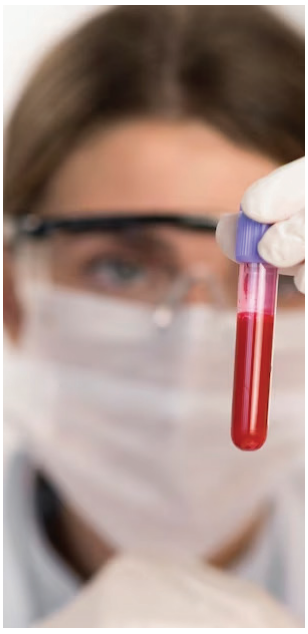
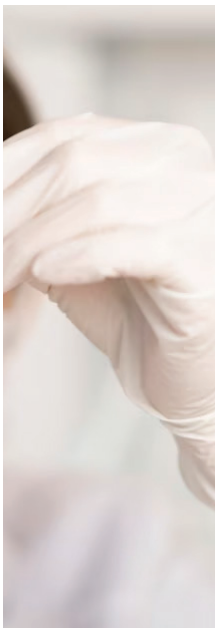
Apollo DIAGNOSTICS®

Expertise. Empowering you.

APOLLO TRUST ASSURED QUALITY AFFORDABLE AAA+





AD Express

FEBRUARY 2023

On the right track, for precise results

Global Accreditations for Quality Testing

CAP ACCREDITED COLLEGE of AMERICAN PATHOLOGISTS

NABL

Apollo DIAGNOSTICS®
Expertise. Empowering you.

Editorial board

Dr. Ramesh Kinha M.D (Pathology)
GM - Technical

Dr. Anita Flynn M.D (Pathology)
ZTC - Karnataka

Dr. Abhik Banerjee M.D (Pathology)
ZTC – West Bengal & East Zone

From the Editors' Desk

Dear Reader,

It gives us immense joy to place the second issue of 2023 in your hands!

The roughest of storms are followed by a placid calm. We wish that the COVID pandemic which taught the healthcare fraternity tough lessons make way to better weather and greener pastures. We also take this opportunity to thank all our patrons for the immense support we have garnered over the years.

In the second edition of this year, we present a case report on Langerhans cellhistiocytosis, a distinct hematological entity which can present with lymphadenopathy. We have two case reports from the department of cytogenetics on two rare numerical chromosomal anomalies. We have included 3 articles which delve into the finer details of lab medicine. The first article sheds light on how the quality of water used in testing can impact the results in a laboratory. The second article reviews the importance of water quality in laboratory testing. The final article enumerates the problems encountered by cytopathologist while reporting cytology material which is handled improperly. In another first time inclusion, we have included a crossword in this edition. We expect our creative tendencies to gravitate towards biochemistry ballads, serology sonnets and musings from microbiology in the forthcoming issues.

We thank the contributors for taking time to put pen to Paper and covering a gamut of topics with aplomb. AD express has gained considerable impetus by the way of your contributions and we welcome more from you all.

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

Wish you all a fabulous New Year ahead!

Best regards,

Dr. Marquess Raj M.D. Dip. N.B (Path), MBA (HM)
ZTC – TN & Pondicherry
marquess.raj@apollodiagnostics.in

Dr. Shalini Singh M.D (Path), IFCAP, DMSC (Endocrine)
Lab Director, Global reference lab, Hyderabad
shalini.singh@apolloclinic.com

Contents

1. The story of a "groovy" cell
Langerhans Cell Histiocytosis: A Case Report

Dr. Shalini Singh
(Lab Director, Global reference lab, Hyderabad),
Dr. Prashanth
(Consultant Pathologist, RRL Bengaluru)
Dr. Devasmita Gain
(Consultant Pathologist, HLM Koshy hospital, Bengaluru)

Case history:

A 29-year-old male presented to the OPD with swelling in the right side of the neck for 5 weeks with no other significant complaints. On examination, 2 lymph nodes were noted in the right cervical region, largest measuring 2.5 x 1.5 x 1cm, level II, firm, mobile non-tender.

Ultrasound neck revealed multiple heterogenous well-defined Ultrasound neck revealed multiple heterogenous well-defined lesions (likely lymph nodes) in bilateral level II and right level V. FNA was performed.

- Cytologically, smears showed numerous atypical histiocytes, admixed with polymorphous population of lymphoid cells, eosinophils, neutrophils and foamy histiocytes. Atypical histiocytes having moderate amount of cytoplasm with ovoid to round nuclei, some showing nuclear grooves and nuclear folds were noted with few binucleate histiocytes.
- Few smears showed presence of Charcot-Leyden crystals. Charcot-Leyden crystals are hexagonal to rhomboid, bipyramidal structures indicative of tissue eosinophilia which played a key factor in the following case and helped in drawing attention to the LCH diagnosis.
- A final cytological impression of Langerhans Cell Histiocytosis was given which was later confirmed by histopathology and immunohistochemistry. Patient was on observation for 6 months, following which chemotherapy was started. Cytrabine was administered (5 cycles/month) for 6 months and the patient is on follow up.

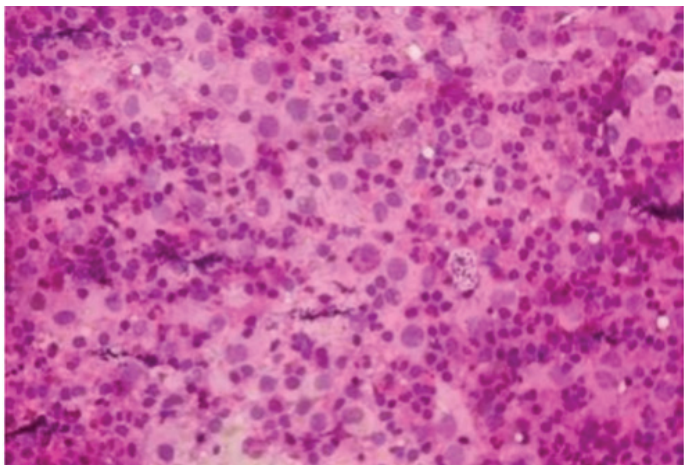


Fig 1: Inflammatory Cells including lymphoid cells, eosinophils, neutrophils, foamy macrophages

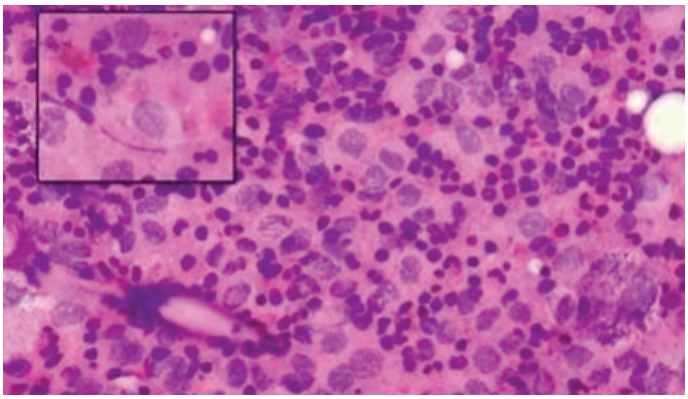


Fig 2: Histiocytes showing coffee bean appearance (40x)

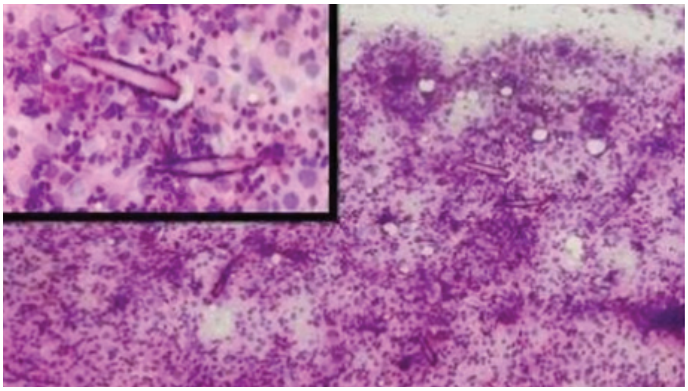


Fig 3: Charcot leyden crystals (40x)

Discussion:

Langerhans cell histiocytosis (LCH) is a rare disease which presents either as a localized, tumour-like process or a disseminated proliferation of Langerhans cells, most commonly noted in children and young adults with an incidence of about 5 per million (1).

In 1953, Lichtenstein used the terminology Histiocytosis X to describe LCH and categorized them into three variants - Eosinophilic granuloma, Hand-Schuler-Christian disease and Letterer-Siwe syndrome (2). In 1987, Histiocyte Society Writing group adopted the term LCH and necessitated the presence ofBirbeck granules on electron microscopy or demonstration of CD1a on immunohistochemistry to confirm the diagnosis of LCH in a typical histology (3).

The revised classification of histiocytosis consists of five groups of diseases and categorises LCH under"L" (Langerhans-related) category (2). Histiocytic/dendritic cell neoplasms are now regrouped and positioned to follow myeloid neoplasms in the latest classification (4).

Pathophysiology remains a mystery with one evidence suggesting an aberrant immunological mechanism, with cytokines playing a major role (5). BRAFV600E mutation and various other alterations in the MAPK signalling pathway were noted in more than half of the LCH cases (6).

BRAF-V600E mutations were noted in the circulating fractions of dendritic cells (DCs) in human blood along with bone marrow CD34+ hematopoietic progenitor cells in high-risk LCH patients which strengthened the notion that LCH cells were more similar to dendritic cell precursors derived from bone marrow, and did not originate from skin Langerhans cells. LCH can be unifocal or multifocal, involving one system or multiple. Involvement of lymph nodes canbe a part of a widespread disease or as an isolated metastatic draining site of a lesion in the bone, skin or lung or as an individual involvement. FNAC is useful in diagnosis of LCH in lymph nodes in up to 85% of the cases (8).

Typical cytomorphology of LCH warranties the presence of typical Langerhans cells which can be identified by their abundant, eosinophilic cytoplasm and vesicular nuclei with prominent nuclear indentations and grooves, which arises due to deep invaginations of the nuclear membrane (5,8,9). Inlesions of bone, lymph nodes and lungs, eosinophils are often numerous, and are accompanied by debris, foamy phagocytizing macrophages and, occasionally, Charcot- Leyden crystals which contain eosinophil membrane protein formed due to rupture of eosinophilic granules (5,10). Langerhans cells show positivity for CD1a, Langerin, S100, CD68, Cyclin D1 on IHC and classical tennisracquet shaped Birbeck granules on Electron microscopy (11).

Differentials of histiocytes or eosinophils in cytology from lymph nodes include sinus histiocytosiswith massive lymphadenopathy (SHML), malignant histiocytosis, Kimura's disease, dermatopathic lymphadenopathy, Hodgkins lymphoma and reticuloendotheliosis with eosinophilia (Omenn's syndrome) (9) . Treatment involves surgical resection/ curettage if a single system is involved and chemotherapy for disseminated lesions.

References:

1. Lee LY, Kang C, Hsieh Y, Hsueh S. Diagnosis of Nodal Langerhans Cell Histiocytosis by Fine Needle Aspiration Cytology. Chang Gung Medical Journal. 2005 Oct 1;28(10):735.
2. Phulware RH, Guleria P, Iyer VK, Bakhshi S, Seth R, Mridha AR, Jain D, Mallick S, Arava SK, Agarwal S, Kaushal S. Cytological diagnosis of Langerhans cell histiocytosis: a series of 47 cases. Cytopathology. 2019 Jul;30(4):413-8.
3. Patne SC, Dwivedi S, Katiyar R, Gupta V, Gupta AK. Langerhans cell histiocytosis diagnosed by FNAC of lymph nodes. J Can Res Ther2015; 11:1028
4. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, Bejar R, Berti E, Busque L, Chan JK, Chen W. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022 Jul;36(7):1703-19.
5. Van Heerde P, Egeler RM. The cytology of Langerhans cell histiocytosis (histiocytosis X). Cytopathology. 1991 Jun;2(3):149-58.
6. Badalian-Very G, Vergilio JA, Degar BA, MacConaill LE, Brandner B, Calicchio ML, Kuo FC, Ligon AH, Stevenson KE, Kehoe SM, Garraway LA. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood, The Journal of the American Society of Hematology. 2010 Sep 16;116(11):1919-23.
7. Berres ML, Lim KP, Peters T, et al. BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups. J Exp Med. 2014; 211:669-683.
8. Kakkar S, Kapila K, Verma K. Langerhans cell histiocytosis in lymph nodes. Cytomorphologic diagnosis and pitfalls. Acta cytologica. 2001 May 1;45(3):327-32.
9. Lee JS, Lee MC, Park CS, Juhng SW. Fine needle aspiration cytology of Langerhans cell histiocytosis confined to lymph nodes. A case report. Acta cytologica. 1997 Nov 1;41(6):1793-6.
10. Kumar N, Sayed S, Vinayak S. Diagnosis of Langerhans cell histiocytosis on fine needle aspiration cytology: a case report and review of the cytology literature. Pathology Research International. 2011;2011.
11. Pileri SA, Grogan TM, Harris NL, Banks P, Campo E, Chan JK, Favera RD, Delsol G, De Wolf Peeters C, Falini B, Gascoyne RD. Tumours of histiocytes and accessory dendritic cells: an immunohistochemical approach to classification from the International Lymphoma Study Group based on 61 cases. Histopathology. 2002 Jul;41(1):1-29.

2. Rare chromosomal abnormalities in products of conception – A review of 2 rare cases reported in Apollo Diagnostics Global Reference Laboratory, Hyderabad.

Dr. Shubhangi Miryala, Dr. Preethi Pattamshetty, Dr. Vasavi Narayanan
Department of Genetics, Global Reference Laboratory, Hyderabad.

Introduction

Chromosomal anomalies are the commonest cause for pregnancy loss. The diagnosis of abnormal chromosomal numbers in products of conception (POC) provides insight into genetic errors playing a part in pregnancy loss. They also help in identifying the risk for future miscarriages or evaluating the genotypeof a child with abnormal features.

In addition to highlighting the necessity for screening couples for chromosomal anomalies, these abnormalities can occur as an accident when the egg or the sperm is formed or during the early developmental stages of the fetus. The age of the parents and multiple environmental factors may play a role in the occurrence of these genetic errors. As such, an abnormal POC cytogenetic result may preclude the need for any further extensive evaluation of bad obstetric history (BOH) cases. In patients where the POC karyotype reveals a structural or numerical rearrangement, parental chromosome analysis is warranted.

The aim of the present article is to share a few abnormal genotypes encountered in POC samples tested in Apollo Diagnostics, Global Reference Laboratory, Hyderabad.

In the present study, we have shown findings of rare abnormal karyotype/FISH results of some POC samples. Trisomy 22, triploidy, trisomy 13 are discussed here, cases where POC karyotyping/ FISH was performed.

Methodology:

01 Samples were referred for cytogenetic analysis of products of conception to Apollo Diagnostics, Global Reference Laboratory, Hyderabad.

02 Tissues from products of conception are cultured to produce Tissues from products of conception are cultured to produce metaphase cells for G-banded chromosome analysis.

03 Long-term cultures were harvested at approximately 14 days and Long-term cultures were harvested at approximately 14 days and then G-banded.

04 Karyotyping and analysis of metaphases was performed as per routine Karyotyping and analysis of metaphases was performed as per routine method.

05 FISH analysis was performed to assess the extent of mosaicism (additional metaphases) wherever relevant FISH analysis was performed to assess the extent of mosaicism (additional metaphases) wherever relevant.

Case 1:

31 year old primigravida presented at 13 weeks of pregnancy. Patient was a known asthmatic

USG findings : Nuchal translucency, absent nasal bone and generalized subcutaneous edema. Ductus venosus agenesis.Single umbilical artery, bladder not seen and hydrops. It also showed a crossed renal ectopia of the left kidney.

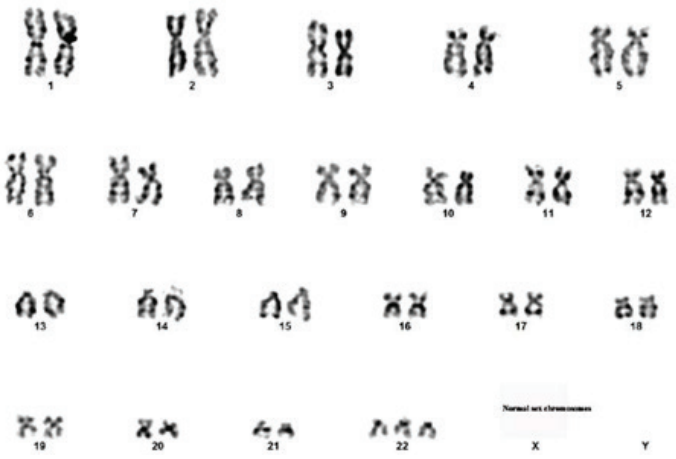
Bits of POC sample material were collected in normal saline, along with the skin tissue and received at the lab in good condition.

Cytogenetic evaluation of the POC material showed trisomy 22 in all the metaphases screened.

Concise discussion:

- Trisomy 22 is a rare chromosomal disorder wherein an extra (third) copy of chromosome 22 is commonly found in patients with repeated miscarriages. It is rarely seen in live-born infants.
- Most affected babies die before or shortly after birth due to severe complications.

  This anomaly is due to an error during the division of reproductive cells in one of the parents or during cellular division after fertilization.
- Most of the trisomy 22 errors (>96%) occur during oogenesis, predominantly during the first meiotic division. Trisomy 22, representing 11–16% of cases, is a common trisomy in spontaneous abortions.
- In contrast, live-born trisomy 22, is rarely seen as severe organ malformations are associated with this condition.
- Common features include midface hypoplasia with flat/broad nasal bridge, dysplastic ears with pits, tags, cleft palate, hypertelorism, microcephaly/cranial abnormalities, congenital heart disease, genital abnormalities, and IUGR [Kobrynski et al., 1993].



Case 1: 31 yr old primigravida for MTP; POC sample for cytogenetic analysis showed 47, --, +22 karyotype indicating trisomy 22 responsible for the fetal loss.

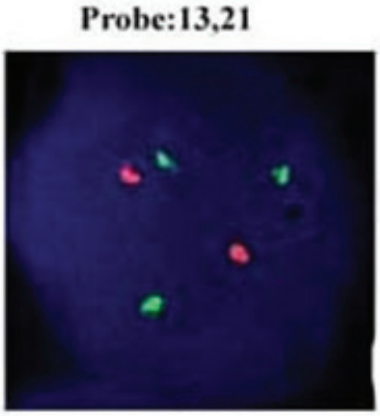
Case 2:

39-year-old female

Ultrasound showing no cardiac activity at eight weeks of pregnancy

POC material was referred to the lab for aneuploidy detection by Florescence in situ hybridization studies (FISH) for 13, 21, 18, X and Y.

The results showed three green signals for the 13, 21 probe used indicating Trisomy 13 or Patau's Syndrome in the fetal material (prevalence of 1 in 8000 births).



Case 3:

POC from a 39-year-old female referred for aneuploidy detection by FISH; results showed Trisomy 13 or Patau's Syndrome in all the cells evaluated.

Concise discussion:

- The presence of three copies of chromosome 13 is most commonly due to non-disjunction at meiosis, said to be occurring more frequently in mothers with advanced age (age greater than 35).
- An unbalanced Robertsonian translocation results in trisomy with two normal copies of chromosome 13 and an additional long arm of chromosome 13.
- In this case, one of the parents may be a Robertsonian translocation carrier.
- Another less common cause is mosaicism, which results in three copies of chromosome 13 in some cells and two copies in the others.5% of trisomy 13 fetuses have a mosaic placenta that may permit survival to the neonatal period due to the compensation provided by the diploid cells in the cytotrophoblast (Kalousek et al, 1989).
- The most common malformations on ultrasound evaluation in case of fetuses with trisomy 13 were craniofacial defects, cerebral malformations and genitourinary tract anomalies. The major defects identified in 50% fetuses with trisomy 13 in the first trimester include holoprosencephaly, omphalocele and megacystis.
- Intra-uterine growth restriction (IUGR) may or may not be apparent in trisomy 13 unlike in trisomy 18 cases.
- Maternal age may not be a factor as it has been reported that ~70% of women with trisomicfetuses were younger than 35 years of age (Kroes et al, 2014.Fetuses with trisomy 13 without any anomaly have been observed in the second trimester (Benacerraf, 2008).

Take home message:

All pregnancies must be evaluated by mid trimester ultrasound scan for fetal structural anomalies.

50–60% of spontaneously aborted products of conception have been detected with chromosomal abnormality

Parental balanced translocations or inversions can produce abnormal gametes and present with recurrent miscarriages.

Hence, karyotype testing is recommended as an important part of genetic evaluation of parents with recurrent pregnancy loss.

Genetic testing in early pregnancy will be essential in all these cases especially where fetal anomalies are recorded.

It is important to counsel these families about recurrence risk after suitable testing, for further pregnancies.

Pre-test counselling is needed to discuss the various limitations and diagnostic scope of specific genetic tests, for optimal benefit to the patient.

References:

1. Lee LY, Kang C, Hsieh Y, Hsueh S. Diagnosis of Nodal Langerhans Cell Hi1. Benacerraf B. Ultrasound of foetal syndromes. 2nd Ed. Philadelphia: Churchill-Livingstone-Elsevier; 2008. pp. 483–496.
2. Kalousek DK, Barrett IJ, McGillivray BC. Placental mosaicism and intrauterine survival of trisomies 13 and 18. Am J Hum Genet 1989;44:338-343.
3. Kroes I, Janssens S, Defoort P. Ultrasound features in trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) in a consecutive series of 47 cases. Facts Views Vis Obgyn. 2014;6(4):245-9.
4. Hyde, K. J. andSchust, D. J. Genetic considerations in recurrent pregnancy loss. Cold Spring HarborPerspect. Med. 5(3), a023119.

3. Water Quality in Medical Laboratories

Dr. Abhik Banerjee
Zonal Technical Chief, East Zone, Apollo Diagnostics, Regional Reference Laboratory, Kolkata

Water Quality in Medical Laboratories

Water quality has always been a very important aspect in ensuring the performance of a medical laboratory. However sadly it is also one of the most neglected or better to say overlooked parameter in day to day quality monitoring in a diagnostic set up.

Requirement of Clinical grade water in a Laboratory:

Clinical grade water is basically required for following purposes in a medical laboratory:

1. Automated analyzers feed
2. High end manual assays like PCR, Next generation sequencing (NGS), HPLC, Liquid chromatography tandem mass spectrometry (LC-MS/MS) based assays
3. Reagent reconstitution, Sample dilution for assay purpose
4. Others like buffer preparation, blank and standard solutions, culture media etc.

The autoanalyzers require high quality water for various purposes, encompassing almost all steps of sample processing as well as pre or post analysis laundry of different parts of analyzers including cuvette wash station, sample and reagent probes, instrument pipettes, internal tank (reservoir) and incubator baths in photometers. High purity water is also required for overall health of the analyzers to ensure robust, optimal and uninterrupted functioning.

Impact of poor quality water in a laboratory

Poor quality water may lead to complete system failure in a medical laboratory either in the form of breakdown of the analyzers or run failure in ongoing assays. According to Clinical Laboratory Reagent Water (CLRW) specifications, there may be 4 key types of impurities in pure water namely ions, particulates, organics and bacteria (including bacterial by-products) (1). Notably, bacteria and ions have an impact across the widest range of applications be it general chemistry, enzyme analysis, molecular testing, EIA or toxicology. Analyzers with ion selective electrodes, analyzers running on colorimetric principles, modern days' immunoassay analyzers and high end analyzers like Next generation sequencer (NGS), LC-MS/MS not only demand high purity water but also consistent and uninterrupted supply in many cases. Water impurity in the form of ions, particulates, organics and bacteria may lead to cuvette contamination, sample/ reagent probe contamination, errors in sample and reagent dilution, poor reagent stability, level sensing error, reduced calibration stability and sensitivity, capillary blocking, scaling and many more. Regarding assays, routine biochemistry assays like calcium, alkaline phosphatase, LDH, amylase, iron, creatinine kinase etc., HPCL based assays, ion exchange chromatography, polymerase chain reaction, heavy metal testing need high purity water without which quality and reliability of these assays are considerably compromised. Molecular diagnostics requires type I nuclease-free water suitable for gene sequencing. Presence of DNase and RNase in water can have a significant impact on genetic analyses rendering characterization difficult or even not possible at all. To avoid interferences, this must be kept free from calcium, magnesium, organics, endotoxin and bacterial nucleases using further purification technologies, such as multiple ion exchange, dual wavelength photooxidation and ultrafiltration. Chromatographic techniques, for example, LC-MS/MS, GC-MS and HPLC in toxicology, require type I water with the lowest possible levels of organic contamination, best achieved by optimal system design with high purity components and dual wavelength photo-oxidation. ICP-MS and ultra-trace IC require water that is virtually free of elemental and ionic impurities, needing a high purity water system with multistage removal of ions using the highest efficiency and purity ion-exchange resins. All these ultimately lead to increased

downtime, unforeseen expenditure in the long run, overdue, erroneous reports, potential misdiagnosis, delay in treatment and poor customer satisfaction affecting business as well as reputation of the lab. At the same time, such situation adversely affects laboratory's cost per test by increasing consumption of reagents due to increased need for re-assays.

Clinical grade water

The primary reference for the use of water in the clinical laboratory is the National Committee for Clinical Laboratory Standards (NCCLS) guideline, "Preparation and Testing of Reagent Water in the Clinical Laboratory," ed 3 (document C3-A3, October 1997) (2). The NCCLS recommends that, water meet specific levels of purity for ionic content (resistivity), bacterial content, pH, and silica concentration. Additionally, the NCCLS indicates that, specific purification technologies be used to control particles and organic contaminants. The water purity is divided into ranges called "types". Type I water is considered ideal for procedures that, are highly sensitive to contamination, such as enzymatic assays, trace analysis and tests involving nucleic acids. These test methods demand minimum interference and maximum precision and accuracy. Type II water is required in the general laboratory for analytical procedures where freedom from organic impurities is of more significance. Type III water is used in washing of glassware, preliminary rinsing of glassware and feed water for production of higher grade water. The NCCLS is now known as Clinical and Laboratory Standards Institute (CLSI) and subsequently the above mentioned gradation or types of water has been replaced with the with the terms Clinical Laboratory Reagent Water(CLRW), Special Reagent Water(SRW) and Instrument Feed Water (IFW). CLRW can replace types I and II water for most applications. IFW meets the type-III requirements from the previous NCCLS classification. SRW may be specified when CLRW purity is unsatisfactory or inadequate and additional parameters are required to ensure optimal water quality necessary for high end applications like HPLC, LC-MS/MS or Molecular testing. The College of American Pathologists (CAP), a global accreditation agency has also endorsed and reinforced the CLRW and CLSI sets recognised standards. CAP recommends that laboratory water should meet the CLSI, Clinical Laboratory Reagent Water (CLRW) grade standard as a minimum.

Laboratory water purification technologies

To achieve the required purity or standard of water specified by CLRW, various water purification technologies are being used by lab water purification systems in modern medical laboratories. Pre-filtration, activated carbon, reverse osmosis, electrode ionisation, deionisation, UV purification are some of the most common and effective technologies used for this purpose. Most of these systems use different technologies in combination to meet the desired result. For example, reverse osmosis, ultraviolet irradiation, and ultra-microfiltration are used to remove bacteria whereas reduction of ionic content in the water may be attempted by combination of reverse osmosis, deionisation along with electrode ionisation (EDI). Removal of DNase and RNase can be achieved by treatment of water with UV light combined with ion exchange media (3).

Choosing a good quality water purification system for lab

Commercially available HPLC grade water typically supplied in bottles are often used by labs. However, when used as an eluent this can lead to poor quality result. This may be due to contamination of the water through storage. An alternative and a better solution to bottled water is using a water purification system which ensures the quality of the water throughout the process. Considering the need of high purity water in a modern, fully automated medical laboratory which demands highly reliable, constant, uninterrupted, high volume ultra-pure water supply, multi-stage water purification system has been introduced by reputed vendors. There are usually three stages in such systems. In the 1st stage, primary treatment of the feed water can be done by pre-filtration, use of activated carbons, reverse osmosis, ion exchange resins or electrode-deionization (EDI). Pre-treatment reduces all the major types of impurities inorganic, organic, microbiological and particulate by over 95%. The better the pre-treatment the higher will be the potential quality of the final ultrapure water. In the 2nd stage, this pre-treated water is stored in a reservoir and finally polishing of this reserved water (3rd

stage) is achieved by combination of two or three different technologies like ion exchange, carbon absorption, ultraviolet photo-oxidation, ultrafiltration and recirculation. Hence a pre-installation site survey, feed water quality check and understanding the requirement of type of water purification technology for the lab, good water purification system design as per requirement, evaluation of vendor especially in view of after sales service, ability of the vendor to maintain, trouble shoot promptly with minimum downtime, supply consumables and spare parts in no time whenever required, user testimonial or feedback and cost of consumables, cost of AMC (annual maintenance contract) should be kept in mind before going ahead for procurement. Conclusion: Quality of water is of prime importance in a medical laboratory. With the growing need of high volume and complex testing, there is an increasing demand of higher purity water requirement by laboratories. Considering importance of good water purification design for reliable test results and long life of analyzers, laboratory should be careful and take utmost care during selection of its water system as it will directly impact the productivity, efficiency and accuracy of workflow in a laboratory.

Conflict of Interest

The author declares no conflict of interest. (Already published in "Journal of Applied Biochemistry and Laboratory Medicine (2020) 01 / JABLM_08", Oct 2020 issue)

References

1. Indian Standard: Reagent Grade Water-Specification (Third Revision): Second Reprint Nov 1996.
2. Stewart MB. The Production of High-Purity Water in the Clinical Laboratory: Laboratory Medicine. 2000; 31 (11): 605-612.
3. Nabulsi R, Al-Abbadi MA. Review of The Impact of Water Quality on Reliable Laboratory Testing and Correlation with Purification Techniques: Laboratory Medicine. 2014; 45 (4): e159-e165

4. The predicament of cell fixation

Dr.Marquess Raj
ZTC Tamil Naduand Pondicherry

Foreword:

Since the phenomenal publication in 1941 by Drs. Papanicolauand H.Traut diagnostic cytopathology developed as parallel but distinctly separate discipline from histopathology.

Procedures such as FNAC and the Pap test when performed by practicing clinicians many a time yield unsuitable material for reporting. These samples are deemed unsatisfactory, not because of lack of skill but because of lack of knowledge in the science of cellular fixation. This write up does not attempt to confine the branch of cytopathology to lab medicine practitioners alone but to explain the perils microscopists face when the procedure of sampling is done by non-practitioners of lab medicine.

The study of cells

Cytopathology is a branch that must be embraced by the entire medical fraternity for the following reason. The ease by which cytopathology procedures can be performed make them ideal tools that can be employed for screening of a myriad of diseases ranging from inflammatory conditions to cancer. Exfoliative cytology as a screening test is not only a predictive test for malignant disease, but may also acquire a diagnostic function.

The practice of cytopathology depends on four fundamental requisites:

1. The sample must be representative of the lesion.
2. Sample must be adequate depending on where the sampling has been done from.
3. Sample must be processed correctly.
4. Relevant clinical and radiological information must accompany the sample.

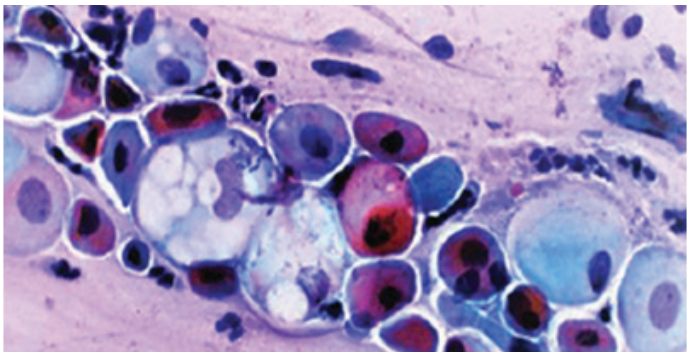
Cell fixation

Procedures such as FNAC andPap smear involve the harvesting of cells from the body and smearing the cells on to glass slides. Preservation of cellular morphology is of utmost importance in cytopathology. Cell morphology changes with the type of processing employed. Two fundamentally different methods of fixation and staining are used in FNAC: air-drying followed by staining with a hematological stain such as Giemsa or alcohol fixation and staining according to Papanicolau.

Textbooks of cytology use the “egg analogy” to explain how different cells look with wet and dry fixation.The effects produced on cells is easily understood if one compares the three-dimensional shape of a fried egg with that of a boiled egg. Air drying causes the cell, both the cytoplasm and nucleus to flatten on the slide just like the egg flattens in a frying pan. Thus, cell and nuclear size are exaggerated in air dried smears. Wet fixation imparts changes to cell morphology that can be likened to a boiled egg. The results obtained with wet fixation are comparable with tissue sections.

The egg fallacy

Letting a Pap smear dry too much while sampling will distort cell morphology and hence compromise reporting. Smears reported as unsatisfactory warrant repeat examination and sampling. This amounts to significant cost burden and loss of precious time to the health care professional. In addition, unnecessary acrimony exists between the lab medicine practitioner and the clinician as a result of the blame game. Information regarding the type of smear whether “fixed” or “unfixed” is central to the processing of slides and reporting.



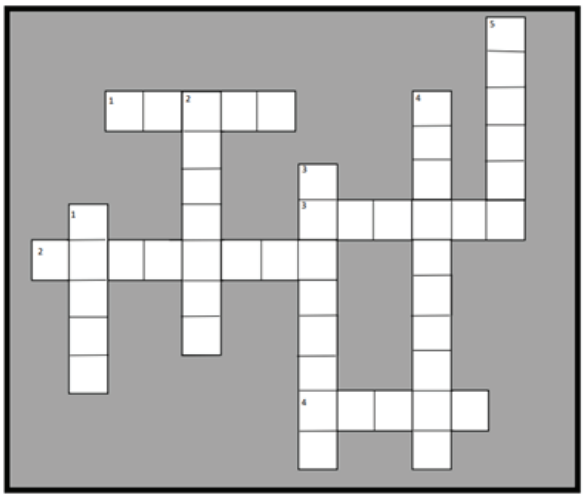
Cytoplasmic vacuolation shown in the above image is a consequence of over drying. Prompt fixation is essential for retention of desired morphology

Final Thoughts:

With medical science expanding and the water tight compartments of clinical branches becoming fuzzy. Pathologists should encourage clinicians to advocate and do cytological procedures. But sharing of knowledge is crucial. Pathologists should hold workshops for clinicians and educate them on cellular fixation. After all, scrambled eggs might not suit everybody's culinary tastes.

5. Crossword

Dr.Devasmita Gain
Consultant Pathologist, HLM Koshy's Hospital,Bengaluru.



ACROSS: (Cytology classification system)

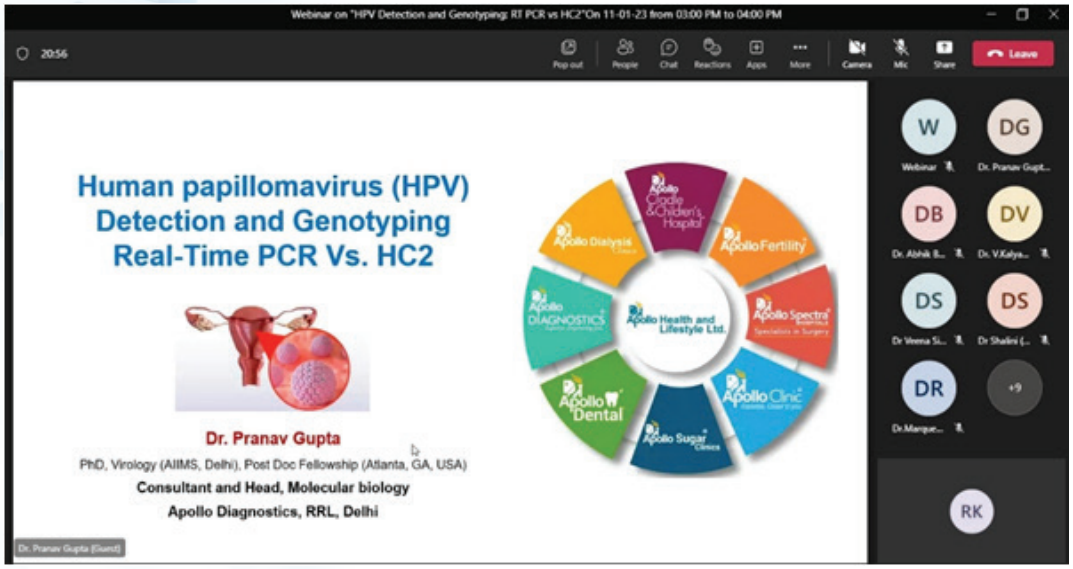
1. Uropathology
2. Pap smear
3. Lymph node
4. Salivary gland

DOWN (Inclusion bodies)

1. Noted in G-6-PD Deficiency
2. Noted in Mott cells in Myeloma
3. Concentric lamellated calcified structures
4. Noted in yellow fever
5. Eosinophilic/ Basophilic. Noted in HSV, VZV,CMV etc

Recent Events:

1. Webinar: Human Papillomavirus (HPV) Detection and Genotyping- Real Time PCR V/s Hybrid Capture- Dr. Pranav Gupta on 11th January 2023.



2. Webinar: Cystitis Glandularis and Malakoplakia- Bening mimickers of invasive carcinoma of Urinary Bladder- Dr. Bharathi Bhushan on 25th January 2023.

3. Felicitation of Dr.Vasavi from Apollo diagnostics from Osmania University recognizing her work in the field of Cytogenetics

4. Various CMEs from Apollo Diagnostic Doctors.


DIAGNOSTICS
Expertise
Empowering
Your Clinical
Decisions

Apollo DIAGNOSTICS®

Expertise. Empowering you.