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AD EXPRESS
2021: ISSUE -1

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WHY APOLLO DIAGNOSTICS

"New Year's Message"

Apollo DIAGNOSTICS®
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Wishing you all a very Happy New Year 2021!!

Dear Readers,

As it has been rightly said "What the New Year brings to you will depend a great deal on what you bring to the New Year.", we believe in the same and therefore we would like to announce the launch of our medical journal "AD EXPRESS". This journal was envisioned and founded to represent the growing needs of laboratory medicine and diagnostics as a science and emerging and increasingly vital field, now widely recognised as an integral part of clinical medicine.

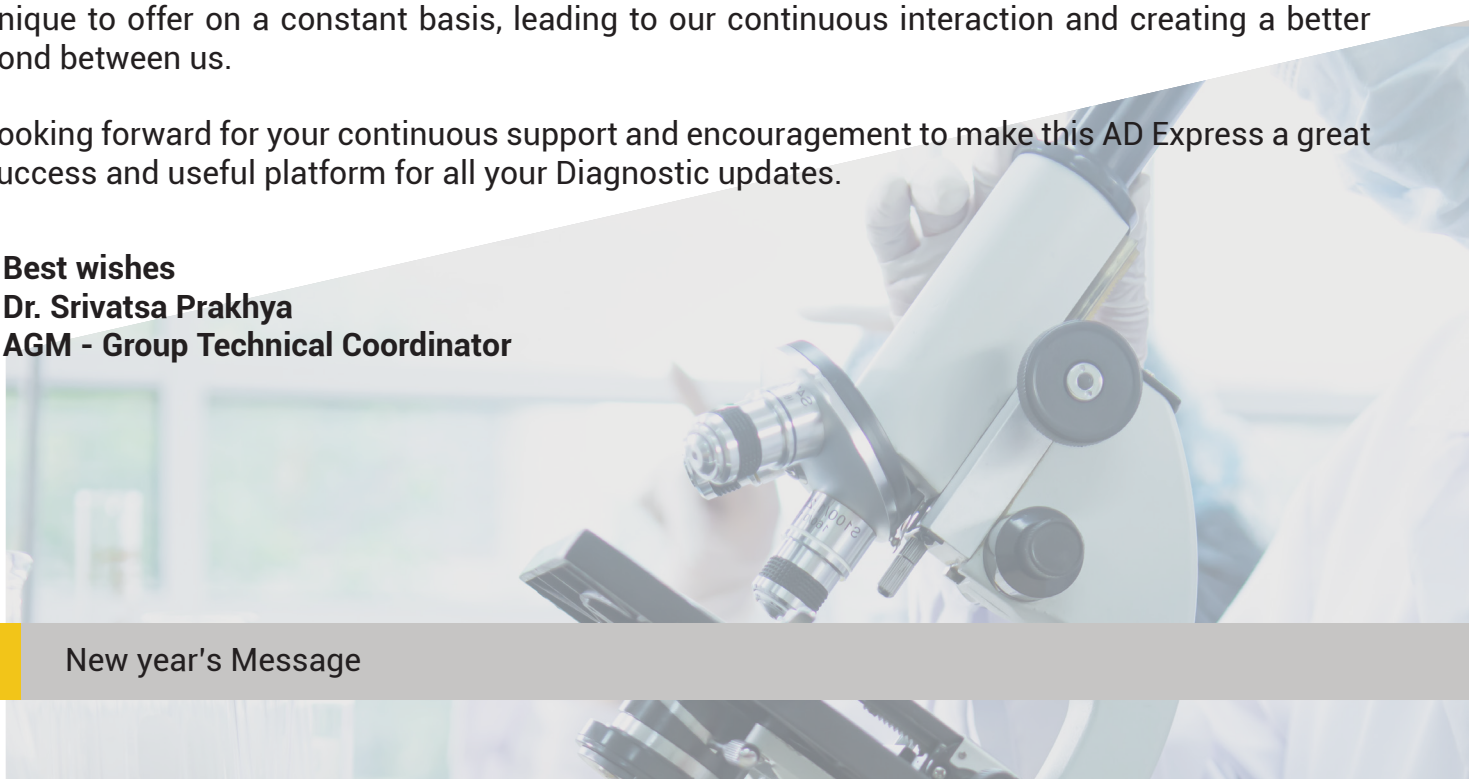
AD Express is ONE STOP destination for laboratory medicine comprising of theoretical aspects, application dependent studies, validation of emerging technologies, key technical affairs in diagnostics and rare case presentations, with high quality information and original data set.

This journal is introduced to represent the growing needs of diagnostics in medical practice. With the mission to become the voice and address researchers, clinicians and diagnosticians, Apollo Diagnostics introduce this platform that allow the clinicians to come forward and share interesting cases or findings in their respective areas of specialization and widens the horizon for other practitioners.

Your continuous support and appreciation always encourages us to get something new and unique to offer on a constant basis, leading to our continuous interaction and creating a better bond between us.

Looking forward for your continuous support and encouragement to make this AD Express a great success and useful platform for all your Diagnostic updates.

Best wishes
Dr. Srivatsa Prakhya
AGM - Group Technical Coordinator



From Editors' Desk

We are happy & proud to place the landmark First Edition (first issue) of AD Express in the hands of our Readers!

Apollo Diagnostics (AD) is indeed an offshoot from the large parent AHLL* banyan. We have achieved considerable pan-India ramification in a relatively short span of time.

AD Express is envisaged as a "printed ambassador" of AD to permit our Technical Team to communicate with the end-user of our services all over India – primarily the Medical Fraternity, and perhaps evolve to cover the Patient and the Care-Giver, in keeping with our emerging "One Apollo" philosophy. A perspective of Laboratory Medicine, it will be aimed to be presented to the reader touching upon hitherto less talked about focuses.

For example, there is a current onus on the early diagnosis of non-communicable diseases (NCD) alongside the earlier infection/infestation related lab-work that needs to be talked about, since significant societal disease burden can be lowered by early diagnosis.

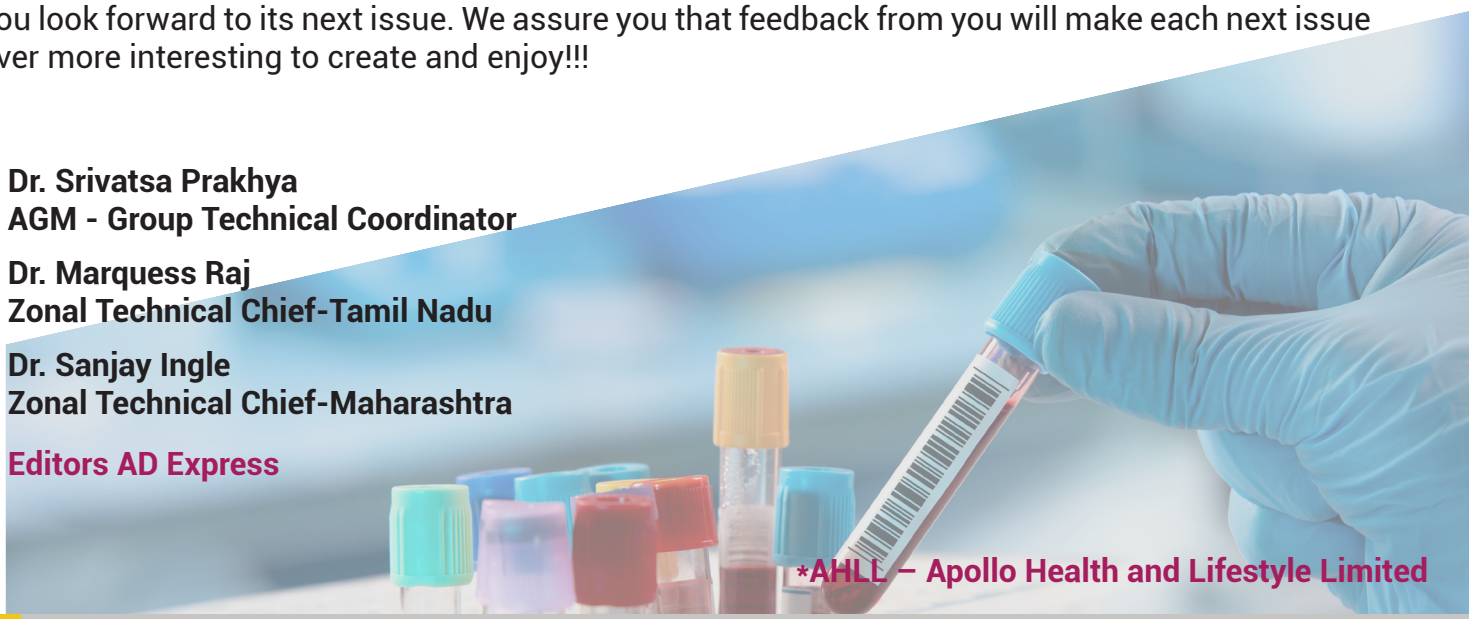
Dear Reader, we look forward to your feedback eagerly!!!
We do not want AD Express to become just another periodical - we look forward to the time when you look forward to its next issue. We assure you that feedback from you will make each next issue ever more interesting to create and enjoy!!!

Dr. Srivatsa Prakhya
AGM - Group Technical Coordinator

Dr. Marquess Raj
Zonal Technical Chief-Tamil Nadu

Dr. Sanjay Ingle
Zonal Technical Chief-Maharashtra

Editors AD Express



*AHLL – Apollo Health and Lifestyle Limited

COVID-19: Testing Strategies and Emergence of Strains

Dr. Sireesha Vaidya, Dr Lakshmi.Addala, B.Ajay Babu, Vasavi Induri, K. Deepika, N. Keshava, K. Kiran, Haju Katravath, Mr. V. Raju, Mr. Ramesh Reddy, Dr. Smita Hiras Sudke

Apollo Diagnostics, Apollo Health Lifestyle Limited, National Reference Lab, Hyderabad.

Introduction:

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a novel evolutionary divergent RNA virus, is responsible for the present devastating COVID-19 pandemic. Public health emergencies (such as COVID-19 pandemic) are stressful situations for people and communities which lead to social stigma, the unknown factors and lack of knowledge on COVID 19 created fear, anxiety, myths and rumours around heightened social stigma.

Hundreds of different strains of corona virus existed and three of them namely SARS-CoV (southern China in November 2002), MERS-CoV (September 2012 in Saudi Arabia), and SARS-CoV-2 (Wuhan, China 2019) posed more risk[1]. All of them originated as animal infections, developed further and eventually transmitted to humans. According to WHO, first case of SARS-CoV-2 was identified in Wuhan, China in December 2019. Initially, the health officials noticed increase in pneumonia cases with an unidentified cause. The cases were attributed to seafood and poultry, but the virus has most likely evolved from animal source though exact source is unknown. In span of 2-3 months, the virus has spread to hundreds of countries mostly due to transmission from person-to-person and WHO named it as COVID-19 - Corona Virus Disease 2019.

Global Incidence of COVID-19 as on 28 December 2020 (WHO report):
Confirmed Cases: 79,673,754 & Deaths: 1,761,381
Cases in India: Confirmed cases: 10,207,871 & Deaths: 147,901

COVID-19 can be symptomatic or asymptomatic
A symptomatic COVID-19 case is a case that has developed signs and symptoms compatible with COVID-19 virus infection. Symptomatic transmission refers to transmission from a person while they are experiencing symptoms.

An asymptomatic laboratory-confirmed case is a person infected with COVID-19 who does not develop symptoms. Asymptomatic transmission refers to transmission of the virus from a person, who does not develop symptoms. The ratio of being asymptomatic is 1:5.

Methodologies:
Different methodologies inclusive of molecular and serology are used for diagnosis of COVID-19

Table 1: Different tests for the diagnosis of COVID -19

	Molecular Test*	Antigen Test*	Antibody Test*
Type of Test	Real time-PCR test*, Nucleic Acid Amplification Test (NAAT), LAMP test, CRISPR test (Lateral flow assay).	Rapid chromatographic immunoassay	Serological test. IgG and IgM
Specimen Type	Nasopharyngeal / nasal or throat swab/ BAL fluid/Sputum/Lower respiratory samples.	Nasal or nasal pharyngeal swab	Serum / whole blood/ finger prick
Target	Detects RNA of virus targeting minimum of two genes, mostly S gene (Spike protein), N gene (Nucleocapsid), RdRP (RNA-dependent RNA polymerase), ORF 1ab (Open reading Frame 1 ab).	Qualitative detection of specific antigens present in human nasopharynx.	Antibodies against SARS-COV-2
Turn Around Time.	8 hours to 48 hours depending on the location.	15 - 30 minutes, depending on the test.	Same day
Interpretation	Diagnoses active corona virus infection	Diagnoses active corona virus infection	Covid-19 IgG & IgM test is intended for use as an aid in identifying individuals with an adoptive immune response to recent or prior infection.
Limitations	Pre-analytical errors leading to false negative and false positive results. Sample collection of naso/oropharyngeal swabs must be collected by trained personnel to avoid false negative results.	A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor quality specimen is obtained.	IgG & IgM test can be used for the serosurveys & not for diagnosis. Diagnose COVID-19 at the time of the test or show that you do not have COVID-19

***Available at Apollo Health Lifestyle Limited, National Reference Laboratory (NRL), Hyderabad.**

Other tests:

HR CT chest scan diagnostic test in clinically suspicious COVID-19 with a negative RT-PCR test.

Advances in molecular testing strategies of COVID-19:

Next Generation Sequencing (NGS) based technology: With recent findings of different strains of COVID 19 in infected population groups, sequence-based assays to elucidate the gene sequence of virus are being applied.

Genetic variants/strains found globally:

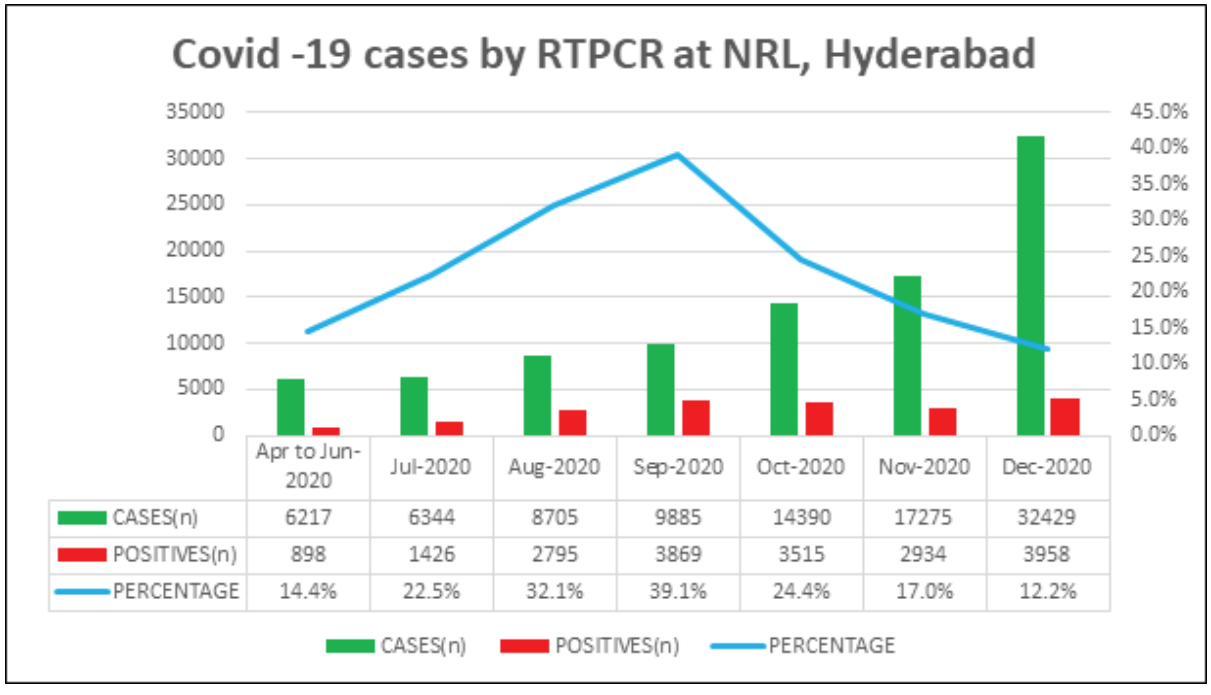
Based on GISAID (Global Initiative on Sharing All Influenza Data)[6], 2020 consortium the geographical distribution of strains world-wide are given (Table 2).

Table 2: Geographic distribution of strains of SARS-CoV-2.

Strain	lineage	Region of emergence / prevalence
L	--	Asia
S	A	Restricted regions of USA, Spain
V	B.2	USA
G	B.1	Europe
	B.1.1.7	UK Strain
GR	B.1.1	Italy, South America
GH	B.1	Germany, France, North America

The L strain (Wuhan strain, December, 2019) first mutated to S strain i.e., beginning of Jan, 2020 and by mid-January 2020, strains V and G emerged. To date, strain G is the most widespread and mutated into strains GR and GH at the end of February 2020. In Asia, where the Wuhan L strain initially appeared, the spread of strains G, GH and GR is increasing[2]. Significantly, recent experimental evidence suggests emergence of highly fit variant strains of 'G' rendering to enhanced transmissibility of virus. This characteristic of virus is decoded to amino acid changes in Spike protein [3,4]. And, the recent findings of new strain called as "UK strain" of SARS-CoV-2 is noted with mutations in Spike protein.

RT-PCR Tests Performed at NRL, Hyderabad from April, 2020 to December, 2020 were **95,245** cases out of which **19,395** were positives showing an average positivity rate of 20.3%.



Overall, results of epidemiological data on SARS-CoV-2 infections revealed that frequency of amino acid mutations was relatively higher in the SARS-CoV-2 genome sequences of Europe (43.07%) followed by Asia (38.09%), and North America (29.64%)[5]. Nevertheless, surveillance programs for constant monitoring of mutations across the globe and vigilance for escape mutants in vaccination program is pivotal.

In view of the dynamic nature of mutational property of the virus, manufacturers should provide updated technology or methodology for accurate diagnosis of various strains of COVID-19.

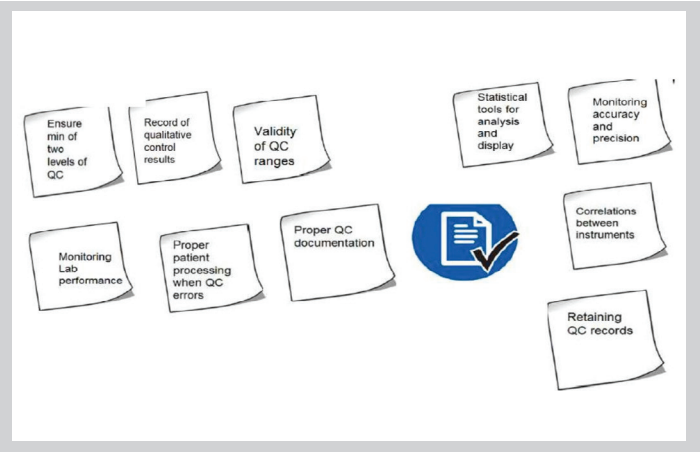
References:

1. "Transmission of COVID-19". European Centre for Disease Prevention and Control. Retrieved 6 December 2020.
2. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Frontiers in Microbiology: Daniele Mercatelli and Federico M. Giorgi; 22 July 2020.
3. Baric RS. Emergence of a Highly Fit SARS-CoV-2 Variant. N Engl J Med. 2020 Dec 31;383(27):2684-2686. doi: 10.1056/NEJMcibr2032888. Epub 2020 Dec 16. PMID: 33326716.
4. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J, Fontes-Garfias CR, Mirchandani D, Scharton D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN, Menachery VD, Xie X, Plante KS, Weaver SC, Shi PY. Spike mutation D614G alters SARS-CoV-2 fitness. Nature. 2020 Oct 26. doi: 10.1038/s41586-020-2895-3. Epub ahead of print. PMID: 33106671.
5. https://www.nature.com/articles/s41598-020-70812-6.
6. https://www.gisaid.org/references/statements-clarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses.

Ensuring Accurate Internal Quality Control (IQC) Results

Narendra Vadlamudi, Praneeth Kumar. M, Hemanta Das, Vandana P.
Apollo Diagnostics, Apollo Health Lifestyle Limited, National Reference Lab, Hyderabad.

Monitoring Internal Quality Control (IQC) is of paramount importance in ensuring accurate patient test results are reported. But while testing instruments, processes and reagents are undoubtedly crucial in the laboratory setting, they are only one part of a bigger quality control picture. The ability to correctly interpret the resulting data arising from internal QC is vital, however in order to do so adequately, laboratories need to mandatorily monitor their performance.



Optimal utilization of Information Technology which is custom modified and user-friendly for laboratory staff is very essential, so that maintaining quality is not a nightmare with multitude of tasks that are undertaken by everyone in the laboratory.

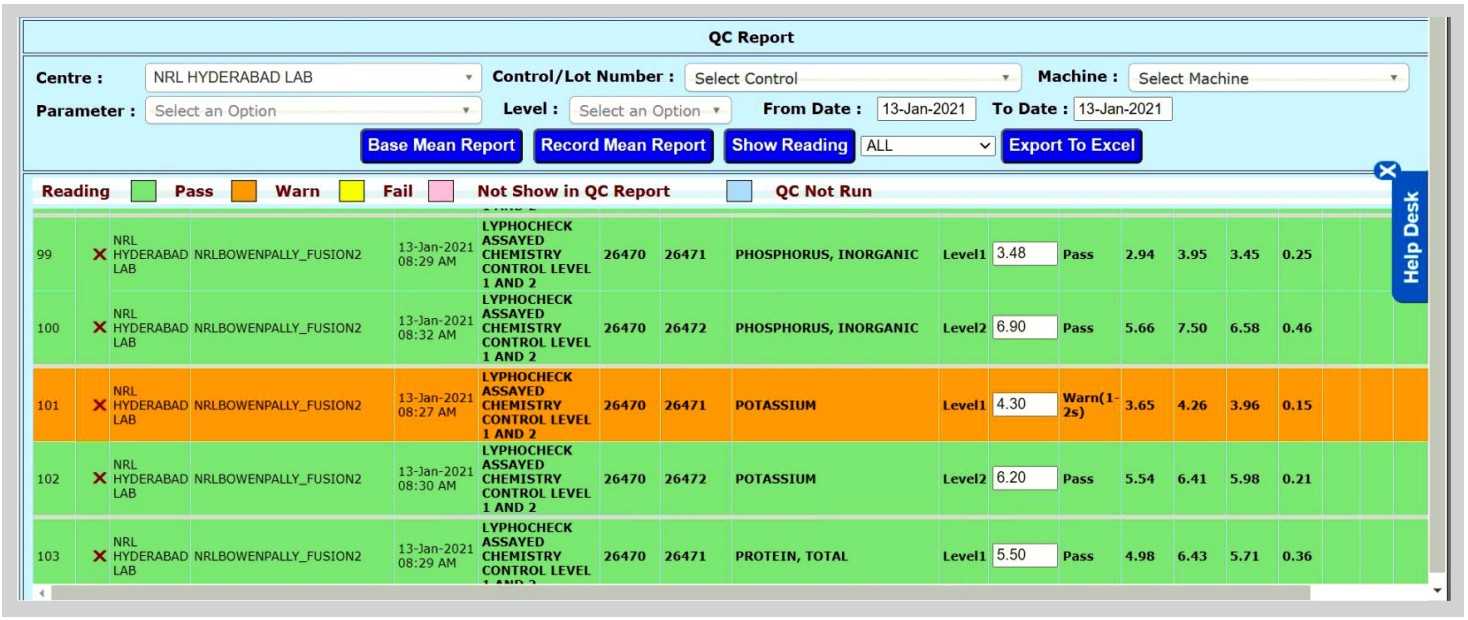
The LIS at Apollo Diagnostics is very dynamic, helping all our labs across India to maintain stringent quality for every report that is released.

For example, a foolproof feature in our LIS is that if the QC results have failed for any test across the lab specialties, the software does not allow the instrument to process patient samples for that test. Also, a simple colour-code system helps to immediately identify at a single glance whether the Internal QC results are acceptable or failed for patient runs, so that immediate corrective action is taken and the testing process continues for that test.

Our QC data management program provides statistical review, documentation and analysis across all controls used in the laboratory.

It is a networkable QC program to cover every section in our laboratories.

It analyzes all quality control data to assist the laboratory staff in completing their QC tasks per lab policies and regulations.



QC Report

Centre : NRL HYDERABAD LAB | Control/Lot Number : Select Control | Machine : Select Machine

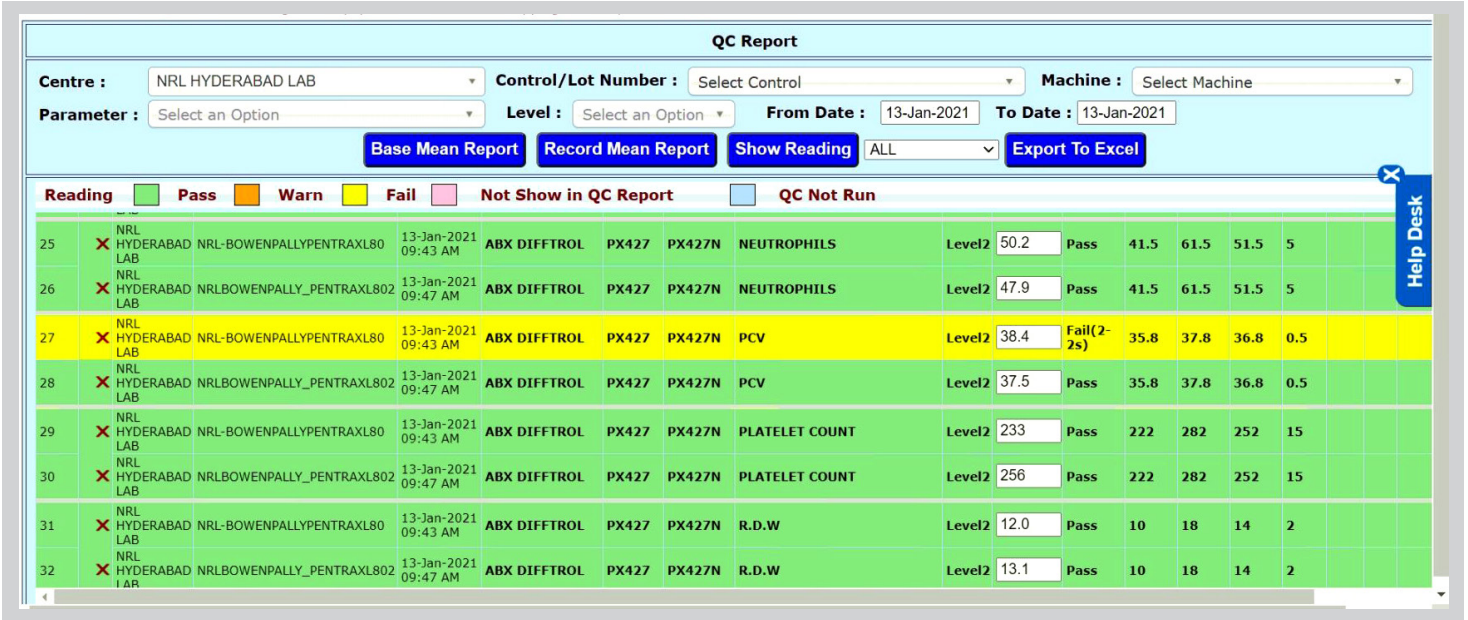
Parameter : Select an Option | Level : Select an Option | From Date : 13-Jan-2021 | To Date : 13-Jan-2021

Base Mean Report | Record Mean Report | Show Reading | ALL | Export To Excel

Reading | Pass | Warn | Fail | Not Show in QC Report | QC Not Run

Help Desk

99	X	NRL HYDERABAD LAB	NRLBOWENPALLY_FUSION2	13-Jan-2021 08:29 AM	LYPHOCHECK ASSAYED CHEMISTRY CONTROL LEVEL 1 AND 2	26470	26471	PHOSPHORUS, INORGANIC	Level1	3.48	Pass	2.94	3.95	3.45	0.25	
100	X	NRL HYDERABAD LAB	NRLBOWENPALLY_FUSION2	13-Jan-2021 08:32 AM	LYPHOCHECK ASSAYED CHEMISTRY CONTROL LEVEL 1 AND 2	26470	26472	PHOSPHORUS, INORGANIC	Level2	6.90	Pass	5.66	7.50	6.58	0.46	
101	X	NRL HYDERABAD LAB	NRLBOWENPALLY_FUSION2	13-Jan-2021 08:27 AM	LYPHOCHECK ASSAYED CHEMISTRY CONTROL LEVEL 1 AND 2	26470	26471	POTASSIUM	Level1	4.30	Warn(1-2s)	3.65	4.26	3.96	0.15	
102	X	NRL HYDERABAD LAB	NRLBOWENPALLY_FUSION2	13-Jan-2021 08:30 AM	LYPHOCHECK ASSAYED CHEMISTRY CONTROL LEVEL 1 AND 2	26470	26472	POTASSIUM	Level2	6.20	Pass	5.54	6.41	5.98	0.21	
103	X	NRL HYDERABAD LAB	NRLBOWENPALLY_FUSION2	13-Jan-2021 08:29 AM	LYPHOCHECK ASSAYED CHEMISTRY CONTROL LEVEL	26470	26471	PROTEIN, TOTAL	Level1	5.50	Pass	4.98	6.43	5.71	0.36	



QC Report

Centre : NRL HYDERABAD LAB | Control/Lot Number : Select Control | Machine : Select Machine

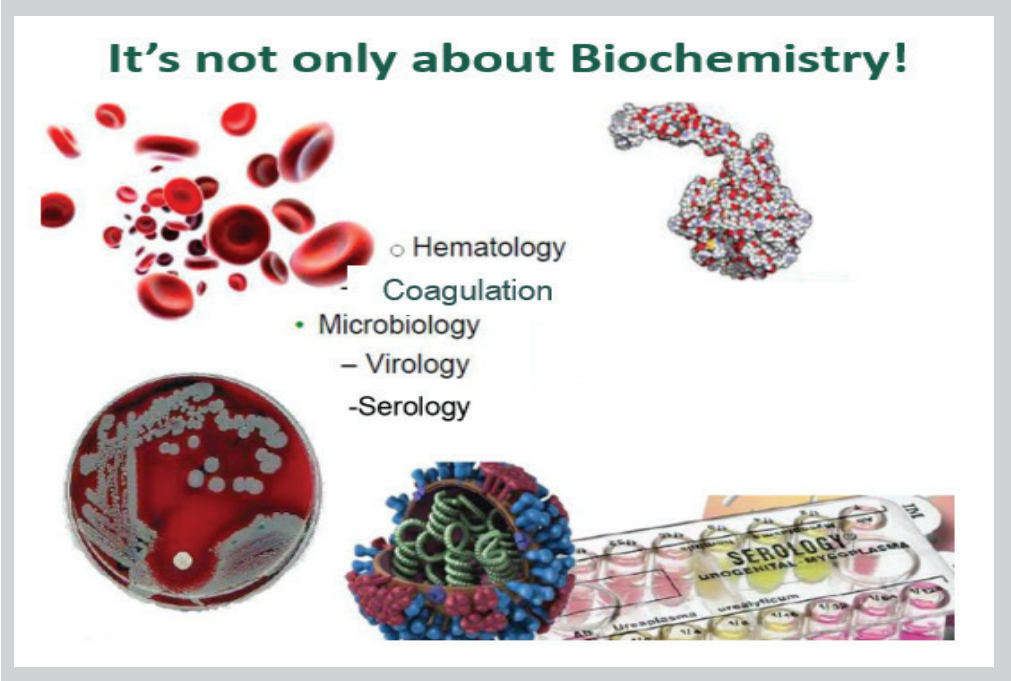
Parameter : Select an Option | Level : Select an Option | From Date : 13-Jan-2021 | To Date : 13-Jan-2021

Base Mean Report | Record Mean Report | Show Reading | ALL | Export To Excel

Reading | Pass | Warn | Fail | Not Show in QC Report | QC Not Run

Help Desk

25	X	NRL HYDERABAD LAB	NRL-BOWENPALLYPENTRAXL80	13-Jan-2021 09:43 AM	ABX DIFFTROL	PX427	PX427N	NEUTROPHILS	Level2	50.2	Pass	41.5	61.5	51.5	5	
26	X	NRL HYDERABAD LAB	NRLBOWENPALLY_PENTRAXL802	13-Jan-2021 09:47 AM	ABX DIFFTROL	PX427	PX427N	NEUTROPHILS	Level2	47.9	Pass	41.5	61.5	51.5	5	
27	X	NRL HYDERABAD LAB	NRL-BOWENPALLYPENTRAXL80	13-Jan-2021 09:43 AM	ABX DIFFTROL	PX427	PX427N	PCV	Level2	38.4	Fail(2-2s)	35.8	37.8	36.8	0.5	
28	X	NRL HYDERABAD LAB	NRLBOWENPALLY_PENTRAXL802	13-Jan-2021 09:47 AM	ABX DIFFTROL	PX427	PX427N	PCV	Level2	37.5	Pass	35.8	37.8	36.8	0.5	
29	X	NRL HYDERABAD LAB	NRL-BOWENPALLYPENTRAXL80	13-Jan-2021 09:43 AM	ABX DIFFTROL	PX427	PX427N	PLATELET COUNT	Level2	233	Pass	222	282	252	15	
30	X	NRL HYDERABAD LAB	NRLBOWENPALLY_PENTRAXL802	13-Jan-2021 09:47 AM	ABX DIFFTROL	PX427	PX427N	PLATELET COUNT	Level2	256	Pass	222	282	252	15	
31	X	NRL HYDERABAD LAB	NRL-BOWENPALLYPENTRAXL80	13-Jan-2021 09:43 AM	ABX DIFFTROL	PX427	PX427N	R.D.W	Level2	12.0	Pass	10	18	14	2	
32	X	NRL HYDERABAD LAB	NRLBOWENPALLY_PENTRAXL802	13-Jan-2021 09:47 AM	ABX DIFFTROL	PX427	PX427N	R.D.W	Level2	13.1	Pass	10	18	14	2	



In a nut shell, a highly intelligent IT enabled Quality Systems at Apollo Diagnostics strive to

- Identify trends, instrument errors or reagent issues as soon as they arise, assuring validity and increasing confidence in the accuracy of results.
- Improve External QA performance by eliminating any undetected bias.
- Minimize false rejections whilst maintaining high error detection through the use of statistical QC procedures required in laboratories.
- Help our laboratories have confidence in reporting of results.
- Facilitate regulatory requirements and meet ISO 15189, NABL & CAP accreditations.

First Trimester Screening: Recent Markers

Dr. Suhasini. D, Dr. Smita Hiras Sudke, Dr. Sujana Reddy, Dr. Lakshmi Addala, Mr. J. Naresh, Mr. K. Naresh, Ms. G. Deshma, Mr. N. Sachin.
Apollo Diagnostics, Apollo Health Lifestyle Limited, National Reference Lab, Hyderabad.

Conventional screening in first trimester includes established biochemical markers PAPP-A & free beta hCG with ultrasound markers NT & NB for risk calculation. Recent studies show that additional maternal markers like placental growth factor (PlGF) and alpha fetoprotein (AFP) measured in the first trimester of pregnancy could be more effective and have higher detection rate than two biochemical markers.

Plgf as a first trimester Trisomy 21 marker*:

Placental growth factor (PlGF) is an angiogenic protein synthesized by syncytiotrophoblasts. PlGF is a particularly important maker for women who present at 12-13 weeks when PAPP-A becomes less informative. PAPP-A is very good if the sample is taken at 9-10 weeks, but less informative when the sample is taken at 13 weeks. Studies show about 50% reduction in the number of unnecessary invasive tests by adding PlGF in 1st trimester screening.

Plgf as a Preeclampsia marker*:

A low PlGF concentration is considered to be a reflection of placental dysfunction, and has been shown to precede the clinical onset of preeclampsia evident from both the first-and second-trimesters of pregnancy. It correlates strongly with time to delivery in women with suspected preterm (<35weeks' gestation) pre-eclampsia [2].

PlGF has been shown to be a good rule-out test for pre-eclampsia in the first trimester with NPVs between 0.95 and 0.97. Other tests used for the prediction of time to delivery measure additionally soluble fms-like tyrosine kinase-1 (sFlt-1) and utilize the ratio of both factors (sFlt-1/PlGF). [3] These tests are advocated by the UK National Institute for Health and Care Excellence (NICE) for use in women with suspected preterm pre-eclampsia, in particular as a 'rule-out' tool.

1st Trimester Quad Test*:

The current restricted availability in India of quality ultrasound NT could be overcome, to a great extent, by the introduction of a first-trimester four-marker serum-only test [1]. 1st Trimester quad test, which includes PAPP-A, free ßhCG, PlGF and AFP is the first biochemistry-only screening protocol for Down syndrome in the first trimester. This test provides high detection rate for 1st Trimester screening of Down syndrome. With AFP in first trimester, there is an opportunity to identify women at high risk for Open Spina bifida using additional ultrasound markers.

***Available at Apollo Diagnostics, Hyderabad.**

Details	Markers	5%FPR
1st Trimester	PAPP-A + free βhCG	66%
1st Trimester – Quad	PAPP-A + free βhCG + PlGF + AFP	74%
2nd Trimester – Quad	free βhCG + uE3 + AFP + Inhibin-A	72-83%

Studies show that when NT was also included, the rates were 95% for 5% FPR.
A protocol of NT plus the four serum markers enhances the performance compared with NT, PAPP-A and free b-hCG. The same cut-off risk of 1 in 250 at term used by other screening protocols in India could be maintained for the new tests.

Performance has not only been shown to be similar to the second-trimester Quad test but also found to facilitate early screening for preeclampsia and open spina bifida.

An overview of Maternal Screens at NRL, Apollo Diagnostics, Hyderabad

	2016	2017	2018	2019	2020	Overall
Trisomy 21						
Low	843	6629	26078	37325	36839	107714
High	47	307	738	757	997	2846
Total	890	6936	26816	38082	37836	110560
Positive Rate	5.28	4.43	2.75	1.99	2.64	3.42
Trisomy 18						
Low	888	6918	26765	38033	37744	110348
High	2	18	51	49	92	212
Total	890	6936	26816	38082	37836	110560
Positive Rate	0.22	0.26	0.19	0.13	0.24	0.21
Trisomy 13						
Low	890	6925	26786	38052	37796	110449
High		11	30	30	40	111
Total	890	6936	26816	38082	37836	110560
Positive Rate	0.00	0.16	0.11	0.08	0.11	0.09

References:

1. Seshandri Suresh, Howard S. Cuckle, Sujatha Jagadeesh, Kushagradhi Ghosh, Gayathri Vemavarapu, Tulika Taval, Sudarshan Suresh. Down's Syndrome Screening in the First Trimester with Additional Serum Markers: Indian Parameters. The Journal of Obstetrics and Gynecology of India (January–Feb ruary 2020) 70(1):12–17 https://doi.org/10.1007/s13224-018-1198-1.
2. F. P. Mccarthy, C. Gill, P. T. Seed, K. Brahmam, L. C. Chappell and A. H. Shennan. Comparison of three commercially available placental growth factor-based tests in women with suspected preterm pre-ec lampsia: the COMPARE study. Ultrasound Obstet Gynecol 2019; 53: 62–67, Published online 5 Decem ber 2018.
3. N O'Gorman etal. Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2017 Jun;49(6):751-755, doi: 10.1002/uog.17399. Epub 2017 May 2014.

Haemoglobin Electrophoresis:

Road Map for Early Diagnosis & Prevention of Haemoglobinopathies

Dr Smita Hiras Sudke, Dr K Ramakrishna, Dr MM Poornima, Dr N Srinivas, Dr P Srivatsa, Dr D Suhasini
Apollo Diagnostics, Apollo Health Lifestyle Limited, National Reference Lab, Hyderabad.

A haemoglobin electrophoresis test is a blood test used to measure and identify normal and abnormal forms of haemoglobin in the bloodstream. [1] Haemoglobin is the protein inside red blood cells responsible for transporting oxygen to tissues and organs. Abnormal types of haemoglobin may be acquired by inheriting gene mutations that are responsible for producing abnormal haemoglobin. Abnormal haemoglobin carries lesser oxygen as compared to the normal haemoglobin. These blood cells also have a shorter life span than that of the normal haemoglobin. This could lead to group of conditions called the haemolytic anaemias.

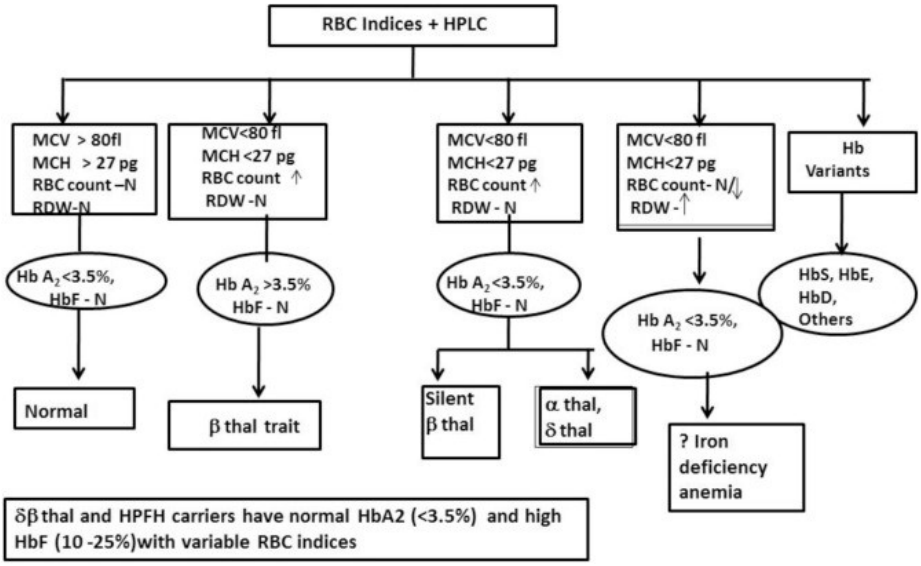
Haemoglobin-A (HbA) is the main form of Haemoglobin in a normal adult. Haemoglobin-F (HbF) is the major Hb in a fetus, and the remainder is HbA2. Approximately 800 different mutant forms of haemoglobin have been identified. Some of these are asymptomatic, especially among heterozygotes. Some may cause major morbid effects, especially in homozygotes. Globins found in different haemoglobins during fetal and adult life are indicated by Greek characters; α, β, γ, and δ. Variations in the amino acid composition of the globin chain cause the hemoglobinopathies. [1]

NORMAL LEVELS OF HAEMOGLOBIN TYPES

Haemoglobin electrophoresis test result refers to the percentages of the different types of haemoglobin that can be found in the blood. However, this is different in babies and in adults:

Age	Type of haemoglobin	Percentage
New-Born	Haemoglobin F	60-80%
>1 year to Adult	Haemoglobin F	1% to 2%
>1 year to Adult	Haemoglobin A	95% to 98%
>1 year to Adult	Haemoglobin A2	2% to 3%

What Does Haemoglobin Electrophoresis investigate?



The test can help us to figure out what type of blood disorder it is (Hemoglobinopathies).
This test is usually done along with other blood tests.

a) **As part of a routine check-up:** Haemoglobin is tested to conduct a follow up on a complete blood test during a routine check-up.

b) **To diagnose blood disorders:** In cases where symptoms of anemia are noticed in the patients, this test will help them find any abnormal types of haemoglobin in the blood. These could be a sign of the disorders including:

- Sickle Cell Anemia
- Thalassemia(alpha & beta)
- Hb-C disease
- Hb-E disease
- Hb-D disease

c) **To monitor the treatment and**

d) **To screen for genetic conditions:** People who have a family history of inherited anaemias such as thalassemia or sickle cell anemia could choose to screen for these genetic disorders before having children. A haemoglobin electrophoresis will indicate if there are any abnormal types of haemoglobin caused by genetic disorders. New-borns are also routinely screened for these genetic haemoglobin disorders.

Genetic counselling & Prenatal Diagnosis:

Both pre and post-test counselling are important particularly for prenatal diagnosis programmes to eliminate the irrational fears among people particularly with respect to stigmatization.[2]
Prenatal diagnosis is ideally done in the first trimester of pregnancy (10–12 weeks gestation) by chorionic villus sampling and DNA analysis which requires well trained obstetricians and sonologists for fetal tissue sampling as well as a competent laboratory for molecular diagnosis. Amniotic fluid can also be used for DNA-based prenatal diagnosis. In India, many couples who are at-risk are identified extremely late and the fetal blood sampling is done by cordocentesis at 18–19 weeks gestation and HPLC based analysis of fetal blood is also done.[2]
Characterization of these mutations is done by allele specific priming (ARMS) or by reverse dot blot hybridization (RDB). Each of these approaches have some advantages and limitations. DNA sequencing is also becoming cheaper and as the β globin gene is not large, direct sequencing is also possible and may ultimately become more cost effective as the repertoire of mutations in

Road Map Ahead:

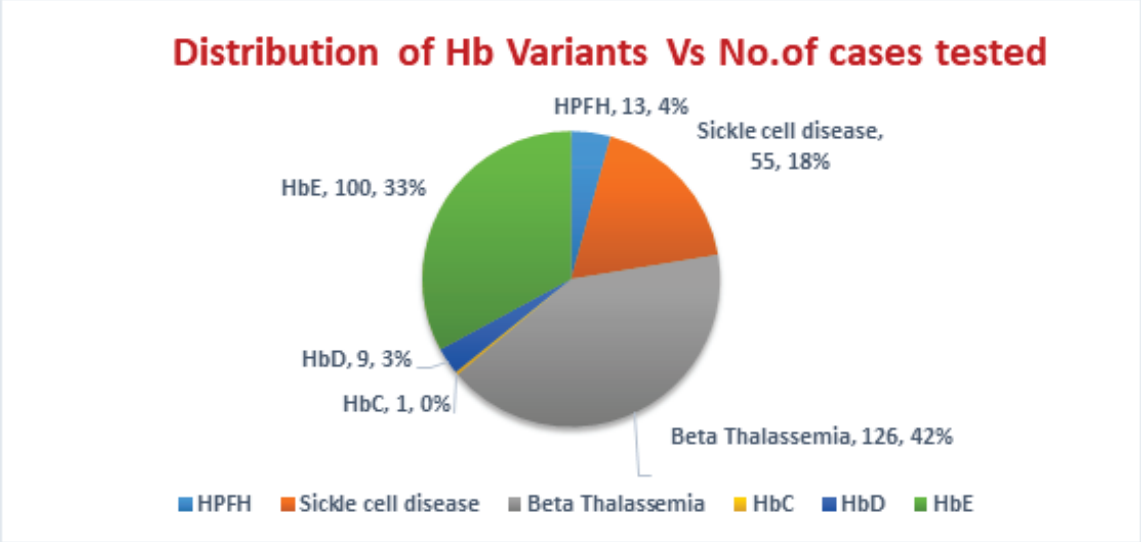
Prenatal diagnosis of β-thalassemia by the RDB or ARMS technique can prevent the birth of an affected child in developing countries in which β-thalassemia is prevalent. [3]

Overview of Haemoglobin electrophoresis cases from Sept'2020 to Dec'2020 at NRL, Apollo Diagnostics, Hyderabad

A retrospective analysis of 1432 cases were tested at NRL during the period September to December 2020, irrespective of age, gender, pregnancy status & region as a part of routine health checks or as a prenatal testing or as a diagnostic tests A microcytic hypochromic picture was predominantly observed in hemoglobinopathies like Hb-C, Hb-D, Hb-E & Thalassemia.

HbA2 levels play a major role in interpreting iron deficiency vs beta thalassemia. Sickle cell disease was confirmed with a positive sickling test and presence of sickle cells (sickle shaped cells) seen on a parallel peripheral smear. Out of 1432cases, 304 were hemoglobinopathies with 70% of them presenting with heterozygous disease; 17% were homozygous and 13% were compound cases with distribution as shown below . We have observed that compound cases are majorly associated with Heterozygous beta thalassemia & HbE.

Hb Variant	Cases	Homozygous	Heterozygous	Compound
HPFH	13	2	9	2
Sickle cell disease	55	17	34	4
Beta Thalassemia	126	2	101	23
HbC	1	0	1	0
HbD	9	1	4	4
HbE	100	31	63	6
Total Cases (n)	304(21%)	53 (17%)	212(70%)	39 (13%)



Limitations:

1. HbA2 and HbF levels should be considered in conjunction with family history and laboratory data including serum iron, TIBC, ferritin, red cell morphology, haemoglobin, HCT, and MCV.
2. Blood transfusion may temporarily obscure or dilute abnormal haemoglobin.
3. Quantitation of haemoglobins is performed optimally after one year of age. [1]

References:

1. Wallach's Interpretation of Diagnostic tests, pg 977-979
2. Burden of Thalassemia in India: The road map for control : Roshan Colah, Khushnooma Italia, Ajit Gorakshakar; Paediatric Haematology Oncology Journal, Volume 2, Issue 4, Dec'2017, pg 79-84.
3. Prenatal Diagnosis in Beta-Thalassemia: An Indian Experience: Agarwal S, Gupta A, Gupta U.R, Sarwai S, Phadke S, Agarwal S.S: Fetal Diagn Ther 2003;18:328–332

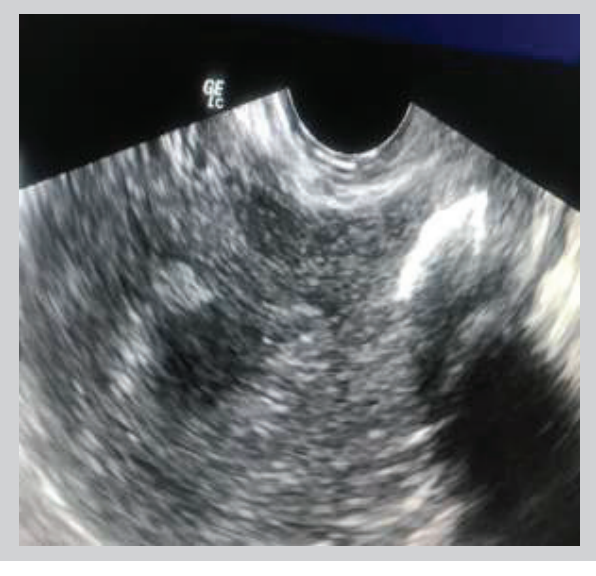
Retained Products Of Conception Causing Secondary Infertility

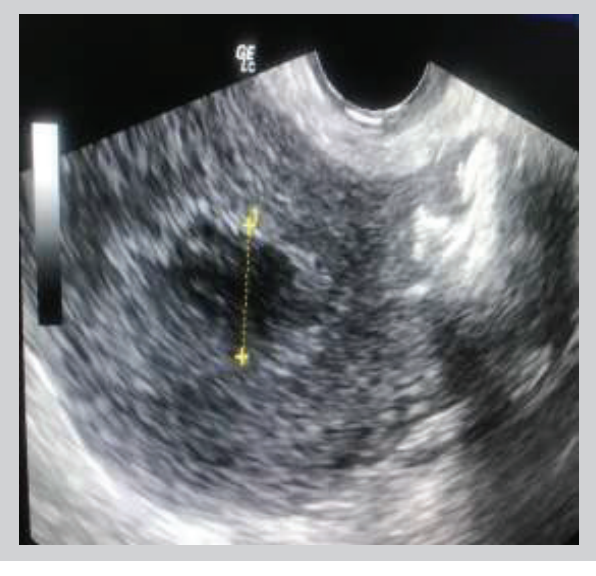
Dr. Kalyan Barmade, Dr Sanjay Ingle
Apollo Diagnostics: Maharashtra

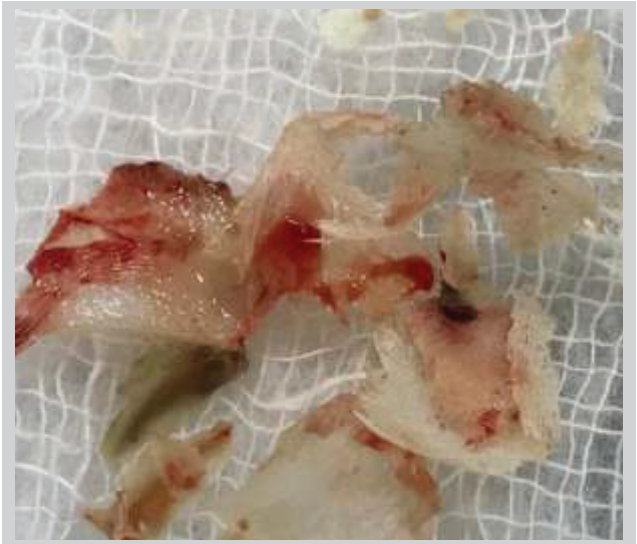
Intrauterine retention of fetal bones is a rare complication of a second trimester miscarriage. These patients present with pelvic pain, abnormal uterine bleeding, dysmenorrhea, dyspareunia and secondary infertility. This article describes two cases of retained fetal bones causing secondary infertility which was diagnosed on Hysteroscopy.

A 30-year woman (P2 L2 A2) presented with secondary infertility. She was unable to conceive, for the last three years despite regular unprotected intercourse. She had regular menstrual periods without significant dysmenorrhea or dyspareunia. During her initial assessment and workup, systemic review and past medical history were of little significance. Her first conception resulted in abortion at 2 months while the next two conceptions resulted in live births with the younger child being 6 yrs old. She had a second trimester MTP done 4 yrs back. Subsequent menstrual cycle was normal. No abnormality was found on general physical examination. Bimanual vaginal examination revealed a normal sized uterus with closed cervical os and normal adnexae. Semen analysis of her husband was normal and so were her serum LH, FSH, and prolactin. Her hysteroscopy revealed calcific shadows in uterine cavity. She was counselled and asked to come for dilation and curettage (D & C) under general anesthesia.

The other case is of a 27 years female with bleeding PV on & off since 3 months and history of second trimester abortion three months back. Her hysteroscopy revealed calcific shadows in uterine cavity. She was counselled and asked to come for dilation and curettage (D & C) under general anesthesia. D & C was performed in both the cases, uterine cavity had a gritty surface which was bony hard in places. Bonney's forceps were used to grasp hard structures protruding into the cavity, assisted with ultrasound, to make sure the forceps were within the uterine cavity. Initially fragments of calcified material were removed and then long slender structures were recovered, which were identified as fetal bones. Procedure was completed without any complication and no heavy bleeding was encountered. Histopathology work up of both the cases were reported as bony fragments.







USG showing Bony spicules

Bony spicules

Prolonged intrauterine retention of any fetal bones can be a cause of secondary infertility. The exact incidence remains unclear. This case report describes two cases with secondary infertility of three years and three months. Workup revealed calcific foci in endometrium on hysteroscopy. After dilation and curettage, fetal bones were retrieved. Unexplained infertility can often be explained if before applying this label, uterine factor is also taken into account. When common causes like tubal blockage, male factor, and ovulatory dysfunction have been excluded, infrequent causes like polyps, adhesions, and in rare instances foreign bodies also should be looked for Second trimester dilation and evacuation is not preferred by Royal College of Gynecologists and Obstetricians (RCOG); rather medical termination is the preferred choice.

D & E procedure at this gestation is likely to be complicated by a number of things, retained products of conception being one of them. This can be eliminated if the procedure is performed under ultrasound guidance. When suspecting retained fetal bones, then transvaginal ultrasound scan (TVS) should be ordered. Echogenic endometrium is the most likely finding in these cases. Differentials for echogenic endometrium include intrauterine contraceptive devices, foreign bodies, calcified submucous fibroids, Asherman syndrome or rare occurrences such as heterotopic bones. In a fertility workup endometrium is mostly neglected as uterine factors are not accounted for according to the RCOG guidelines in an otherwise normal couple. HSG, which is used in uncomplicated cases to assess the tubes, is unlikely to diagnose retained bones.

Evacuation in second trimester should ideally be supplemented with ultrasound scanning to ensure complete removal of retained products. If ultrasound is provided at a later date, repeat operation and instrumentation remain possible risks in case of
retained products.

References:

- Lanzarone VF, Pardey JM. Retained intrauterine fetal bone as a rare cause of secondary infertility. Aust NZ J Obstet Gynaecol 2009;49:700-1
- Cameron ST, Glasier A, Johnstone A, Dewart H, Campbell A. Can women determine the success of early medical termination of pregnancy themselves? Contraception 2015; 91:6-11.

  Timor-Tritsch IE, Masch RJ, Goldstein SR, Ng E, Monteagudo A. Transvaginal ultrasound- assisted gynecologic surgery: Evaluation of a new device to improve safety of intrauterine surgery. Am J Obstet Gynecol 2003; 189:1074-9.
- Topçu HO, SimSek BS, Tasdemir U, Güzel Al, Doganay M. Retention of fetal bones 8 years following termination of pregnancy. J Exp Ther Oncol 2014; 10:267-9. National collaborating centre for women's and children's health on behalf of the National Institute for Health and Clinical Evidence (NICE). Fertility: Assessment and treatment for people with fertility problems. Clin Guidel CG 2013; 156

Liquid Based Cytology-The Clinician's Perspective

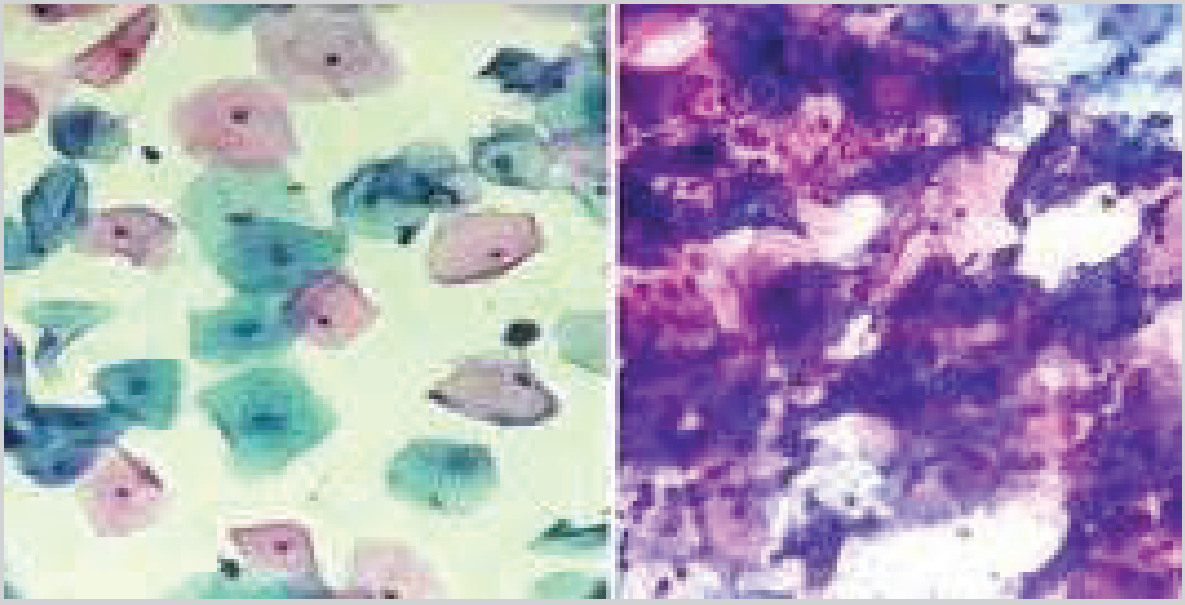
Dr. Marquess Raj
Apollo Diagnostics: Tamil Nadu

The Emergence of LBC PAP test

The PAP test has come a long way since it was developed in the early decades of the nineteenth century. Though new advances for cervical cancer screening such as computer imaging and molecular markers are in the evolving phase, the PAP test continues to be the most cost effective method for cervical cancer screening. Recently, Liquid Based Cytology (LBC) has been developed, which is an automation based technique. With every passing year the LBC market continues to grow with many more physicians advocating the test. However from a large perspective there is definitely more potential with overall coverage in developing countries being far lower than developed countries.

Common doubts that may linger from the clinical perspective

The most common challenge that practitioners encounter is the correct however overcome with simple demonstration of the correct technique & also informing the user about potential pitfalls that can occur during sampling. Another prevalent doubt is regarding the difference that the new technology makes. It is certainly difficult to completely do away with conventional PAP smears as significant numbers of patients do not have access to LBC. Considering that the choice of test literally lies in the hands of the physician. We would certainly emphasize that LBC makes a paramount difference in the quality of sample & report subsequently. The challenges with processing & reporting conventional PAP smears range from fixation artifacts to slides sticking to each other. Also transporting LBC samples are far easier when compared



The picture elucidates the clearer fields that LBC presents for microscopy :

The LBC sample can also be subjected to molecular evaluation for common HPV genotypes. HPV is implicated in cervical cancer & more than 33 genotypes exist. Mixed infections with different combinations can also occur. Although it is generally agreed that the genotype 16 is implicated as the harbinger of cervical cancer in India, conflicting evidence has emerged from other investigators around the globe (3) Supplementing LBC with HPV testing improves the risk prognostication Any doubts if LBC eliminates the percentage of unsatisfactory samples can safely be put to rest as it does reduce the same. The following study by a lone investigator over a ten year period bears testimony to this fact. (2)

Pap method	Total Pap Smears	Total % HSIL	Total % Unsatisfactory
Conventional Pap	128,630	0.28	**0.30**
ThinPrep™	88,575	0.38	**0.64**
BD SurePath™	92,875	0.50	**0.17**

The Apollo Diagnostics advantage:

Apollo Diagnostics employs a platform which is widely recognized as better amongst the existing technology for LBC. Our teams of expert pathologists screen every slide meticulously before they are reported. Since inception, our team has come a long way reporting over 20000 LBC samples per year & are continually improving. With many more patients & physicians preferring LBC over conventional PAP test, LBC is bound to become the 'way to go' in cervical cancer screening.

References:

- **Coverage of cervical cancer screening in 57 countries: low average levels and large inequalities.**
- **Gakidou E, Stella N, Ziad O. PloS Med. 2009;5:e132 Evolution of Pap testing at a community hospital: a ten year experience. Nance. www.ncbi.nlm.nih.go v/pubmed/17304530**
- **Molecular evaluation of common HPV genotypes in LBC samples.GMJ.Gehad Gamal Kamel et al.**

Rare Down's Syndrome Entity - A Cytogenetic Case Study

Dr.Vasavi Narayanan & Preethi P, Harika M
Apollo Diagnostics, Apollo Health Lifestyle Limited, National Reference Lab, Hyderabad.

Partial trisomy 21 is relatively uncommon. It is rare to find the extra partial chromosome 21 translocated to another chromosome in a non-centric fusion translocation. We present this interesting cytogenetic finding in a case referred to our department.

Background - Down's syndrome (DS) is a genetic disorder caused by the presence of the whole or a part of the third copy of chromosome 21. Screening for DS is offered to all pregnant women irrespective of age. Although the probability of having a DS baby increases with maternal age, 70% of these children are born to women 35 years of age and younger, simply because younger people have more children.

95% of cases of DS are the result of three copies of chromosome 21 in every cell of the body. In cases that are mosaic for DS, individuals have a mixture of cells, some having two copies of chromosome 21 and some having three copies. Then, there are cases of DS with a translocation of the extra, whole or partial chromosome 21, translocated to another chromosome.

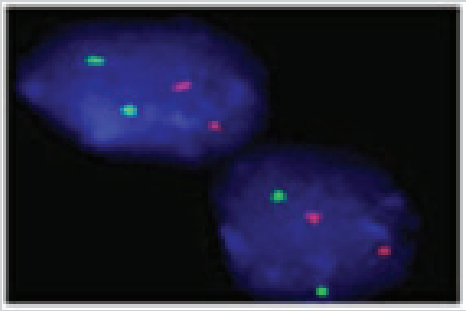
Case Presentation: A 2-day old male child was referred to the Department of Cytogenetics for evaluating the case for Down syndrome by FISH Method. We received 2ml neonatal blood along with the TRF showing the details: Broad nasal bridge, low-set ears, undescended testis, simian palmar crease.

Result and Discussion: FISH evaluation showed 92% cells normal for chromosome 21 and ~8% cells showing an extra chromosome 21. FISH signal, however was not very clear. We spoke to the referring clinician and were given to understand that the DS symptoms were definitely there. Hence a resample of the case was taken to rule out any mosaicism; this time we told the clinician that karyotyping of the sample would add to the information at hand.

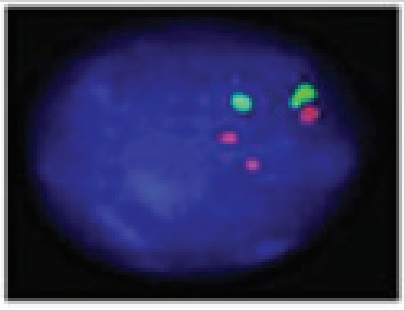
Chromosomal studies revealed an interesting finding – there was an extra G band (q21) of chromosome 21 that was translocated to chromosome 11. That is, a part of chromosome 21 was in excess dose, found attached in a non-centromeric fusion with chromosome 11. This is a rare finding – otherwise, usually a translocated trisomic chromosome 21 would show up as a Robertsonian translocation fused at the centromere of one of the acrocentric chromosomes.

In the present case, the FISH probe used, did not encompass the partial extra 21 region hence did not give a clear partial trisomy result. (In a small percentage of cells (<10%) there was a trisomic signal initially not very clear, confirmed subsequently on a metaphase bearing the extra signal bound to chromosome 11, (Image 1).

Image 1



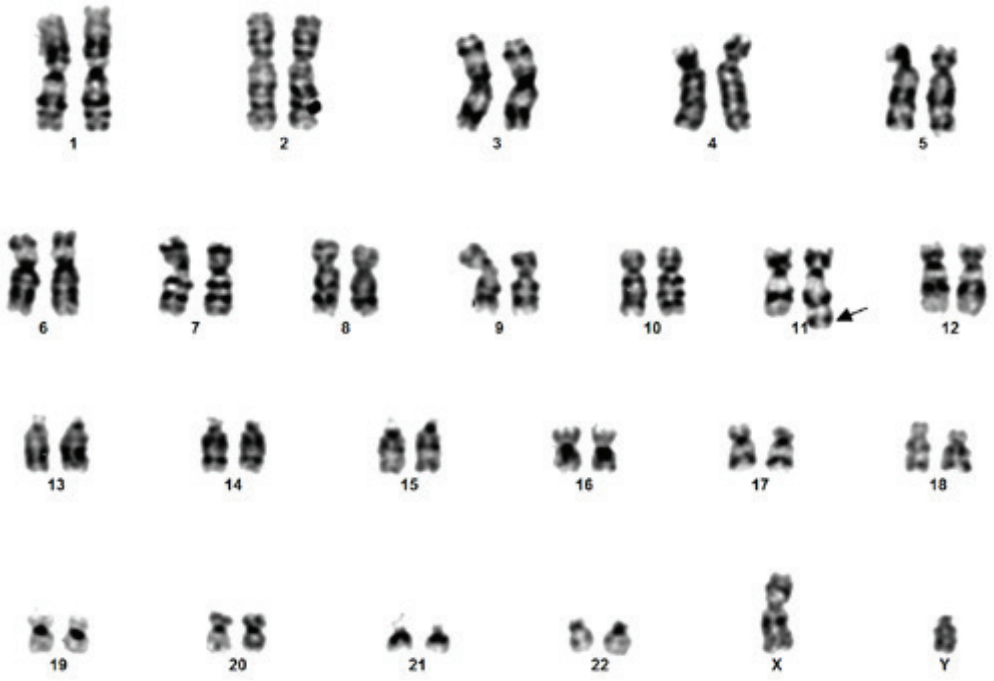
Nuclei showing Two Red Signal i.e normal for chromosome 21



Nuclei showing Three Red Signal i.e trisomy 21

Hence, using both the techniques along with adequate clinical information from the referring physician, this case was found to be 46,XY,dup(21)(q21),ins(11;21)(q23;q21) indicating a partial trisomy 21 with an insertional translocation in chromosome 11 (See image 2 below).

Image 2



Each person with Down syndrome is unique and the severity of symptoms varies greatly among individuals. A diagnosis is not only important in these cases (translocation involving chromosome 21) for the patient but also for the parents who may be at the risk of having further affected offspring. Translocation DS can sometimes be inherited from an unaffected parent.

Methods used: Fluorescence in-situ hybridization using Kreatech probes (21q22) that binds to specific region of chromosome 21 for identifying trisomic condition. Probe was applied after processing the cells and scoring done (Leica microscope with Cytovision software). Cell culturing: 2 ml peripheral blood sample was collected again in a sodium heparin vaccutainer. Lymphocyte culture was done and G-banded metaphases were prepared for analysis and chromosomal study.

War On Waste - The Apollo Diagnostics Way

Dr. Marquess Raj
Apollo Diagnostics: Tamil Nadu

Foreword:

Lean management is centered on making obvious what adds value & reducing everything else. Ideal lab management should include principles of lean. A lean laboratory is one which is focuses on reducing waste, improving outcomes & in the process optimizing cost. Apollo diagnostics (AD) definitely applies the principles of lean in the laboratory workflow. Five such systems that are in place are described in this write up.

1.Make each tube count :

AD aims at utilizing testing products and materials to deliver results in the most efficient way in terms of cost or speed or both. Blood collection tubes are perhaps the most common consumable used in lab testing.AD has a process for sharing of samples in the laboratory.

2.Pen free lab :

With the healthcare industry embracing environment friendly measures, paperless processes are being recognized in the industry. Paper contributes to clutter, burden & stagnation in the lab work process. AD software systems being robust eliminate the use of worksheets unless warranted. Reduction in the printing of worksheets definitely reduces cost & also streamlines work flow process. As many a time in systems which include a worksheet, sample processing may get delayed because of the absence of the

Example:

The blood collection tube with red top contains no anticoagulant & can be used for both biochemical analysis & serology.AD process employs a sample colour coding system by which blood collection tubes are shared across biochemistry & departments. This significantly reduces the cost incurred on tubes & therebyeliminates waste.

3.Interfacing

AD had already been awarded for the “patient safety initiatives” put in practice. Interfacing of patient results without manual transcription, ensures that error of the human hand is eliminated. This ensures that patient safety is assured in the postanalytical phase. This apart from being a seamless system that saves time & energy builds client confidence.

4.Patient EMR

The LIS supports a patient EMR to which raw data can be uploaded for patient access. Though reports are being uploaded as part of practice, the EMR can be used to upload any value added service we provide.

5.eArchival of tubes

Archival of sample containers is a cumbersome chore in the laboratory. Ensuring that a scanning mechanism & defined process exists in the laboratory ensures easy retrieval of samples. AD has such a process which facilitates the easy retrieval of sample tubes. This ensures that time is spent on productive work in the laboratory.

Example:

Graphs of HbA1C or CBC histograms can be uploaded for the patient's reference. This ensures that all the efforts put in ensuring quality results are documented.

After word:

Elimination of waste in the laboratory can be achieved by improving on existing processes & applying the principles of lean right from the grassroots level. By reducing cost, time & by directing work hours towards more productive outcomes, Apollo diagnostics is waging a perennial war on waste

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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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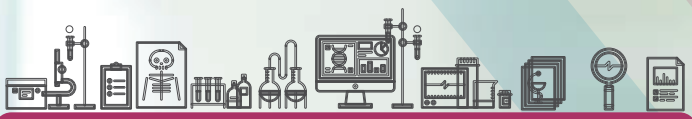

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