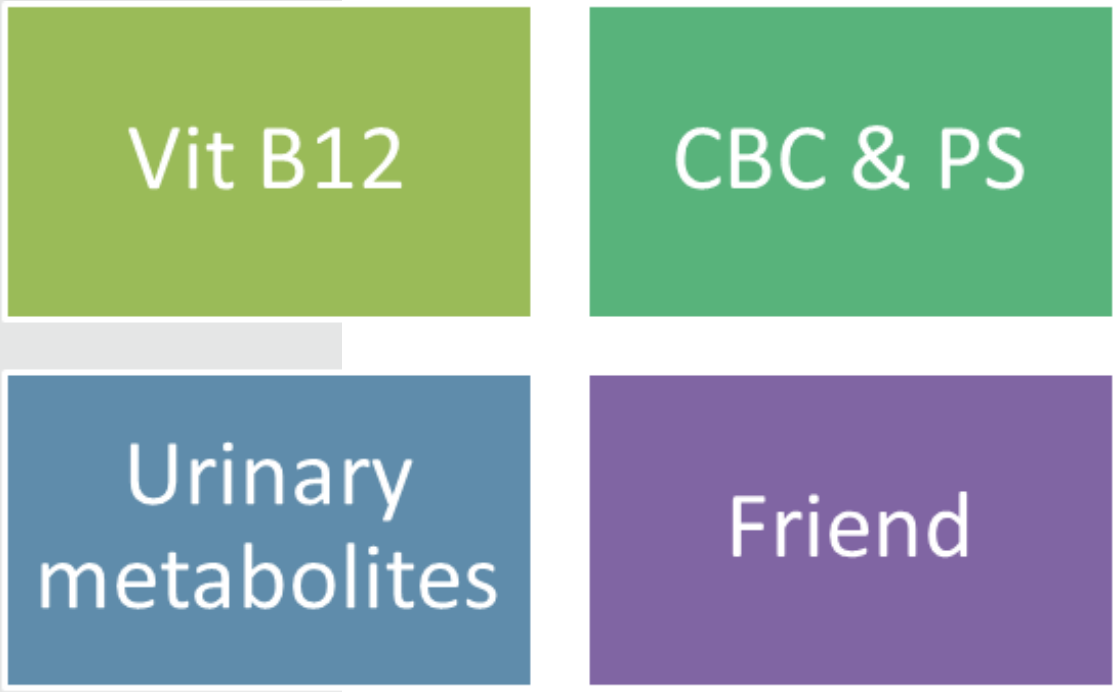

Figure 2 : Strategies for evaluation of Vitamin B12 deficiency

B 12 levels are affected by decreased GFR, hypothyroidism & even by lifestyle modifications such as food fads,profoundly.Hence estimation of active vitamin B12 can give a true estimate of the deficiency to the clinician.

Vitamin B12 has a wide biological reference range & many a time different manufacturers quote different ranges (Expected Range: 138 - 914 pg/ml).There is a propensity to under diagnose Vitamin B12 deficiency because of this. In these indeterminate states the role of active B12 /holo TC is considered as an early indicator of B12 deficiency and is possibly a marker of cobalamin malabsorption. (2)

Active-B12 contains the biologically available cobalamin because only transcobalamin-bound B12 promotes the uptake of the cobalamin therein by all cells, via specific receptors. The markedly shorter half-life of Active-B12 compared to Holohaptocorrin makes,a decrease of Active-B12 as one of the earliest marker of cobalamin deficiency. (3)

There are studies in general populations and in patients with different clinical conditions such as pernicious anemia, alcoholism, Alzheimer's disease, pregnancy & gastrectomised patients, showing the advantage of active B12 compared to conventional total B12 assessment. Cell damage can reduce intracellular B12, thus significantly elevate plasma B12. Falsely increased B12 can be seen in

myeloproliferative disorders, liver diseases, intestinal bacterial overgrowth, congenital TC II deficiency.
This can also result from the increased binding of B12 to HC, which has an inhibitory effect on B12 binding to TC thus reducing the functional B12 status. In these conditions the true picture may be masked if total B12 alone is used and active B12 seems to be better marker.

With advanced platforms such as LCMS being easily available, active vitamin B12 estimation is becoming easier than ever.

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Article – 2

Did you know - Is fasting a requisite for thyroid function tests?

Dr. Abhik Banerjee MBBS (CU),
MD Pathology (MUHS) Zonal Technical Chief,
East Zone Apollo Diagnostic, RRL Kolkata

Thyroid function tests (TFT) are used to evaluate thyroid function and they are one of the most commonly requested tests in in day to day clinical practice. The usual and initial blood tests done for evaluation of thyroid function are,Thyroid stimulating hormone (TSH), Thyroxin (T4) and not always but definitely sometimes tri-iodothyronine (T3). However physicians & patients alike may harbor doubts regarding the importance of time of sample collection or the state of the patient being fasting or non-fasting & their effect on thyroid function test results.

There are no definite clinical or laboratory guidelines for thyroid function testing (unlike lipid profile) emphasizing the time of phlebotomy or the fasting or non-fasting status of the patient.

> *However in last few years a few studies have indicated a statistically significant postprandial decline of TSH occurs (not T3 or T4) in comparison to the fasting values.*

Unfortunately, whether this finding has any major and statistically significant clinical relevance in test interpretation is till now a matter of debate. Few of these studies have raised concerns that, such difference in values might be significant in diagnosis and rational management of subclinical hypothyroidism (SCH, mild thyroid failure with mild elevation of TSH but normal T3 and T4) and also in certain clinical scenarios (e.g. Pregnancy, sub-fertility) where even marginal variations in TSH is important to get detected and thereby managed judiciously.

Interestingly, according to one school of thought and perhaps the majority, this post prandial decline of TSH value is more related to time of sampling or phlebotomy rather than food related alteration. Circulating Thyroid stimulating hormone (TSH) is known for its normal circadian rhythm with a peak around midnight whereas a nadir during late afternoon. Hence TSH level is subject to circadian variation and as per literatures, this variation is of the order of even up to 50% especially in between 8:00-9:30 am. Obviously a TSH measured in the early morning will be different than a TSH level measured later in the same day and with same subject.

To conclude, currently in absence of larger studies considering and addressing all possible interfering factors (e.g. Interference by other drugs) including differences in testing platforms, let us observe this space for some time before labeling the fact that, fasting /non fasting state has definite impact on TSH results and thereby a fasting sample is mandatory.

It will be prudent to conclude that, the preferred sample for thyroid function test especially TSH should be collected at the same time of the day (and preferably at morning to rule out the effect of decline in later half of the day) each and every time he or she comes back for follow up. For fresh cases also, where a patient is coming for initial evaluation, just to rule out the possibility of missing cases of SCH, morning sampling can be preferred and recommended. It is high time that, the physicians and clinical laboratories should reach a consensus regarding this as lack of uniformity in the time of sampling for TSH may lead to unnecessary repetition of tests resulting in anxiety and deferred treatment in patients.

Disclaimer:
This discussion paper solely represents the author's personal opinion and do not necessarily reflect the views of common man and experts in the said field

Article - 3

Knotty questions – FAQ's on COVID antibody

– Dr.Mir Salmaan Ali , MBBS MD,
HOD of Microbiology,Global Reference laboratory, Apollo Diagnostics,Hyderabad

1. What is COVID Antibody ?

Antibodies are proteins made by the immune system to fight infections like viruses and may help to ward off future occurrences by those same infections. Antibodies can take days or weeks to develop in the body following exposure to a SARS – CoV - 2 (COVID 19) infection and it is unknown how long they stay in the blood .

2.Are antibody tests used to diagnose COVID-19?

No. An antibody test does not detect the presence of the SARS-CoV-2 virus to diagnose COVID-19. These tests can return a negative test result even in infected patients (for example, if antibodies have not yet developed in response to the virus) or may generate false positive results (for example, if antibodies to another coronavirus type are detected), so they should not be used to evaluate if you are currently infected or contagious (ability to infect other people).

3. If antibody tests cannot be used to diagnose COVID-19, what tests are available for that?

Currently, there are two types of diagnostic tests for COVID-19:

- Molecular (RT-PCR) tests, which detect the virus' genetic material
- Antigen tests that detect specific proteins on the surface of the virus

4. Why Antibody Tests?

Antibody Testing Is Critical in Overcoming the COVID-19 Pandemic—Now and in the Future .Antibody tests play an important role throughout the patient care pathway and are vital for the management and surveillance of the virus. They are critical in determining the full scope of the disease, combating the pandemic, and rebuilding public confidence. Highly accurate antibody tests help inform clinical and public health decisions as we look towards safely opening our communities.

5.Which antibody tests?

When an individual is infected with the SARS-CoV-2 virus, unique antibodies will develop at different stages of the infection. SARS-CoV-2 Total antibody tests detect both antibodies (IgM and IgG) that are present during current infection or early during the immune response. SARS-CoV-2 IgG antibody tests specifically detect IgG antibodies that persist and are the basis for an individual's longer-term immune response.

6.What does a positive antibody test mean?

If you have a positive test result on a SARS-CoV-2 antibody test, it is possible that you have recently or previously had COVID-19. There is also a chance that the positive result is wrong, known as a false positive. False positive tests may occur: Because antibody tests may detect coronaviruses other than SARS-CoV-2, such as those that cause the common cold.When testing is done in a population without many cases of COVID-19 infections. These types of tests work best in populations with higher rates of infection.

7.Does a positive antibody test mean that I am immune to COVID-19?

A positive antibody test does not necessarily mean you are immune from SARS-CoV- 2 infection, as it is not known whether having antibodies to SARS-CoV-2 will protect you from getting infected again. It also does not indicate whether you can infect other people with SARS-CoV-2.

8.What does a negative antibody test mean?

A negative result on a SARS-CoV-2 antibody test means antibodies to the virus were not detected in
your sample.
It could mean:

- You have not been infected with COVID-19 previously.
- You had COVID-19 in the past but you did not develop or have not yet developed detectable antibodies. It is unknown if all infected individuals will develop a detectable antibody response.
- The result may be wrong, known as a false negative. This occurs when the test does not detect antibodies even though you may have specific antibodies for SARS-CoV-2.

9. What if I get different results on two tests from two different laboratories? Which one should I believe?

The test results from different laboratories may vary depending on several factors such as the accuracy of the test itself and also how long it may take for your body to develop antibodies after you had the coronavirus infection, if you were in fact infected. For this and other reasons, you should always review your test results with your health care provider.

10. What is Spike Protein & Neutralization ?

Humans produce antibodies against both the nucleocapsid and spike protein, as well as other proteins but key question to answer are "Are they neutralizing antibodies ?" and " Can they protect you from reinfection ?"
A common part of the immune response is for humans to neutralize or block the binding of the virus to ACE2 receptor. If an antibody interferes with binding, you may have some level of protection. Different antibodies will target different proteins in the viral structure

Spike (S1 and S2) Protein
The S1 protein is the head of the spike protein and contains a receptor binding domain (RBD). The S1RBD is instrumental for allowing the SARS–CoV-2 virus to reproduce by attaching to and infecting host cells. The S2 protein is the stalk of the spike protein and allows the SARS–CoV-2 virus to remain fused to host cells.

Nucleocapsid (N) Protein
The N protein is located inside of the capsid (the viral protein shell) , along with RNA of SARS – CoV-2 . This protein functions in the dispersal of genetic material during viral reproduction . The SARS–CoV-2 N protein is highly similar in structure to that of the SARS coronavirus (SARS-CoV)

11.What is the Role of Neutralizing Antibodies in Protective Immunity ?

Protective immunity is multifaceted, Antibodies can be binding or neutralizing Binding (non-neutralizing) Abs

Produced at high levels, but unable to independently prevent infection Bind and flag pathogen as 'invader'. They are good markers of prior infection
Neutralizing Abs (NAbs)

NAbs bind virus leading to loss of infectivity and blocking viral entry into host cells Function independent of other immune system components. Commercially available assays do not distinguish NAbs from non-NAbs

Testing for NAbs is challenging Classically detected using plaque reduction neutralization tests (PRNTs) with live virus, SARS-CoV-2 requires BSL-3 for culture

Increasingly, BSL-2 methods are being developed using pseudo typed Vesicular Stomatitis Virus (VSV) expressing SARS-CoV-2 spike protein

12.What are the different methodologies available for SARS-CoV-2 testing ?

- Lateral flow assays
- Enzyme immunosorbent assays (ELISA)

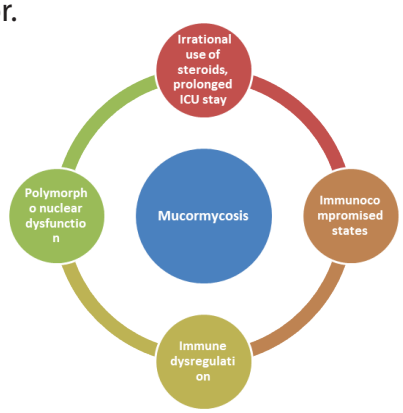
Article - 4

Return of the bread mould - Re-emergence of mucormycosis – Dr.Ramakrishna Reddy HOD of Histopathology,Global Reference laboratory, Apollo Diagnostics,Hyderabad

Mucor ,the common bread mould made a resurgence in recent times. A significant increase in mucormycosis was noted in Covid – 19 patients during hospitalisation or after discharge as sequelae of COVID 19.

Mucormycosis is an opportunistic fungal infection caused by members of the class Zygomycetes, order Mucorales which is normally seen in soil and decaying vegetation. Most common organisms responsible are Rhizopus and Mucor.

Pathogenesis :



Clinical presentation: Patients can present with facial pain, pain over sinuses, pain in teeth and gums, paraesthesia, nasal crusting and nasal discharge which could be blackish or blood tinged, conjunctival redness, periorbital swelling, diplopia. Worsening of respiratory symptoms, hemoptysis, chest pain, alteration of consciousness, headache can also be present.

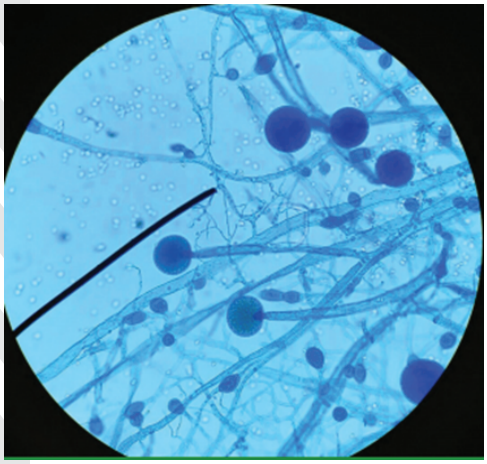


Fig.1.Mucor in lactophenol cotton blue

Laboratory Diagnosis:

Broad aseptate/pauciseptate hyphae with right angled branching in histopathological examination of debrided tissue favours diagnosis of Mucormycosis.

Other tests which can be done include KOH staining, Calcofluor white stain, Lactophenol cotton blue method and molecular methods like PCR.

Treatment:

Combination of surgical debridement and antifungal therapy is the mainstay of the treatment. Liposomal Amphotericin B is the treatment of choice.

Advanced diagnostics – Clinical Exome Sequencing

– Dr. Srivatsa Prakhya
DGM – Technical and Advanced Diagnostics

Foreword :

The human genome is a complete set of nucleic acid sequences encoded as DNA within the 23 pairs of chromosomes in the cell nucleus. The haploid human genome (23 chromosomes) is very large and is composed of about 3 billion base pairs & contains about 30,000 genes. It contains both protein-coding regions known as exons and non-coding DNA sequences known as introns.

Genome sequencing can provide information on genetic variation that can lead to disease or can increase the risk of disease development, even in asymptomatic people. Thus genome sequencing can be used as a tool to predict disease development & help in taking preventive measures against undiagnosed disease. For people experiencing a health-impacting condition, DNA sequencing can provide a precise diagnosis which might affect the medical management of symptoms, or provide treatment options.

Ideally, whole-genome sequencing (WGS) is prescribed to look for genetic aberrations (Eg, single nucleotide variants, deletions, insertions and copy number variants).Changes in the noncoding sections of DNA within genes, called introns, can also be determined by WGS. Although under normal conditions, introns are removed by RNA splicing during a post-transcriptional process; alterations in these regions can be important to assess whether the DNA is transcribed into RNA or potentially results in a truncated, non-functional protein. However sequencing the whole genome is a costly practice and it consumes a lot of time. Hence in order to efficiently answer this problem a new genetic technique has been developed known as Exome sequencing which has proved to be an efficient strategy to diagnose the genetic basis of various diseases.

The complete set of coding regions are collectively called as Exome that constitute about 1,80,000 exons which is about 1% of the total human genome. It is most widely studied area as believed to be associated with 85 % of mutations that have a large effect on disease. However, in recent years, to further reduce the cost and turnaround time more defined and targeted subset of whole exome sequencing known as clinical exome or targeted exome sequencing is being regularly used in clinical settings which covers usually 3000 to 6000 genes, eventually reducing the complexity and cost compared to whole exome sequencing. The subset of genes are chosen based on various studies about the disease associations, prevalence/frequency of mutations in these genes. However, the list of disease-associated genes has been expanding with newer research and publications every week resulting in variations in the clinical exome sequencing (CES) gene set of different labs. While the CES is relatively cheaper and easier for the labs, the underlying assumption is that the non-covered genes/regions are not important. Many a time, this may lead to missing of a vital mutation. CES is largely popular in Asian countries and price-sensitive markets. Whereas, the rest of the world is using WES commonly and gradually exploring Whole Genome Sequencing (WGS) which would cover the non-protein coding regions as well.

Clinical applications:

1. Identification of rare genetic disorders.

CES can be used to identify causal variants of rare genetic disorders such as Bartter syndrome, Alzheimer's disease, Parkinson's disease, Miller syndrome. In 2009, the responsible gene, MYH3, was identified in Freeman-Sheldon syndrome (FSS). Exome-sequencing strategy also has been applied in primary immunodeficiency diseases: STIM1 T cell deficiency was discovered in a child with Kaposi sarcoma. Diagnosis of rare form of X linked lympho proliferative disease 2, exhibiting a novel inflammatory bowel disease 2 like manifestation caused by loss of tolerance to commensal organisms in the digestive system (bowel disease in an infant).

2. Discovery of Mendelian disorders

In Mendelian disorders of large effect, findings thus far suggest one or a very small number of variants within coding genes underlie the entire condition. Because of the severity of these disorders, the few causal variants are presumed to be extremely rare or novel in the population, and would be missed by any standard genotyping assay. CES provides high coverage variant calls across coding regions, which are needed to separate true variants from noise. A successful model of Mendelian gene discovery involves the discovery of de novo variants using trio sequencing, where parents and proband are genotyped.

3. Cancer treatment and its pre diagnosis

CES is particularly very beneficial for cancer diagnosis and treatment. The presence of some gene mutations can confer sensitivity or resistance to a given drug. Exome sequencing has been used extensively to diagnose novel diseases and find novel causative mutations for known disease phenotypes. Another advantage of genome sequencing is that information regarding drug efficacy or adverse effects of drug use can be obtained. In addition to reaching a diagnosis, finding the causative mutation can allow for alteration of treatment, prevention of further invasive testing, accurate prognoses, and confirmed diagnoses, which are essential for eligibility for benefits and access to clinical trials and in the future may allow for more targeted treatment.

4. Rare variant mapping in complex disorders

Current association studies have focused on common variation across the genome, as these are easiest to identify with our current assays. However, disease-causing variants of large effect have been found to lie within exomes in candidate gene studies, and because of negative selection are found in much lower allele frequencies and may remain un typed in current standard genotyping assays. Whole genome sequencing is a potential method to assay novel variant across the genome. However, in complex disorders (such as autism), a large number of genes are thought to be associated with disease risk. This heterogeneity of underlying risk means that very large sample sizes are required for gene discovery, and thus whole genome sequencing is not particularly cost-effective. This sample size issue is alleviated by the development of novel advanced analytic methods such as Exome/Clinical Exome sequencing.

CES is useful in human medicine for diagnosis of particularly difficult-to diagnose patients, diagnosis of young patients who may not yet exhibit a full spectrum of symptoms, prenatal diagnosis, and early diagnosis of debilitating disease. In addition to reaching a diagnosis, finding the causative mutation can allow for alteration of treatment, prevention of further invasive testing, accurate prognoses, and confirmed diagnoses, which are essential for eligibility for benefits and access to clinical trials and in the future may allow for more targeted treatment.

Benefits of Exome sequencing and early diagnosis

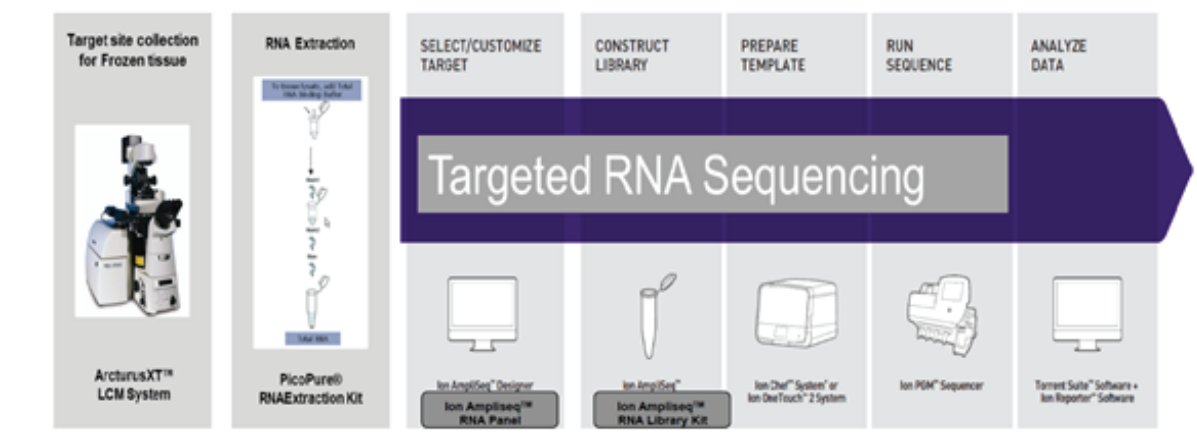
1. Establish a molecular diagnosis of the disease.
2. Low cost as compared to whole genome/Exome sequencing
3. It consumes less time as compared to whole genome/Exome sequencing
4. It can diagnose genetic disorders before its occurrence occurring in life.
5. It is the efficient tool available to diagnose certain diseases which are not detected by conventional blood tests.
6. It has also been found to be more effective than other methods such as karyotyping and microarrays. This distinction is largely due to the fact that phenotypes of genetic disorders are a result of mutated exons. Since these variants may lead to various genetic disorders which we aim to diagnose at an early age. In addition, since the CES only comprises 0.1-0.2% of the total genome, this process is more cost efficient and fast as it involves sequencing around few million bases rather than the 3 billion base pairs that make up the genome.

Exome sequencing platforms

There are several platforms available, which are constantly being updated and improved. The major providers of exome capture platforms are Ion Torrent (Thermo fisher Scientific), Nimble Gen, Agilent, and Illumina, and each have different designs and strengths.

Ion Torrent Next Generation Sequencing:

Work Flow Overview



Ion Torrent Applications:
Cancer research

Enabling NGS analysis and discovery of multi-biomarker types (fusions, insertion/deletions (indels), single nucleotide variants, and copy number variations), Oncomine assays are part of an end-to-end workflow that includes simple, scalable sequencing with optimized bioinformatics and reporting—designed for cancer research.

- Drug and companion diagnostic development
- Immune repertoire solutions
- Immuno-oncology solutions
- Liquid biopsy clinical research
- Targeted sequencing for oncology research

Infectious disease and microbial research
Uncover microbial diversity, study pathogen outbreaks, and identify mutations that may be associated with antibiotic resistance. Take advantage of increased throughput, higher accuracy, and longer reads to produce rapid and accurate sequencing of microbes with streamlined sample preparation and a simple, scalable, and optimized data analysis workflow.

- Bacterial typing
- de novo microbial sequencing
- Metagenomics studies
- Microbial sequencing
- Viral typing

Complex disease
Our complete NGS solutions are uniquely suited to research in understanding how the combination of genetics and the environment influence development of complex diseases such as autoimmune disorders, neurodegenerative diseases, and many others.

- Gene expression profiling
- Methylation analysis
- Small RNA and miRNA sequencing
- Targeted DNA sequencing
- Targeted RNA sequencing
- Targeted transcriptome sequencing
- Whole-exome sequencing

Aneuploidy and CNV analysis for reproductive health
Copy number variation (CNV) analysis is a next-generation sequencing method that can be used to analyze chromosomal aberrations like aneuploidy. Ion Torrent targeted NGS is a simple, rapid technique that is designed to reliably deliver easy-to-interpret data.
Human identification (HID)
Adopting targeted NGS for forensic DNA analysis and phenotyping in your laboratory is simpler than ever when you combine the Ion Chef System and Ion GeneStudio S5 Systems with optimized Precision ID library preparation, template preparation and sequencing kits, and forensically relevant panels.

Targeted DNA sequencing
Targeted sequencing is a rapid and cost-effective alternative to whole genome sequencing. Ion AmpliSeq technology transforms this application by enabling researchers to rapidly and simply amplify thousands of targets using as little as 1 ng of DNA.
Genotyping by sequencing

Human genetic variation is present in many different forms in the genome, ranging from large, structural, chromosomal changes to single nucleotide polymorphisms (SNPs). We offer a broad range of products for analysis of genetic variation and genomic profiling.
RNA sequencing

Transcriptome sequencing, or RNA sequencing (RNA-Seq), provides fundamental insights into how genomes are organized and regulated. RNA sequencing relies on next-generation sequencing (NGS) methodology and techniques.

- Small RNA and miRNA sequencing
- Targeted RNA sequencing
- Whole-transcriptome RNA-Seq

Exome sequencing
Exome sequencing is a targeted sequencing approach that interrogates only the disease-causing exonic regions of the genome. We enable flexible, and simple exome sequencing.

Advantages offered by Ion Torrent work station (Thermo Fisher Scientific)
1. The Ion Torrent work station exome sequencing workflow is a simple and reliable solution, requiring less than 60 minutes of hands-on time.

2. It currently sequences two exomes with an Ion PI™ Chip (v1 or v2), providing coverage of over 90% of bases at over 20x depth, quickly providing annotated filtered variants.

3. The Ion Torrent work Exome Kit contains about 300,000 primer pairs across 12 pools, and requires a very low amount of input DNA (usually ~50 ng).

4. It has been observed that constructing the library, preparing the template, sequencing, and analyzing data can be performed in just a few days, a remarkable improvement over traditional methods.

Limitations of Clinical Exome Sequencing

1. It is possible that this test will not find a reason for the patient's signs and symptoms. About 75% patients tested do not receive a diagnosis from CES.

2.CES is not a perfect test. Our current understanding of the human exome is limited. This technology is new. Some parts of the exome are not examined.

3. CES does not detect certain types of mutations. For any of these reasons, it is possible that CES will not find some mutations that are actually present in the DNA.

4. CES may reveal information that is unexpected or unwanted and might upset some people.

Apollo diagnostics has collaborated with SRMC, Chennai & we have completed exome sequencing of 2 clinically significant organisms

1.Enterococcus faecalis strain LREF-1, whole-genome shotgun sequencing project is completed. "Enterococcus faecalis strain LREF-1 chromosome, complete genome", accession number JAHZSK000000000. 1 has been successfully released into GenBank.
You could get the details of the entire sequence information by clicking the WGS.

https://www.ncbi.nlm.nih.gov/nuccore/JAHZSK000000000

2.Escherichia coli strain CREC-1, whole-genome shotgun sequencing project is completed.We are working on the manuscript of E.fecalis LREF -1 & E.coli CREC -1 along with SRMC. Will keep you posted on the same.

Escherichia coli strain CREC-1 chromosome, complete genome", accession number JAIGYR000000000. 1 has been successfully released into GenBank.
You could get the details of the entire sequence information by clicking the WGS.

https://www.ncbi.nlm.nih.gov/nuccore/JAIGYR000000000.1

Advanced diagnostics

Clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) Applications in disease diagnosis

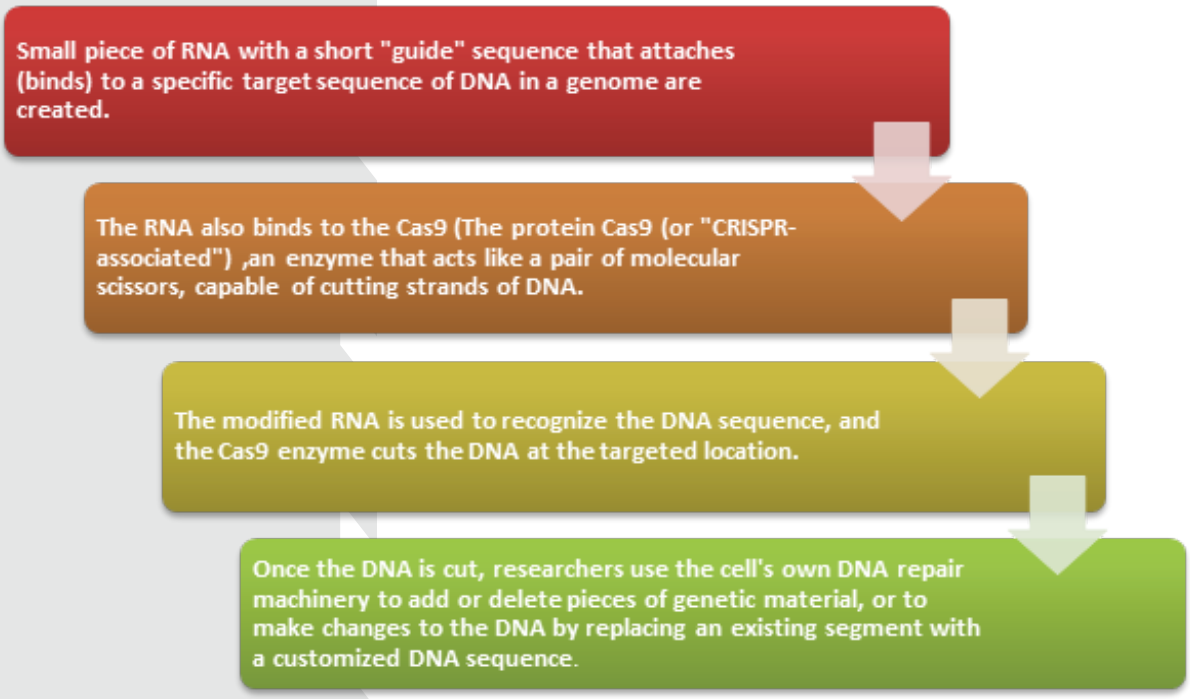
- Dr. Srivatsa Prakhya
DGM Technical and Advanced Diagnostics

Introduction:

This technology allows genetic material to be added, removed, or altered at particular locations in the genome. Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. It is a specialized region of DNA with two distinct characteristics: the presence of nucleotide repeats and spacers. Repeated sequences of nucleotides — the building blocks of DNA — are distributed throughout a CRISPR region. Spacers are bits of DNA that are interspersed among these repeated sequences.

CRISPR-Cas9 was adapted from a naturally occurring genome editing system in bacteria. The bacteria capture snippets of DNA from invading viruses and use them to create DNA segments known as CRISPR arrays. The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones). If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays to target the viruses' DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart, which disables the virus.

Methodology:



Advantages of Method:
It is faster, cheaper, more accurate, and more efficient than other existing genome editing method called **TALENS**.

Ethical concerns arise with genome editing, using technologies such as CRISPR-Cas9, is used to alter human genomes. Most of the changes introduced with genome editing are limited to somatic cells, which are cells other than egg and sperm cells. These changes affect only certain tissues and are not passed from one generation to the next. However, changes made to genes in egg or sperm cells (germline cells) or in the genes of an embryo could be passed to future generations. Germline cell and embryo genome editing bring up a number of ethical challenges, including whether it would be permissible to use this technology to enhance normal human traits (such as height or intelligence). Based on concerns about ethics and safety, germline cell and embryo genome editing are currently illegal in many countries.

Uses:
Genome editing is of great interest in the prevention and treatment of human diseases including **single-gene disorders** such as

•Cystic fibrosis
•Hemophilia and sickle cell disease.

It also holds promise for the treatment and prevention of more complex diseases, such as –

•Cancer
•Heart disease
•Mental illness and
•Human immunodeficiency virus (HIV) infection.

Applications of CRISPR-Cas9 in Cancer diagnostics

The occurrence and development of cancer is a highly complex process with multi-gene and multi-path interactions. Using specifically designed synthetic sgRNA, **CRISPR** systems can be used to detect nucleic acids involved in both infectious and non-infectious diseases.

CRISPR is being evaluated in the development of portable diagnostic tests for malignancies.
Presently, CRISPR technology is used to investigate the genetic mechanisms in almost all areas of cancer, from prevention to prognosis and treatment.

Conclusion:
The CRISPR/Cas9 system is becoming a widespread, practical, and useful tool against many types of diseases and it promises to accelerate as a cancer diagnostic tool. Combining CRISPR & functional genetic screening is a powerful approach to validate alternative genes associated with a specific phenotype.Targeting such underlying mechanisms opens up new avenues for treatment.

Advanced diagnostics - LC-MS/MS for in vitro diagnostics

-Dr. Abhik Banerjee MBBS (CU),
MD Pathology (MUHS) Zonal Technical Chief, East Zone Apollo Diagnostic, RRL Kolkata

LC-MS/MS technology combines high performance liquid chromatography (HPLC), a powerful analytical separation technique with mass spectrometry, a highly sensitive detection technique. This technology provides the opportunity for 'high-sensitivity detection' of conventional as well as specialized clinical chemistry analytes in areas of therapeutic drug monitoring, detection of inborn errors of metabolism (newborn screening by Tandem mass spectrometry), estimation of 25 Hydroxy Vitamin D, measurement of steroids, hormones and many more. LC-MS/MS technology addresses the increasing requirements on 'maximum detection' and innovations in diagnostics industry.

The technology is known for its ultra-high-speed detection, high sample throughput, extreme sensitivity, high molecular specificity and multiplexing capability resulting in accurate and consistent results for numerous and variable parameters. This cutting edge technology along with stringent quality control enables physicians to accurately diagnose and effectively manage congenital metabolic disorders by means of newborn screening, efficiently prevent graft rejection in case of organ transplantation and to treat auto-immune disorders by means of therapeutic drug monitoring of Immunosuppressant's and accurately diagnose Vitamin D deficiency or to monitor the outcome of Vitamin D supplementation.

New horizons and trends indicate a bright future for mass spectrometry in in vitro diagnostics and clinical laboratories. The number of potential clinical applications of mass spectrometry is varied and is definitely going to evolve in near future. LC-MS/MS-based methods and applications may soon be accepted even for routine clinical use in addition to its expanded and specialized tests for diagnosis and treatment. We need initiatives from major diagnostic laboratories to bring this technology to open a new direction in clinical pathology and research in India

Advanced diagnostics - Artificial intelligence in health care

- Dr. Srivatsa Prakhya
DGM – Technical and Advanced Diagnostics,
- Dr.Prerna Agarwal
Manager,technical operations.

Artificial intelligence in health care – COVID-19 has greatly accelerated the use of tele-health resources. In April of 2020, 43.5% of Medicare primary care visits utilized tele-health methods rather than in-person visits. One of the major benefits of tele-health over in-person alternatives is that it reduces contact between patients, healthcare workers, and other patients. Wearable devices enable healthcare workers to have real-time information on patient data while they remain at home.More importantly, tele-health's growth appears likely to continue even after the pandemic is over. This boom in tele-health seems likely to break $185.6 billion by 2026.

AI in diagnosis

With the advent of digital pathology, algorithms can provide aids in diagnosing complex situations. AI is poised to become a transformational force in healthcare by combining inputs from machine learning. AI helps in developing next generation tools to drill down to the pixel level of imaging thereby providing clearer data to analyse. In other applications such as neurology and radiology, AI is being used to enhance neural network simulation to make informed decisions faster to save lives and provide better treatment. Smart monitoring devices attached to patients can monitor, scan and evaluate data and provide suggestions to clinicians on a faster note regarding impending sepsis, mental deterioration, possibilities of a stroke and also immunotherapy options by analysing responses to cancer treatment. Electronic health records are fool proofed for data integrity and quality issues by negating biases. By powering a new generation of tools and systems that make clinicians aware in decision making processes, AI shall transform the medical field to the better for patient care. The major concern of data privacy and regulatory compliance from the social angle is debatable in all affairs concerning the use of data science in healthcare.

AI & the IOT industry

Internet of medical things – By 2025, the IoT industry will be worth $6.2 trillion. The healthcare industry has become so reliant on IoT technology in 2020 that 30% of that market share for IoT devices will come from healthcare. Almost all consumers have access to devices with sensors to collect healthcare data. Smart phones, tablets, wearable devices, collect data from an individual and submit for analysis for appropriate actions to be taken for the individual's benefit. Big data analytics help in storing and statistically analysing the data for informed decisions to be taken by a medical professional. On-going research also suggests that ingested nanoparticles can gather information on occult cancers, impending cardiac plaques and critically low blood levels of compounds and submit the same to healthcare experts from one patient so treatment can begin at the earliest.

AI & Predictive analytics – The global predictive analytics in healthcare market was valued at $1,806 million in 2017, and is estimated to reach $8,464 million at a CAGR of 21.2% from 2018 to 2025. It helps doctors and healthcare workers make treatment related decisions based on current data available regarding a consumer's health. It describes a methodology wherein insights are possible into future events to answer questions such as "What might happen in this case?"This entertains tailor made treatment strategies thereby shortening time taken for cure to occur. Predictive analytics finds a pattern in historical and transactional data and uses it to identify risks and opportunities for future. Based on the available descriptive data, predictive analytics uses different techniques, which include machine learning, statistical techniques, and predictive modeling to evaluate and determine the probable future. The purpose of predictive algorithms in healthcare is:

- To find the correlations in the patient's data;
- To find associations of the symptoms;
- To find familiar antecedents of the symptoms;
- To explore the impact of different factors (genome structure, clinical variable, et al.) on the course of treatment;
- To examine the possible influence of past and current diseases.

Depending on the goal of the analysis, a predictive algorithm can produce assumptions based either on available data directly from a given patient or general medical data from the public health datasets. It's important to remember that predictions are, in fact, nothing more than assumptions and probabilities.

DELIVERING EXCELLENCE

CERTIFIED QUALITY ASSURANCE

1 CAP Accreditation (College of American Pathologist)

- **National Reference Lab, Hyderabad**

18 NABL Accreditation

(National Accreditation Board for Testing and Calibration Laboratories)

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- **Emerging Diagnostics Company in Asia by CMO Asia**
- **Best Patient Safety Initiative by CMO Asia**
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Emerging Retailer of the Year – 2017
by Franchise India

Service Excellence Award 2017 (Healthcare) by Assocham

Service Excellence Award 2018 (Healthcare) by Assocham

Award for Excellence in Franchising and Business Development by Franchise India 2018

PATIENTS SPEAK

Apollo Diagnostics came highly recommended by my friends. I was particularly impressed by their cordiality, time management and process competence. Another must mention is the hygiene factor that is impeccable. Given the quality delivered, this is certainly a value for money experience. Thanks a lot Apollo Diagnostics...keep up the great work!

- Ms. A. Madhavi
(Warangal)

Finding the best place for a diagnostic test is as important as consulting the best doctor. The Apollo name was what guided me to Apollo Diagnostics and I am indeed glad that I came here. From attending us on time to sample collection, everything flowed so smoothly. I was particularly impressed by the hygiene.

- Mrs. Ferha Jabeen
(Old Bowenpally, Secunderabad)

I have always had a great experience with Apollo Diagnostics. The calls are unfailingly answered. The scheduled appointment is kept to the minute. The phlebotomist is well-trained in both technique and putting the patient at ease. Apollo Diagnostics undoubtedly delivers value for money. They will always be my top option for all the diagnostic needs of my family.

- Mr. Prashanta Kumar Jena
(JP Nagar, Bengaluru)

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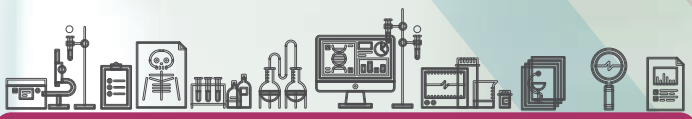
Expertise. Empowering you.



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