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Feto-Maternal Diagnostics

آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi

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COLLEGE of AMERICAN PATHOLOGISTS

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From the Editor's Desk

Dear Readers

It gives me immense pleasure to present the first issue of LABRAD in the post COVID 19 era. While the threat of the pandemic is not entirely over, its intensity has significantly abated globally. The world has opened and is learning to adapt to the new normal. As things begin to become normal around the world, we all need to realize that the reason the pandemic has been controlled so quickly and we could enter the "post-pandemic era "is due to strict application of scientific knowledge. It was only due to the tremendous advancements in science and technology that an effective vaccine was developed in record time, which led to the rapid control of the pandemic. The pandemic resulted in an extremely high mortality and morbidity all over the world. We need to salute the health care workers especially the personnel working in clinical laboratory settings around the world who worked throughout on the front lines in combating the pandemic. Now that the situation has returned to normal "and the dance of death" which claimed hundreds and thousands of lives around the world has practically ended, it felt appropriate that we celebrate the miracle of life and my team thought we could do this best by dedicating this issue of LABRAD to the celebration of the beginning of life. We decided that this issue should be on mother and child diagnostics pertaining to screening and diagnostic tests which are essential in ensuring the safety and good health of the mother and baby, not just before, but also after the baby is born. Thus, we aim to provide the latest diagnostic updates for the "beginning of life ". A compilation of articles covering this topic are being presented in this issue. These include some interesting write-ups on TSH antibodies in pregnancy and newborns, AADC deficiency, radiological screening for cranial anomalies, red cell screening, maternal infections, gestational trophoblastic tumors, and others.
I hope that you will find this issue interesting and informative. My team will appreciate your valuable opinion and feedback as well as constructive suggestions to make future issues better, more informative, and useful.

Dr Sidra Arshad
Editor, LabRad

LABRAD Star Contributors for the Year 2021

Ms. Nazneen Islam
Manager Molecular Pathology

Ms. Iffat Arman
Senior Technologist, Chemical Pathology

Warmest congratulations to Ms. Nazneen Islam and Ms. Iffat Arman on your hard work and outstanding contributions to the LABRAD. This demands time and energy, and we deeply appreciate your efforts to make it an immense success. Also, we encourage everyone to contribute more to the issues to become star contributors of the LABRAD for 2022.

Clinical Relevance of Measuring Thyroid-Stimulating Hormone Receptor Antibodies in Pregnancy and Newborns

Mr Masab Azeem and Dr Hafsa Majid
Clinical Chemistry

Graves' disease occurs before pregnancy in 0.4–1 percent of women and in 0.2–0.4 percent during pregnancy, representing the most common cause (85 percent) of either overt or subclinical hyperthyroidism in women of reproductive age. A more frequent and peculiar form of hyperthyroidism in pregnancy is the gestational transient thyrotoxicosis (GTT) whose prevalence in the Asian population is 5.5–11 percent. GTT is defined as transient thyrotoxicosis caused by the stimulating effect β-HCG has on the TSH receptor toward the end of the first trimester of gestation and is frequently associated with hyperemesis gravidarum and twin pregnancies.

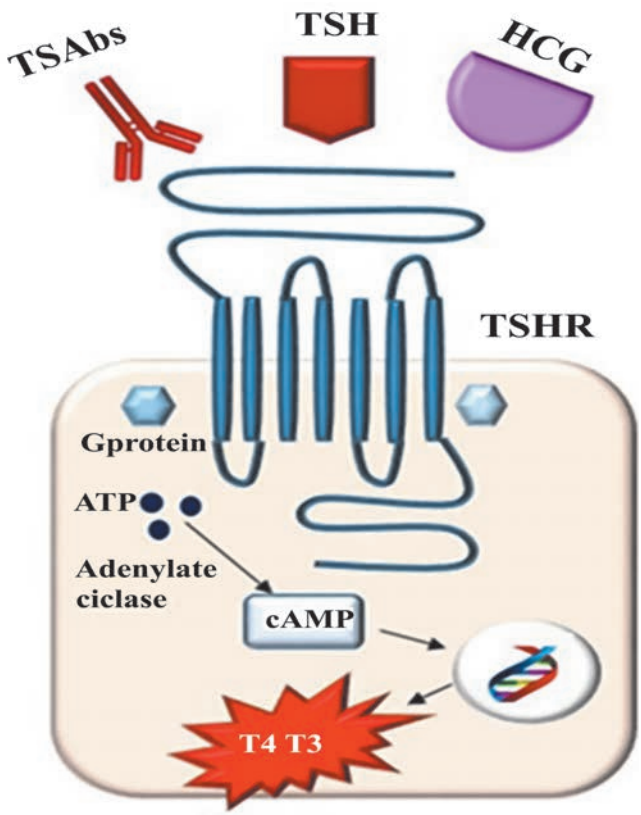

Figure 1: The stimulation of TSH receptor (TSHR) in pregnancy (Bucci I et al.)

The detection of TTRAbs is helpful in the identification of the etiology of thyrotoxicosis and is a hallmark for the diagnosis of Grave's disease. TRAbs can act on Thyroid Stimulating Hormone Receptor (TSHR) to have a stimulating, blocking, or a neutral effect.

During pregnancy the Thyroid antibodies can cross the placental barrier and affect the fetal thyroid to cause fetal hyperthyroidism. Untreated maternal Grave's disease can lead to fetal hyperthyroidism, perinatal mortality, premature delivery, congestive heart failure, craniosynostosis, microcephaly, and psychomotor disabilities. After parturition, newborn is tested for TRAB to evaluate for fetal hyperthyroidism.

The drug of choice for overt hyperthyroidism in pregnancy is Antithyroid drugs. Radioactive iodine is contraindicated in pregnant women. Surgery is only indicated when there are severe side effects of ATDs or uncontrolled thyrotoxicosis. However, serum T3 and T4 must be monitored closely as the side effect of these treatments is maternal hypothyroidism. The sequalae of maternal hypothyroidism is two pronged: maternal adverse effects include pre-eclampsia and placental abruption, fetal adverse effects include spontaneous abortion, preterm birth, low birth weight, intrauterine growth restriction.
In Pakistan, the prevalence of overt and sub clinical hyperthyroidism is reported to be 5.1 percent and 5.8 percent, respectively so for better maternal and fetal outcome there is a need to monitor maternal and neonatal Thyroid-Stimulating Hormone Receptor Antibodies of high-risk pregnancies.

Reference:
Bucci, I., Giuliani, C., & Napolitano, G. (2017). Thyroid-Stimulating Hormone Receptor Antibodies in Pregnancy: Clinical Relevance. Frontiers in endocrinology, 8, 137. https://doi.org/10.3389/fendo.2017.00137

Aromatic l-amino Acid Decarboxylase (AADC) Deficiency- An Ultra-rare and Underdiagnosed Neurometabolic Disorder

Iffat Arman and Dr Sibtain Ahmed
Clinical Chemistry

AADC deficiency is a very rare genetic disorder characterized by decreased activity of aromatic l-amino acid decarboxylase, an enzyme involved in the building (synthesis) of neurotransmitters (dopamine, serotonin, norepinephrine, and epinephrine.), which are responsible for the communication between neurons in the nervous system. The first case was reported in 1990 and since then approximately 135 patients have been reported in global literature. The disorder is characterized by, significant reduction or complete loss of AADC enzyme activity, leading to severe combined deficiency of serotonin, dopamine, norepinephrine, and epinephrine due to mutations in the dopa decarboxylase (DDC) gene. Signs and symptoms onset usually occurs during first year of life as depicted in Table 01.

Table 1: Signs and Symptoms of AADC Patients

Sign in AADC	Symptoms in AADC
Axial hypotonia ❖ Limbhypertonia ❖ Developmental Delay ❖ Dystonia ❖ Hypokinesia ❖ Bradykinesia ❖ Tremor ❖ Athetosis ❖ Myoclonic jerks ❖ Blepharospasm ❖ Oculogyric crises ❖ Feeding/swallowing problems.	❖ Autonomic dysfunction/instability ✓ Ptosis ✓ Excessive sweating ✓ Nasal congestion ✓ Arterial hypotension ✓ Temperature instability ❖ Mood instability ❖ Irritability ❖ Sleep disturbance ✓ Insomnia and/or hypersomnia ✓ Changes in melatonin levels

From diagnostic perspective, urine organic acid (UOA) analysis serves as a vital diagnostic modality with specifically elevated vanillactic acid and N-acetylvanilalanine. This first clue further guides the way for other investigations including CSF analysis for neurotransmitters, AADC enzyme activity and genetic testing for the confirmation of AADC deficiency. Moreover, urine organic acid (UOA) is a relatively simple and non-invasive test compared to CSF sampling which is technically challenging as well. The exact prevalence of AADC deficiency in Pakistan is unknown, however the availability of UOA analysis at AKUH clinical laboratories emphasizes the need for increased clinical awareness and empowered diagnostic suspicion, intending to deliver better care to Pakistani patients.

Role of Flowcytometry & Kleihauer - Betke Test in Detection and Quantification of Fetomaternal Haemorrhage

Dr Maria Owais and Dr Anila Rashid
Haematology & Transfusion Medicine

Fetomaternal haemorrhage (FMH) despite being a rare contributing cause of obstetric haemorrhage with a global occurrence of three in 1000 live births, is still a detrimental cause of intra-uterine fetal demise and neonatal death. In RhD negative females, calculating the size of FMH after events such as delivery, abortion or any other invasive procedures is a crucial step in determining the proper dose of RhD immunoglobulin. The Kleihauer Betke (KB) acid elution test, which involves microscopic estimation of the fetal red cells on a glass slide and exposing it to an acidic pH solution, has long been used for detecting and quantifying fetal red cells in the maternal circulation. Because haemoglobin A is acid-soluble, the maternal red blood cells become the "ghost" cells whereas, the fetal red cells remain pink as haemoglobin F is resistant to acid elution (Figure 1). However, KB test has several limitations which includes, underestimation of FMH due to improper staining, inadequate drying, short buffering time and inter/intra-observer variation. Additionally, ABO incompatibility and alloimmunization shortens the lifespan of fetal red cells in maternal circulation as well as the use of a pre-determined maternal blood volume of five litres in the routine calculations when determining the volume of an overweight pregnant woman can be a cause of FMH underestimation. False over-estimation can result in underweight pregnant woman and during advanced gestational age where fetal cells in the maternal circulation are increased by seven percent. Difference in FMH volume is also affected by variation in the formulas used for the calculation.

Therefore, with such limitations posing concerns over the detection and estimation of FMH volume, the paradigm has shifted to alternative and more accurate technique of flow cytometry which is based on the use of monoclonal antibodies against fetal red cells which are then quantified by fluorescence intensity. Flowcytometry is more accurate, reliable, reproducible, and less labor intensive compared to KB test. Flow cytometry can also detect large bleed more accurately in comparison to the KB test, therefore, allowing optimal administration of anti-D immunoglobulins.

Both tests help in calculating the dosage of anti-D immunoglobulin that needs to be administered to RhD negative mother when the delivered baby is RhD positive or after every potential sensitizing event.

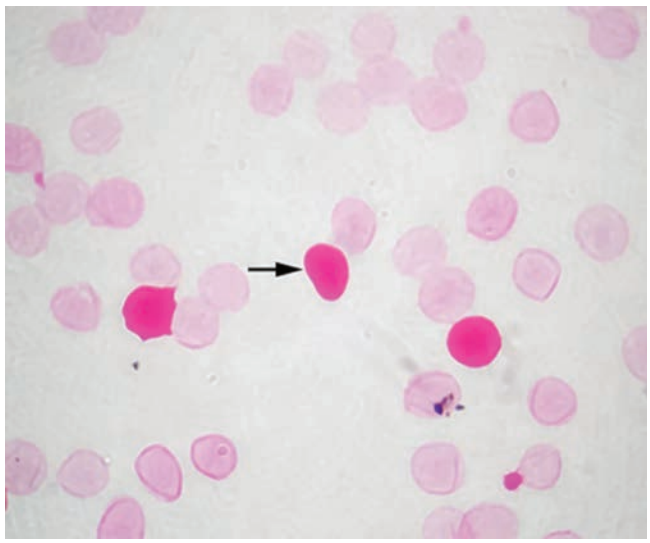

Figure 1: Dark pink fetal red cell (arrow) with background "ghost" maternal red cells.
Source of Image: 2022 UpToDate, Inc. and/or its affiliates.

Gestational Trophoblastic Diseases: An Overview

Dr Romana Idress, Dr Qurratulain Chundriger and
Dr Manahil Khan
Histopathology

Gestational trophoblastic disease (GTD) comprises of a spectrum of disorders varying from hydatidiform moles and gestational trophoblastic neoplasia (GTN). GTN is further classified into hydatidiform mole (HM), invasive mole, choriocarcinoma, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT).

The most common types of gestational trophoblastic disease occur when a sperm cell fertilizes an empty egg cell or when two sperm cells fertilize a normal egg. GTTs are characterized by trophoblastic differentiation of the tumor tissue and production of human chorionic gonadotropin (Hcg). This disease becomes evident six to ten weeks after conception. Most GTD tumors are benign, but some have the potential to turn malignant.

Risk Factors

Maternal age: younger than 20 or older than 35 years
History of previous molar pregnancy
History of miscarriage

Hydatidiform Moles (molar pregnancy)

Partial mole: In partial mole the fertilized egg contains the normal set of maternal DNA but double the number of paternal DNA. Because of this, the embryo only partially develops and does not become a viable fetus. Most partial moles have a triploid or near-triploid DNA content. The patients present with vaginal bleeding or with missed abortion. Macroscopically it shows tiny cysts measuring one to three mm in diameter. Microscopically partial moles show large irregularly shape hydropic villi admixed with smaller fibrotic normal looking villi. Some enlarge villi exhibit cistern formation and trophoblastic lined invaginations and inclusions. Presence of fetal blood vessels and nucleated RBCs are helpful clues in differentiation from complete mole. Immunohistochemistry for p57 shows nuclear staining in cytotrophoblasts and villous stromal cells. Partial moles are managed by suction curettage and close follow-up to exclude persistent mole. The disease status is monitored by serum HCG levels.
Complete mole: A complete mole develops when an empty egg is fertilized by one or two spermatozoa resulting in two complete male derived haploid chromosome sets. The fertilized egg has no maternal DNA and instead has two sets of paternal DNA (46XX). A fetus does not form. The patient usually presents with abnormal vaginal bleeding, anemia, severe nausea or vomiting, abdominal swelling, and high blood pressure at an early point in the pregnancy. Macroscopically it shows diffuse villous swelling resulting in appearance of grapes, measuring up to two cm. Microscopically there is markedly enlarge, hydropic villi with central cistern formation. There is circumferential trophoblastic proliferation around the villi, in addition there may be cytological atypia of the separately lying cytotrophoblasts and syncytiotrophoblasts (Figure A). Immunohistochemistry for p57 shows absent staining in cytotrophoblasts and villous stromal cells. Differential diagnosis of complete mole includes partial mole and early gestations. Early complete moles with no risk factors may be treated on the same lines as partial mole with close follow-up. High risk lesions require single agent chemoprophylaxis and if disease persists then these are managed by

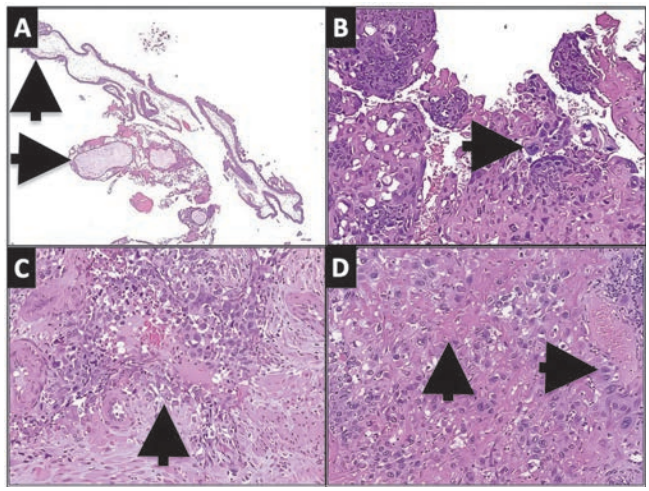

Figure A: Complete mole, showing cisternal dilatation of the villi (↑) and circumferential proliferation of trophoblastic cells around the villi (→).
Figure B: Choriocarcinoma, showing trimorphic trophoblastic population, with cytotrophoblasts, intermediate trophoblasts and villous syncytiotrophoblasts (→).
Figure C: Placental site trophoblastic tumor, shows characteristic infiltrating growth, replacing the vascular wall (↑).
Figure D: Epithelioid trophoblastic tumor shows growth of epithelioid trophoblasts which resemble squamous cells. There is deposition of pink hyaline like material (↑) and tumor cells surrounding the blood vessels (→).

combination chemotherapy or rarely hysterectomy is needed. The treatment response of the disease and disease status is monitored by serum HCG levels. Complete hydatidiform mole carries a 15-20 percent risk of persistent gestational trophoblastic disease and a two to three percent risk of gestational choriocarcinoma.

Invasive mole: Invasive moles are locally invasive, characterized microscopically by invasion of the myometrium by trophoblastic tissue with identifiable villous structures. These may be preceded by either complete or partial molar pregnancy. They are usually diploid in karyotype but may be aneuploid. Microscopically, these lesions are characterized by markedly edematous villi with associated sheets and clusters of atypical trophoblasts within the myometrium. Villi may be present in the vessel and once vascular invasion has developed, it may embolize to distant sites such as vagina, lungs, and brain. Invasive moles have more aggressive behavior than either complete or partial HMs, therefore treated with chemotherapy or hysterectomy. Morbidity or mortality can result, generally related to hemorrhage. The disease status is monitored by serum HCG levels.

Gestational Trophoblastic Neoplasia

There are several types of gestational trophoblastic neoplasia:

Choriocarcinoma: Choriocarcinoma is a malignant tumor which may occur after molar pregnancy, abortion, ectopic pregnancy, or normal pregnancy. Most choriocarcinomas have an aneuploid karyotype, and about three-quarters of them contain a Y chromosome. Macroscopically it shows well circumscribed hemorrhagic nodules. Microscopically the lesion is characterized by admixture of cyto, intermediate and syncytiotrophoblasts showing marked degree of cytological atypia and nuclear pleomorphism. In the background extensive hemorrhage and necrosis seen (figure B).

Characteristically no villi present and presence of villous alert the pathologist for an alternative diagnosis. On immunohistochemistry the tumor cells show positivity for HCG, hPL and SALL4.Ki67 is more than 90 percent. The major differential diagnosis includes persistent molar tissue, placental site trophoblastic tumor and undifferentiated carcinoma. Choriocarcinoma is managed by chemotherapy with an overall disease-free survival is close to 90 percent. Choriocarcinoma may metastasize to lungs, brain, liver, pelvis, vagina, spleen, intestines, and kidney. The treatment response of the disease and disease status is monitored by serum HCG levels.

Placental-site trophoblastic tumor: PSTT disease mostly presents in reproductive age group usually secondary to a term delivery, abortion, or molar pregnancy. Most women present with irregular bleeding. Macroscopically PSTT presents as discrete mass or as polypoid appearance. Microscopically PSTT has an infiltrative growth pattern and comprised of sheets and aggregates of large polyhedral to round cells with abundant amphophilic cytoplasm and infiltrate into the myometrium. Pleomorphic trophoblasts are also commonly seen (figure C). Most tumors have fewer than five mitoses per 10 HPFs. Ki67 index is usually less than 10 percent. They are generally resistant to chemotherapy. Therefore, hysterectomy is the basic primary treatment if the tumor is confined to the uterus. However, about 35 percent of PSTTs have distant metastases at diagnosis. The option of adjuvant chemotherapy is reserved for patients who present with metastasis. Common sites of metastasis include the lungs, pelvis, and lymph nodes, brain, renal, and liver. The treatment response of the disease and disease status is monitored by serum HCG levels.

Epithelioid trophoblastic tumor: ETT is a subset of PSTTs and arises from chorionic-type intermediate trophoblasts. Morphologically, it shows nests and cords of tumor cells surrounded by areas of necrosis and calcification. The tumor cells are round to polygonal with moderate to abundant eosinophilic cytoplasm, distinct cell membranes and round nuclei. Eosinophilic hyaline like material is seen in the center of tumor nests which is an important diagnostic clue. The tumor cells are usually seen around the vessels and may resemble squamous cell cancer (SCC) of the cervix therefore it is important to consider this entity in mind when looking at SCC of cervix. Mitotic activity is usually low (figure D). Differential diagnosis includes PSTT, choriocarcinoma and squamous cell carcinoma. Immunohistochemistry shows positivity for p63 and inhibin. Ki67 is usually less than 10 percent. Hysterectomy is the preferred treatment. Chemotherapy is reserved for resistant disease or with evidence of metastasis. Its clinical behavior appears to be closer to that of PSTT than to choriocarcinoma. It has a spectrum of clinical behavior from benign to malignant. About 10 percent of cases behave in a malignant manner and may metastasize, usually in the lungs.

FIGO Stages of GTN:

I: Disease confined to the uterus
II: Gestational trophoblastic tumor extends outside

of the uterus, but limited to the genital structures (adnexa, vagina, broad ligament)
III: Gestational trophoblastic tumor extends to the lungs, with or without known genital tract involvement
IV: All other metastatic sites

Prognostic Factors:
The prognosis for cure of patients with GTDs is good even when the disease has spread to distant organs, especially when only the lungs are involved. Therefore, the traditional staging system has limited prognostic value. The probability of cure depends on the following:
Histologic type (invasive mole or choriocarcinoma).
Extent of spread of the disease
largest tumor size.
Level of serum beta-hCG.
Duration of disease from the initial pregnancy event to start of treatment.
Number and specific sites of metastases.
Nature of antecedent pregnancy.
Extent of prior treatment.

Acrania-Exencephaly-Anencephaly Sequence/ Spectrum: Radiological Features on Antenatal Screening

Dr Sundas Basharat and Dr Shayan Anwar
Radiology

The term **Acrania-Exencephaly-Anencephaly sequence** encompasses separate pathological entities which are in fact interchangeably diagnosed at different periods of gestation and may be difficult to distinguish.

Definitions and Pathophysiology

Acrania: refers to non-development of cranial vault, associated musculature and dura secondary to defective migration of mesenchymal cells beneath the ectodermal layer after closure of anterior neuropore around fourth week of gestation.

Exencephaly: With acrania regarded as an antecedent, absence of cranial vault and associated structures impair the normal differentiation of cerebral parenchyma resulting in relatively morphologically normal appearing to disorganized cerebral tissue referred to as exencephaly.

Anencephaly: Associated with acrania characterized by complete absence of cerebral parenchyma occurring as a result of defective closure of anterior neuropore around 4th week of gestation at the level of forebrain and midbrain. Normal fusion is however seen at hindbrain and cervical cord level.

Acrania-Exencephaly-Anencephaly sequence refers to the progression of acrania with exencephaly, resulting in degeneration and progressive destruction of cerebral parenchyma secondary to mechanical trauma and exposure to the amniotic fluid with almost complete disintegration of cerebral tissue after 14th week referred to as anencephaly. Disintegrated brain tissue may be visible within the amniotic fluid as echogenic floating particles.

Radiological features on antenatal scans

Acrania: Antenatal diagnosis of acrania is established after 11 weeks onwards since the ossification of fetal cranial vault typically starts after nine weeks. Characterized by absent ossification of predominantly frontal bones on axial and coronal sections, cerebral hemispheres may be seen surrounded by a thin membrane. (Figure 1-3)

Exencephaly: Considered as an antecedent to anencephaly. Characterized by absent fetal cranial vault with exposed fetal brain seen floating within the amniotic fluid, loss of cerebral tissue is seen to variable extent. Absence of nasal bone may also be seen. (Figure 1)

Anencephaly: Considered at the extreme end of

neural tube defects spectrum incompatible with life. Characterized by absent fetal calvarium and brain tissue cephalad to the orbits. “Frog eye” appearance may be seen secondary to absence of brain tissue cephalad to the bulging orbits in coronal plane. Variable presence of brainstem and cerebellum may be seen. (Figure 2,3)

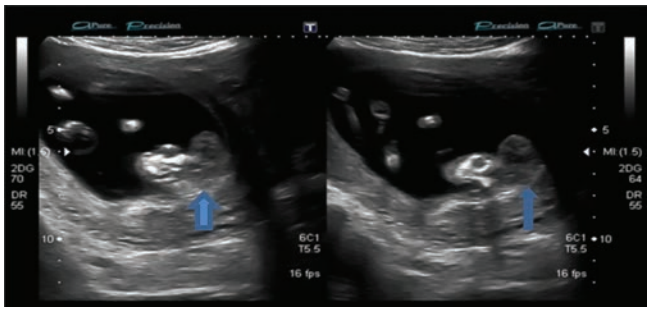

Figure 1: Image from antenatal scan reveals acrania with dysmorphic brain (Blue arrows) above the fetal face suggestive of acrania with exencephaly.

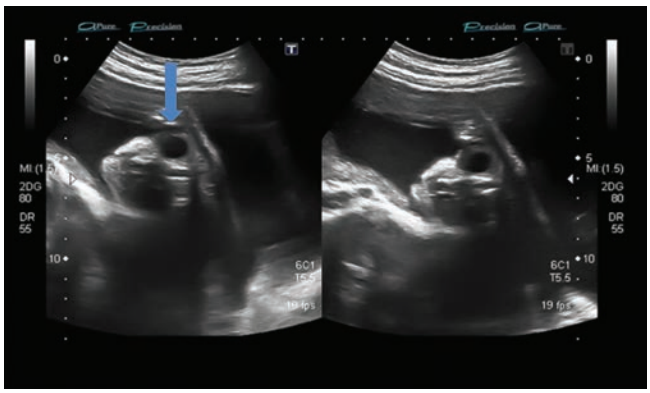

Figure 2: Image reveals absence of cranial vault and brain with protruding orbital structures giving “ frog eye appearance”(blue arrow) suggestive of acrania with anencephaly.

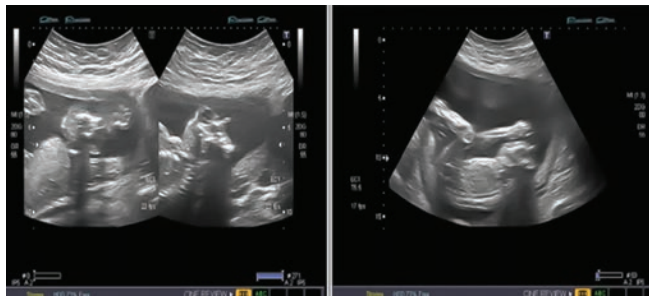

Figure 3: Another example of acrania with absent brain parenchyma suggestive of anencephaly.

Prognosis and Treatment:

Considered as a cranial neural tube defect, incompatible with life.

Most cases result in still birth, however infants who survive usually die within hours, days or few weeks after birth. Early diagnosis is therefore essential for counseling of the parents and planning for early termination of pregnancy.

Prevention:

With an increased risk of recurrence in future pregnancies, folic acid therapy may be considered essential for prevention.

Conclusion:

Sonographic diagnosis of anencephaly and its antecedents in the **Acrania-Exencephaly-Anencephaly Sequence** is possible with the help of high-resolution sonography as early as 11th week of gestation with an accuracy reaching almost 100 percent at 14th week of gestation.

Minimally Invasive Tissue Sampling (MITS) A New Way to Investigate Cause of Death In Resource-constrained Countries

Dr Saman Amin and Zeeshan Uddin
Histopathology

Minimally invasive tissue sampling (MITS) consists of a series of post-mortem punctures using fine biopsy needles aiming to obtain tissue samples and body fluids from a corpse within the first hours after death, which are then submitted for a thorough pathological, molecular and microbiological investigation of the underlying cause of death (Figure 1).

MITS is proposed as an alternative to complete diagnostic autopsy (CDA). CDA is a complex procedure and needs resources, infrastructure and pathology expertise. It is also limited by deaths occurring outside of formal health system premises

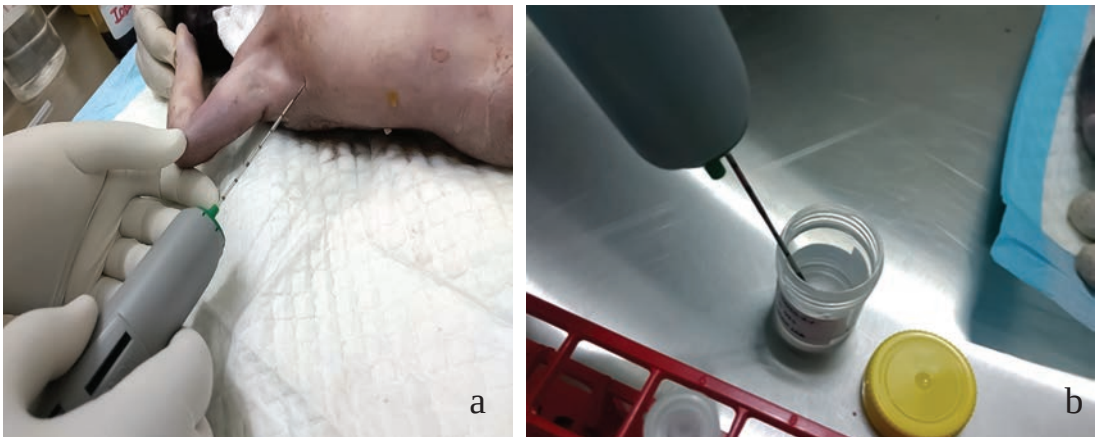

Figure 1: Right lung is being sampled by Bart monopty biopsy needle (a). The sample for histopathology is being sent in formalin (b).

and lack of interest from clinicians and relatives.
It is important to identify cause of death so that government and health institutions can better plan and prioritize their efforts, ultimately saving lives around the world.
MITS was developed in 2013 by the Barcelona Institute for Global Health (ISGlobal). It is carried out by trained pathologists and/ or technicians using fine needles to collect small amounts of tissue and fluid from key organs.
MITS has many advantages such as:

- Non-disfiguring, compared to CDA.
- Relatively low cost.
- Performed quickly.
- Technically much more feasible – Simple and easy to perform by minimally trained personnel in remote areas.
- Relatively accurate – Validated through observational studies across all age groups.

The minimally invasive tissue sampling (MITS) procedure, formerly known as the minimally invasive autopsy (MIA), was developed as an approach to reduce uncertainty around cause of death determination in high mortality settings, where clinical and postmortem diagnoses are nearly always unavailable, and where performance of complete diagnostic autopsy would not be feasible. The procedure uses biopsy needles to obtain post-mortem samples of lung, brain, liver and other key organs for histopathologic, microbiologic, and molecular examination to help determine cause of death.

Childhood Mortality and Role Of Mits:

Despite reductions over the past two decades, childhood mortality remains high, particularly in low-income settings in sub-Saharan Africa and south Asia. In lower- and middle-income countries (LMIC), individuals often die without having been seen by qualified medical personnel and are frequently buried or cremated without a documented medical history, before an evaluation of the cause of death can be conducted; many times, these deaths are not counted at all. As a result, overall mortality is underestimated, and because of a variety of assumptions and dependence on non-specific data, calculations of the local, regional and global burden of specific causes of mortality result in dramatically conflicting numbers and substantial uncertainty. Credible and accurate mortality data would enable policymakers, stakeholders, donors to effectively target the leading causes of childhood mortality for interventions, a critical component for achieving the Sustainable Development Goal for eliminating preventable childhood deaths.

Child Health and Mortality Prevention Surveillance (CHAMPS) An international disease surveillance network focused on child mortality funded by the Bill & Melinda Gates Foundation and coordinated by Emory University and the US Center for Disease Control (CDC). The CHAMPS network is tracking track the causes of under-5 mortality and stillbirths at sites in sub-Saharan Africa and South Asia through epidemiologic surveillance of under five deaths and still births utilizing MITS, laboratory diagnostics including conventional and advanced histopathology and molecular screening of various pathogens, verbal autopsy, and available clinical and demographic data. Recently Pakistan has been selected as a site for CHAMPS with Aga Khan University leading the project.

Another project called The Project to Understand & Research preterm Pregnancy Outcomes and Stillbirth: South Asia (PURPOSe) was launched to determine the main causes of death for still births and preterm (Less than 37 weeks gestation) infants admitted to the NICU or delivered and cared for in selected hospitals in Pakistan and India study sites. Target sample size for Pakistan was 350 stillbirths and 350 preterm neonatal deaths. With a neonatal mortality rate of 15-20 percent, it was estimated that we need to recruit 2,500

women with preterm live births in order to evaluate 350 preterm neonatal deaths.

PURPOSe – MITS acceptance rate was 74 percent for stillbirths and 36 percent for preterm neonatal deaths. Success rate in obtaining the pathology samples was comparable to other reported studies. Because of size, location and bilaterality, obtaining lung samples was in general more successful than other organs. Several histopathological findings were seen in the MITS samples of PURPOSe particularly in lungs including amniotic fluid aspiration, intra-alveolar hemorrhage, hyaline membrane disease, bronchopneumonia/ pyogenic pneumonia etc (Figure 2). Liver showed cases with extramedullary hematopoiesis, ductal plate malformation and a few showing features of storage disorder (Figure 3). Brain samples were mostly unremarkable histologically. Like other studies, lung tissue was found to be the most valuable for determining the COD. The sample preservation was adequate in most cases except in a few cases of still births where the samples were autolyzed due to marked maceration of fetus.

In conclusion, MITS is a reliable alternative to CDA for determining the cause of death in resource constrained settings and relatively conservative countries like Pakistan. Since it is based on blind sampling of various key organs, its accuracy may be further increased by adding radiological support.

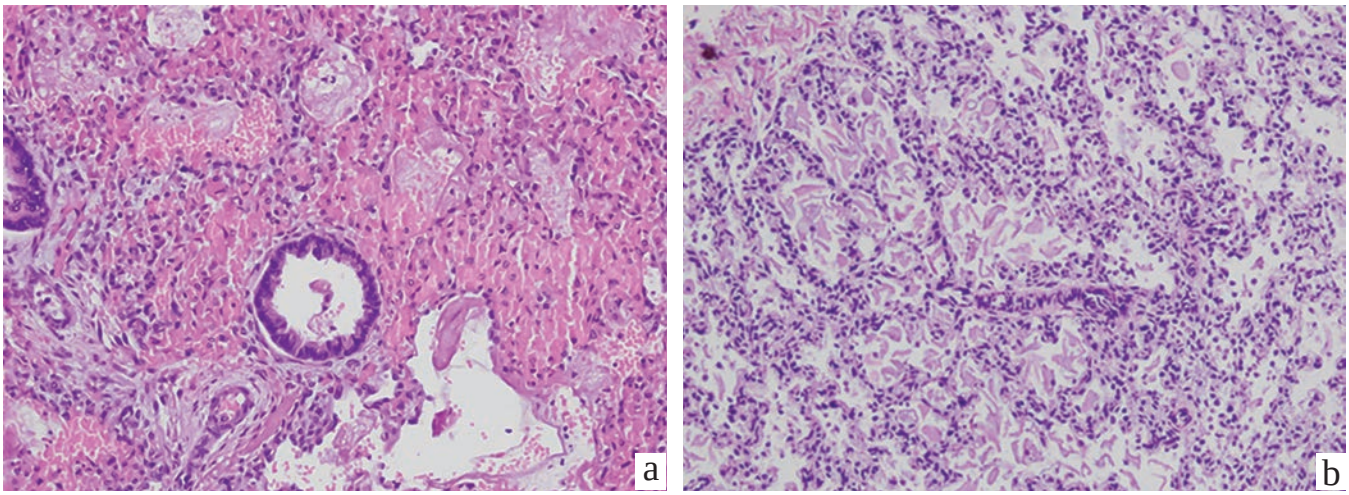

Figure 2: MITS histopathology sample of lung showing cases of hyaline membrane disease (a) and amniotic fluid aspiration (b).

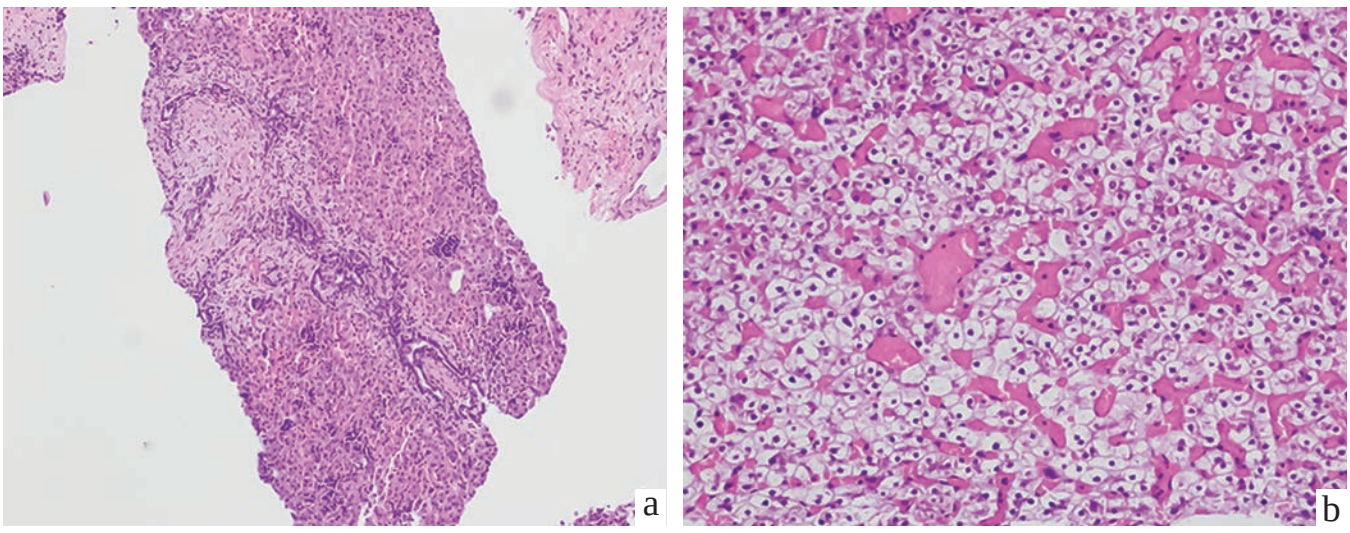

Figure 3: MITS histopathology sample of liver showing examples of ductal plate malformation (a) and storage disorder (b).

Laboratory Guideline for Detection, Interpretation and Reporting of Maternal Cell Contamination (MCC) in Prenatal Analysis

Samina Ghani
Molecular Pathology

The potential presence of maternal cell contamination (MCC) in chorionic villus or amniotic fluid samples poses a serious preanalytical risk for prenatal misdiagnosis. Association for Molecular Pathology (AMP) guidelines available for the entire testing process within a clinical laboratory, from preanalytical, through the analytical and postanalytical phases of analysis. Together with good laboratory practices and existing standards and guidelines for molecular genetic testing, these guidelines can help provide accurate genetic information and minimize the potential of diagnostic error.

Prenatal Guidelines

1. It is recommended that MCC analysis be performed in parallel with diagnostic testing, regardless of the genetic disorder or its mode of inheritance.
2. All pertinent intake information including clinical, family, and testing history, should be provided to the laboratory, along with the prenatal sample.
3. Information regarding multiple gestations should be available to the laboratory, along with the prenatal sample, due to the risk of potential co-fetal contamination from a twin.
4. Only a maternal peripheral blood is strictly required for MCC testing. Paternal samples are not helpful for MCC analysis.
5. Backup cultures should be readily available for repeat or confirmatory testing if MCC is detected in an uncultured specimen.
6. Every effort should be made to include the prenatal sample(s) in the earliest available assay setup and to prioritize the prenatal samples into testing.
7. It is important that only one prenatal test sample be handled at any one time to ensure accuracy and prevent a potential mix-up of samples at this stage of analysis.

Technical Guidelines

1. Maternal specimen should be tested and analyzed for MCC concurrently within the same analysis to allow for a direct comparison of results.
2. The performance characteristics of these assays must be validated for the intended purpose of MCC studies in each laboratory before implementation into clinical testing.
3. Tetranucleotide/pentanucleotide markers are preferable monitors for MCC over smaller repeat markers due to the superior fidelity, robustness of PCR amplification, accurate measurement of repeat units by fragment analysis, distinguishable alleles with a high discriminative capacity, and intergenerational stability among individuals in the general population.
4. PCR stutter peaks (caused by replication slippage during PCR amplification) that are the size of the repeat unit of a maternal allele should be considered noninformative for MCC
5. It is recommended that two to three informative microsatellite markers reflecting clearly definable, separate maternal and fetal genotypes from among a panel of approximately seven to ten markers be used to assess the presence of MCC in a prenatal sample.
6. At least five percent MCC must be routinely detectable by the clinical laboratory. This percentage is recommended as the upper limit based on the fact that erythrocyte admixture of just a few percent is readily visible by eye, and that incorrect interpretation of a PCR-based diagnostic prenatal test has been reported even at a level of one percent to two percent MCC, six with increasing likelihood at higher percentages.

Reporting Guidelines

1. The prenatal diagnostic results should not be released before the MCC analysis is completed.
2. The sensitivity of the MCC assay should be indicated on all issued MCC reports.
3. The presence of very small peaks, possibly indicating low-level MCC at or below the validated level of detection for the MCC assay or below the threshold of sensitivity of the clinical assay, may require acknowledgment on the reports. Comments in the context of such reports may include phrases such as "significant maternal cell contamination not detected," or "maternal cell contamination is unlikely to have interfered with the reported fetal result."

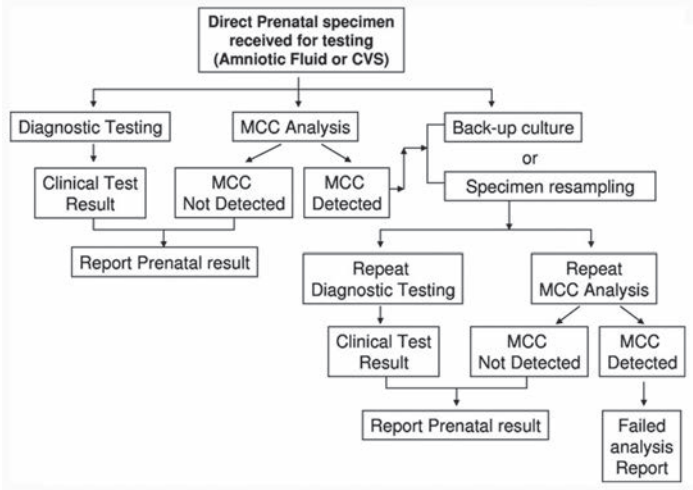

Figure: A practical testing algorithm for prenatal samples.

Importance of Antenatal Red Cell Screening for Assessment of Maternal Alloimmunization

Dr Fatima Farhan and Dr Hasan Hayat
Haematology & Transfusion Medicine

Screening is an important component of initial health-risk assessment with major objective of reducing morbidity and mortality. The aim of antenatal screening is to ensure safety of the mother and the fetus by identifying the need for timely intervention and management of the pregnancies at risk. One of the main hematological emergencies that arise during pregnancy is hemolytic disease of the fetus and newborn (HDFN) due to immune mediated causes like Rh or ABO blood group incompatibility and non-immune cause due to hemoglobin structural variants or hemoglobinopathies. Antenatal screening has become an important component of the standard routine laboratory panel testing nowadays that is recommended at the first antenatal (booking) visit for every pregnant woman.

HDFN arises due to alloimmunization against fetal red cell antigens such as A, B, Rh antigens (anti-D, anti-E and anti-c), Kell, Kidd and Duffy which are recognized as foreign antigens by the mother, leading to formation and transplacental passage of maternal IgG antibodies and destruction of the fetal red blood cells causing severe anemia and hemolysis. Most of the cases of severe HDFN are caused by anti-D antibody affecting 1 in 1200 pregnancies, leading to kernicterus, severe edema, ascites, heart failure and death (hydrops fetalis). ABO incompatibility is more commonly encountered in almost 15 percent of all pregnancies but HDFN occurs in only one percent of such pregnancies and is caused by IgG antibodies of blood group O mothers.

The antenatal red blood cell antibody screening protocol recommends screening of all pregnant women for blood group (ABO and Rh) at the first antenatal clinic visit in the first trimester. Non-sensitized Rh-negative mothers who screen negative for antibodies are screened for antibodies again at 28th week of gestation and at delivery. In mothers with positive antibody screen, the antibody titers are determined and then serially monitored throughout the pregnancy. The non-sensitized Rh-negative mothers who screen negative for Rh antibodies are given 100 microgram of anti-D immune globulin prophylaxis at 28th and 34th week of gestation to prevent maternal red cell alloimmunization. Another approach is to give single dose of 300 microgram at 28th week of gestation. If primary immune response to the D antigen has occurred, maternal administration of anti-D immune globulin will not prevent a rise in titer, and anti-D immune globulin

should not be administered to sensitized women.

Interpretation of antibody titration results by Indirect Antiglobulin Test

The antibody titer is commonly determined by indirect antiglobulin test (IAT). It is performed by incubating the maternal serum with cells having antigen against which mother has developed antibodies (for example Rh-D positive red blood cells in case of RhD alloimmunization) and IgG antiglobulin reagent at 37oC. The critical antibody titer is 1:16 – 32 for anti-D and 1:8 for anti-K. If titration is 1:16, repeat titration at 18-20 weeks of gestation. If titer is < 32, it should be repeated at four weekly intervals, starting from 16-20 weeks gestation. If titer is ≥32, the fetal anemia should be assessed for HDFN by velocimetry of fetal middle cerebral artery by color Doppler imaging after 16 weeks of gestation. Paternal zygosity of the at-risk fetus for being affected by HDFN should be confirmed by noninvasive method of detecting cell-free DNA in the maternal plasma by PCR.

The introduction of routine antenatal red cell screening has changed the overall spectrum of HDFN in the last few decades. An effective, timely, reliable, cost-effective antenatal screening programme can help improve patient outcomes. This has allowed patients to make reproductive choices and to monitor the pregnancy for complications. The timely prophylaxis with Rh immune globulin has decreased the overall incidence of Rh-D alloimmunization in pregnancy and reduced the need for repeated intrauterine blood transfusions. The utility of diagnostic techniques, starting from detecting allo-immunized pregnancy by maternal blood group typing, antibody screening/ identification and titration, to the quantification of feto-maternal hemorrhage by Kleihauer-Betke testing or by flow cytometry has helped achieved the main outcome of management, which is a successful pregnancy and delivery of a healthy newborn.

Maternal Screening for the Prevention of Early Onset Group B Streptococcal Disease in Neonates

Dr Syeda Rija, Dr Mehreen Shahid, Dr Adan Zubair and Dr Moiz Khan
Microbiology

Group B streptococcus (GBS) is a facultative pathogen found in gut and vaginal tract of some women. The primary reservoir is the gastrointestinal tract which provides the source for vaginal colonization. GBS is considered as the most frequent cause of early onset infections in newborn infants ranging from asymptomatic infection to pneumonia, septicemia and meningitis. Early onset of disease is usually related to maternal colonization of GBS in the genitourinary tract, with increased chances of vertical transmission during labor or after rupture of membranes.

The overall prevalence of GBS vaginal colonization in pregnant women ranges from 17 percent to 22.6 percent. The risk factors associated with transmission of infection include GBS bacteriuria during pregnancy, intrapartum fever, preterm labor at <37 weeks of gestation, previous infant with GBS infection or prolonged rupture of membranes for >18h5. Universal prenatal screening of mothers through vaginal/ rectal cultures coupled with targeted intravenous intrapartum antibiotic prophylaxis has shown efficacy in preventing neonatal GBS infections.

For the detection of GBS colonization culture technique remains gold standard for identification of infection however, this method requires 18-72 hours for final organism identification. Therefore, culture-based technique is only feasible for antepartum screening and not for intrapartum testing. Another modality for the detection of GBS infection is Real time Polymerase chain reaction (RT-PCR) which has shown to be more sensitive and specific than culture techniques. Recently, an Xpert GBS assay (Cepheid), which is a commercial RT-PCR test has become available which is more sensitive and specific than culture screening.

Culture based method:
A culture of lower vagina and anorectal swab is the most employed method for GBS screening. These

swabs are placed in non-nutrient transport media after collection and should be kept at ~4oC. Enriched culture media such as Todd-Hewitt broth with nalidixic acid and colistin, are recommended which can be sub-cultured on chromogenic agar or selective media.

Molecular methods:
RT-PCR has been the conventional molecular method for GBS screening. Recently a modification of this method has been introduced namely the Xpert GBS assay test with sensitivity of around 89 percent according to a randomized control trial. The test is fully automated involving DNA intensification, cleaning of nucleic acids and detection of the required cfb gene sequence with a reporting time of 50 minutes.

In conclusion, GBS colonization of mothers can be screened through the conventional culture technique which is the gold standard. However, owing to recent advances newer diagnostic methods such as RT-PCR and Xpert GBS assay offer prompt recognition of GBS infection which are more sensitive and specific than conventional culture-based screening.

Molecular Analysis of Alpha Thalassemia by MLPA

Nazneen Islam, Kahkashan Imam, Sonana Riaz,
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Molecular Pathology

Introduction

The Alpha thalassemia is the inherited disorder of ά globin gene, which encompasses all those conditions in which deficit production of ά globin chain of hemoglobin (Hb) which contain normally two ά-like and two β like globin chain (HbA). Due to underproduction of ά globin chain, γ and β globin chain synthesized excessively for compensation during fetal and adult life respectively, called Hb Bart's (γ4 tetramers in fetus) and HbH(β4 tetramers in adult).Individuals having mutation in one allele of globin gene on chromosome ,are said to have ά thalassemia trait, they may associated with mild anemia or with normal hemoglobin. Compound heterozygotes and some homozygotes for ά thalassemia have moderately severe anemia characterized by presence of HbH disease. In neonatal period some individual who make very little or no α globin chains have a very severe form of anemia which, if untreated, causes death called the Hb Bart's hydrops foetalis syndrome.

Genetics of Alpha Globin

Alpha globin gene present on chromosome16 (16p 13.3) whereas Beta globin gene present on chromosome11 (11p 15.4). There are two functional alpha genes present in chromosome 16, which are arranged as following described in figure 1. The size of cluster gene is 70 kb which consist of three pseudo gene ψ, έ,ά2, and ά1 and four functional genes, έ,ά2, ά1 and φ, in alpha gene there are 3 exon and 2 introns. There are about 10 variable number of tandem repeats are located around the alpha globin gene locus. Two alpha genes lie adjacently to each other. As each diploid cell contains two sets of each chromosome, hence there are four alpha globin alleles are present in normal individual.

Epidemiology

The prevalence of alpha thalassemia is also like others common globin gene disorder (β thalassemia and sickle cell trait), it has high frequencies throughout all tropical and subtropical of the world. In some areas, the carrier frequency of α thalassemia may be as high as 80-90 percent of the population, almost at fixation. Of all globin disorders, α thalassemia is the most widely distributed and therefore many individuals in these areas have interacting combinations of these variants (e.g. both α and β thalassemia). Due to differences in the interactions between the various molecular defects underlying α thalassemia, HbH disease is predominantly seen in South East Asia, the Middle East and the Mediterranean. Similarly, the Hb Bart's Hydrops fetalis syndrome is predominantly seen in South East Asia.

Etiology of ά thalassemia on Molecular Basis
Alpha globin synthesis in normal healthy individual is regulated by four ά globin genes two on each copy of chromosome and this one genotypical express as άά/άά. These genes expression is dependent on remote regulatory elements (named Multispecies Conserved Sequences or MCS-R1 to R4) located far upstream of the globin genes in the introns of a flanking, widely expressed gene. Deletion of genes one (-α) or both (--) α genes from the chromosome is most frequent cause of alpha thalassemia. Occasionally point mutations in critical regions of the α2 (α r α) or α1 (αα r) genes may cause, so-called, non-deletional α thalassemia. Very rarely, α thalassemia results from deletion of the MCS-R regulatory elements (written as (αα)r), in all of these deletions MCS-R2 is always removed and thus appears to be the major regulatory element. When a mutation(s) completely abolishes expression from a chromosome this is called α0-thalassaemia and when the mutation(s) only partially down regulate expression from the chromosome this is called α+-thalassemia.

Genotype/Phenotype Correlations

There is up till now more 128 different genetic defects reported,14 which causing ά thalassemia and there is increasing number of potential interacting mutations, the clinical phenotypes (broadly classified as α thalassemia trait, HbH disease and Hb Bart's hydrops foetalis) resulting from the interactions between these various molecular defects can be simply summarized as in Table 1. The severity of the clinical phenotype correlates very well with the degree of α globin chain deficiency. An important additional point is that, in general, interactions involving non-deletional forms of α+-thalassemia result in a more severe phenotype than in those with deletional forms of α+-thalassemia.

α Thalassemia trait

Those individuals are carriers (heterozygotes) of α thalassemia, whatever the molecular basis, are clinically asymptomatic and the diagnosis is often established during a regular health check or during antenatal screening.

HbH disease

HbH disease is most frequently seen in patients who are compound heterozygotes for two different mutations or less frequently homozygotes for a moderately severe molecular defect. The severity of the clinical features is clearly related to the molecular basis of the disease. Patients with non-deletional types of HbH disease are more severely affected than those with the common deletional types of HbH disease.

Hb Bart's Hydrops Foetalis Syndrome

Infants with the Hb Bart's hydrops foetalis syndrome have the most severe deficiencies in α globin expression. While it most frequently results from the inheritance of no α globin genes from either parent, in some cases it results from the inheritance of a severe nondeletion mutation from one parent and no α genes from the other. Patients on the borderline between severe HbH disease and Hb Bart's hydrops foetalis syndrome are said to have HbH hydrops syndrome.

Molecular analysis

Last few decades it has becomes increasingly possible to diagnose alpha thalassemia very accurately and identify the precise defects underlying these disorders using a variety of molecular techniques. Ultimately, most α globin chain rearrangements have been characterized by MLPA and DNA sequence analysis. However, for today's diagnostic demands these techniques are far too laborious to apply in each case, and from the original work defining these mutations, rapid screening assays have been developed. For suspected but currently unknown rearrangements, Southern blotting or MLPA analysis may be used. Southern blot is the classical method to detect deletions causing α-thalassemia [15-19]. More recently Multiplex Ligation-dependent Probe Amplification (MLPA) is used, based on ligation of multiple probe-pairs hybridized across a (usually large) region of interest (figure 2), followed by semi-quantitative amplification using universal tag PCR primers and subsequently fragment analysis.

Methods

Multiplex Ligation-dependent Probe Amplification (MLPA) is performed on blood after DNA extraction, for analysis alpha thalassemia we identified deletion and duplication of ά globin gene, it consists on four steps procedure which amplify its tested product in two days. The steps are follows:

1) Hybridization Reaction (Day-1)
2) Ligation Reaction (Day-2)
3) PCR Reaction Day-2)
4) Separation of Amplified product by Capillary Electrophoresis

Phenotype	Genotype
Hemoglobin Bart hydrops fetalis (Hb Bart) syndrome	Loss of all 4 α-globin genes
Hemoglobin H (HbH) disease	Loss of 3 α-globin genes
α-thalassemia trait	Loss of 2 α-globin genes either in cis (--/αα, α0 carrier) or in trans(-α/-α)
α-thalassemia silent carrier	Loss of 1 α-globin gene (-α/αα, α+ carrier)

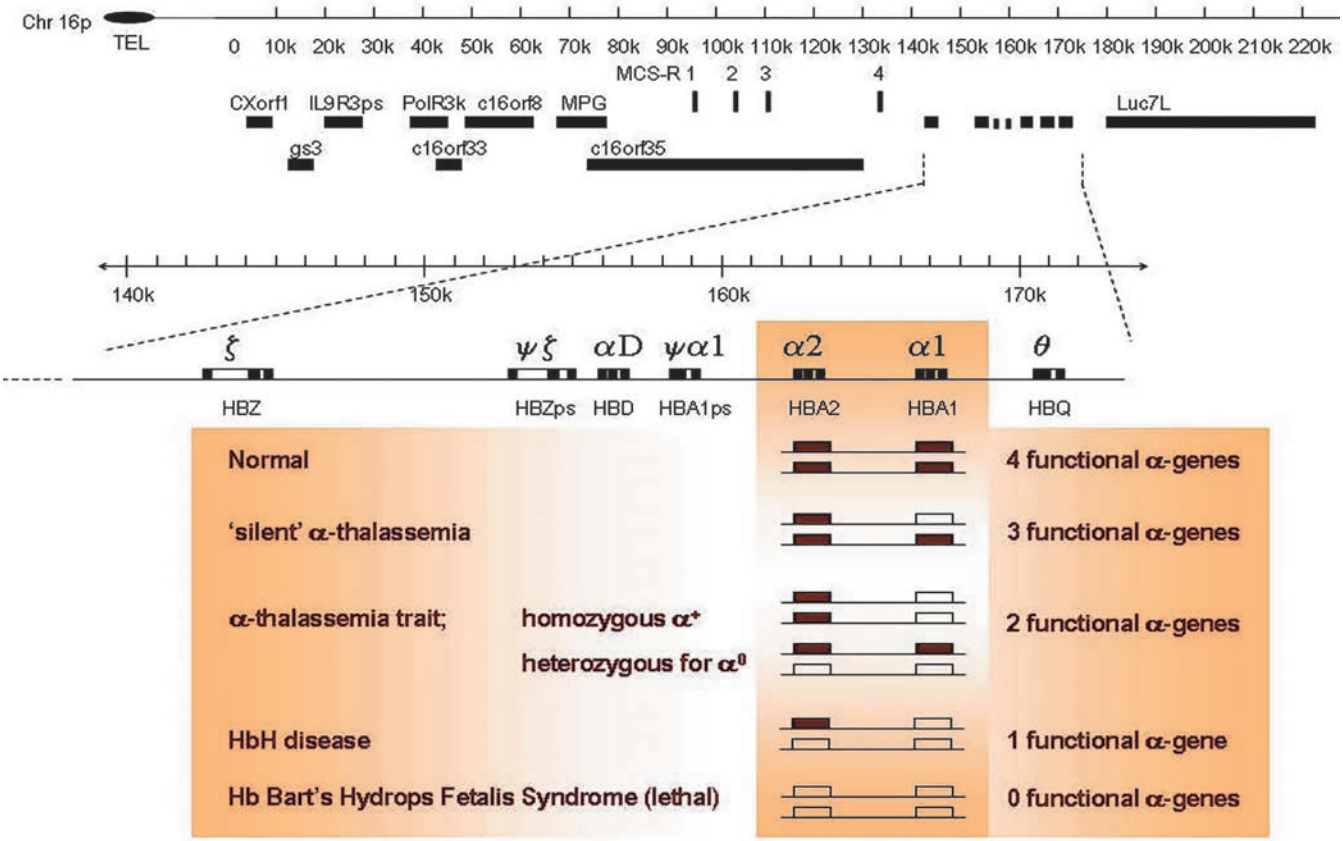
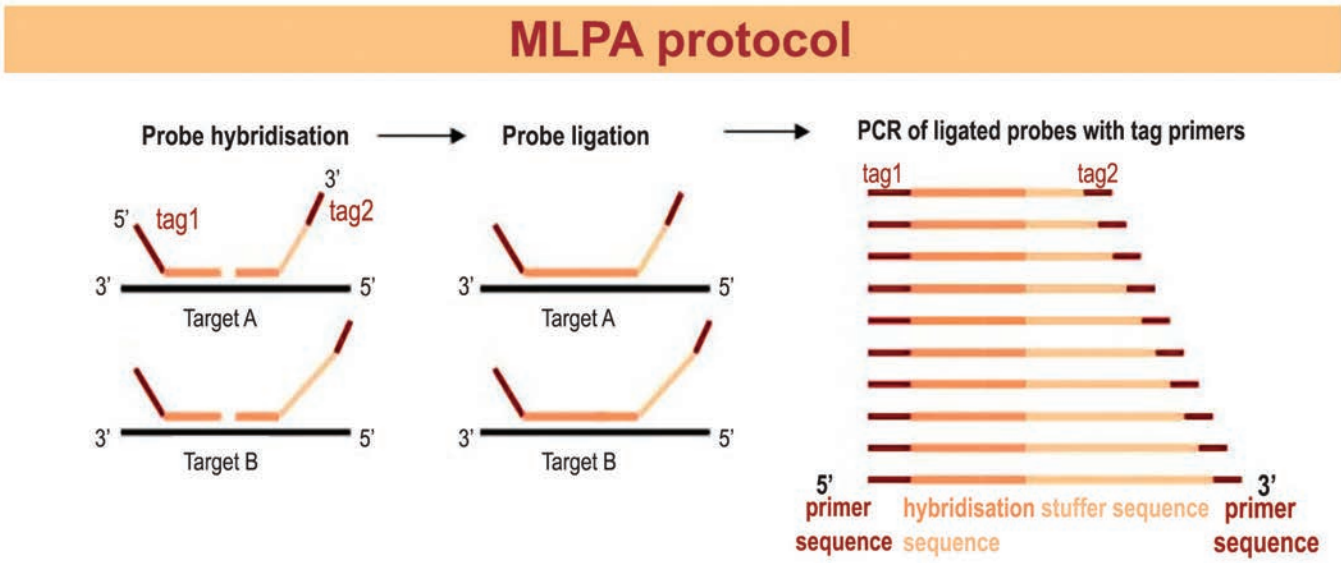


Figure 1: The structure of the α-globin gene cluster on chromosome 16.
Adopted from Harteveld and Higgs Orphanet Journal of Rare Diseases 2010, 5:13



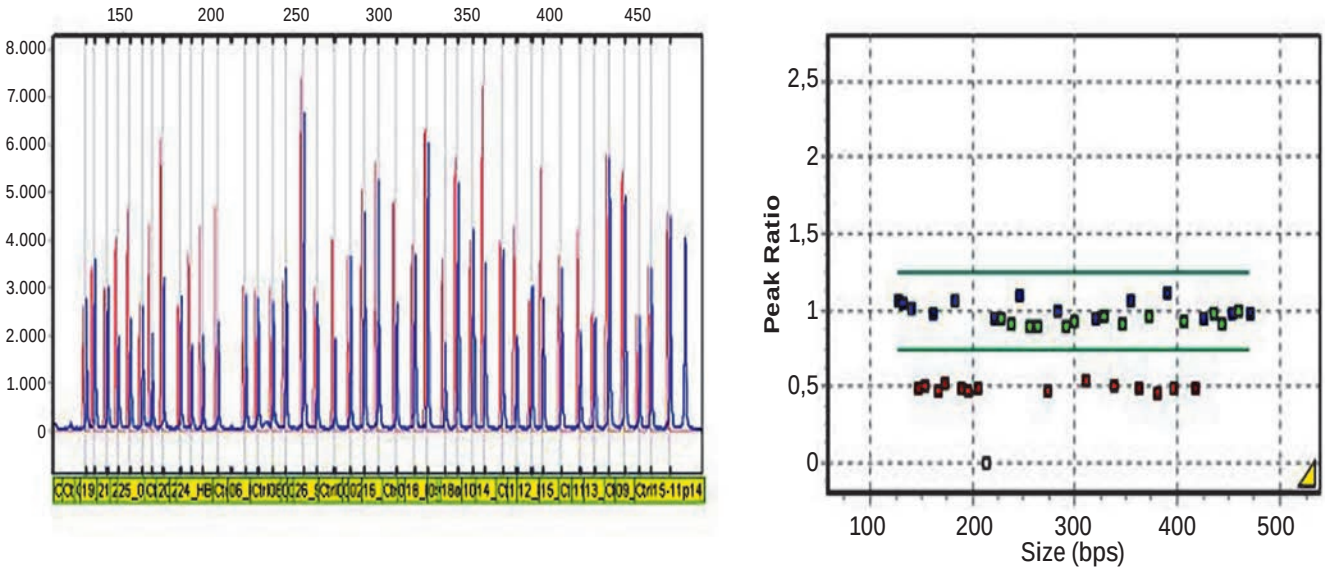


Figure 2: The principle of Multiplex Ligation dependent Probe Amplification (MLPA). Adopted from Harteveld and Higgs Orphanet Journal of Rare Diseases 2010, 5:13

Interpretation

Allele copy numbers	Probe ratios	Analytical Interpretation
2	1	No deletion/Duplication
00	00	Homozygous deletion
1	0.5	Heterozygous deletion
3	1.5	Heterozygous duplication
4	2	Heterozygous triplication/homozygous duplication

Table 1: Alpha-thalassemia mutations in different ethnic groups
Adapted from Barbara J. Bain, Haemoglobinopathy Diagnosis 2nd edition 2006 {Bain, 2006 126/id}

Ethnic Group	Type of Thalassemia	Mutation(s)	Occurrence
India	α+	- α3.7	Common
		- α4.2	Less common
		α Koya Dora α	Relatively rare
		α IVS I-117 α	Relatively rare
	α+ - α0	α PA3(AATA- -) α	Also found in Hindustani from Surinam
Middle East	α0	- - MED 1	Common in Iran, Palestinians, Arab population
	α+	- α3.7	Common in Iran, Palestinians, Arab population
	α+ - α0	α PA1(AATAAG) α	Relatively common in Arab population
South-East Asia	α0	- - SEA	Most common deletion among Asians world wide
		- - FIL	Mainly in Philippinians
		- -THAI	Common among Thai
	α+	- α3.7	
		- α4.2	Relatively common
		ά Constant Spring α	Relatively rare
		α Suan Dok α	One of the most common non-deletion variants in Chinese
		α Quong Sze α	Highly unstable α-chain
		α Pakse α	Highly unstable α-chain
		α init A-G α	Highly unstable α-chain, found in Thai, Laotian
		α init -TG α	Common in Vietnam
			Common in South-East Asia

Kleihauer – Betke Test: An Important Yet Underrated Tool for Prevention of Erythroblastosis Fetalis

Dr Rabiya Jawed
Haematology & Transfusion Medicine

All human beings are born with a blood group (A, B, AB or O), which can be Rh positive (+ve) or Rh-negative (-ve). It may happen that a Rh-negative mother has a baby with an Rh-positive father, resulting in an Rh-positive baby. By chance, the cells of the baby can enter maternal circulation and can trigger an immune response, resulting in antibodies against Rh-positive fetal blood cells. In the subsequent pregnancy, these maternal antibodies can attack the fetal cells when they cross the placenta, and destroy the Rh-positive fetal red blood cells. Destruction of fetal red blood cells can cause fatal anemia and jaundice in the neonate, a condition termed erythroblastosis fetalis, or Hemolytic Disease of the Fetus and newborn (HDF/N).

The fetal and maternal blood circulations run separate to each other in the placenta, however this fetal blood can also enter the maternal circulation in conditions such as abdominal trauma, anterior placental location or uterine tenderness. Termed fetomaternal hemmorhage (FMH), this unique complication of pregnancy can harm the fetus in Rh negative mothers. The presence of Rh positive fetal blood can induce anti-D antibodies in maternal circulation. These anti-D antibodies can enter fetal circulation and destroy the Rh-positive blood cells of the fetus.

The Kleihauer Betke Test is used to quantify the presence of fetal blood in maternal circulation. This test detects fetal blood in maternal circulation at a sensitivity of 5mL, that is if the quantity of fetal blood in maternal circulation is less than 5mL, then the KB test is not able to detect it. Rather than positive or negative, the Kleihauer Betke test reports the total amount of fetal blood in maternal circulation. The working principle of this test is based on the different types of hemoglobin that exists in maternal (HbA, HbA2) as compared to fetal blood (HbF). Fetal hemoglobin is much more resistant to acid elution than maternal hemoglobin, hence maternal hemoglobin washes away when exposed to acid. The slide is then washed, stained and viewed under the microscope. If fetal cells are present, they will appear red as compared to maternal blood cells, which have lost their hemoglobin. The visualised cells are then counted to quantify the fetal blood. A laboratory technologist counts up to 2000 cells, typically in eight to ten high power fields

The counted values are reported as a percentage as follows:

$$\%\text{fetal red blood cells} = \frac{\text{Fetal red blood cells counted in slide}}{\text{Total Maternal Cells}} \times 100\%$$

The volume of FMH i.e., the number of fetal cells with ratio to total maternal circulation is then calculated as follows:

$$\text{Volume of FMH} = \frac{\text{Number of fetal cells} \times 5000 \text{ (maternal blood volume)}}{100}$$

Apart from quantifying fetal blood cells in maternal circulation, this test can also be used to assess the dose of prophylactic Immunoglobulin (RhIg) to be administered to a pregnant Rh-negative mother, within 72 hours of delivering a Rh-negative fetus. The dose of RhoGAM (Anti-D antibody) is calculated as follows:

$$\text{Vial requires: } \frac{\text{Volume of FMH}}{30 \text{ (ml for whole blood)}}$$

The administration of anti-D antibody is essential for the prevention of fatal anemia and jaundice of the newborn during subsequent pregnancies in a Rh-negative mother. While Hemoglobin F quantification by Flow Cytometry is considered more sensitive for HbF detection in maternal blood, the relatively low cost of Kleihauer Betke Test makes it an important tool for assessment of FMH and administration of Anti-D antibodies in mothers belonging to lower socioeconomic brackets in the world.

Study of DMD Gene for Exon/s Deletion/ Duplications by Multiplex Ligation-Dependent Probe Amplification in Prenatal Samples

Sonana Riaz, Sana Sajid, Dr Asghar Nasir and
Dr Zeeshan Ansar
Molecular Pathology

Prenatal testing plays a vital role in determining during pregnancy if the fetus has a likelihood to be born with a genetic condition or congenital disability. It helps the clinician select the best available option for the management of the pregnancy and for the baby's benefit. Today, clinical laboratories offer several types of prenatal testing platforms depending upon which trimester of pregnancy the mother is and the condition being referred. Molecular testing for fetal diagnosis is performed on amniocentesis or chorionic villous biopsy samples obtained during pregnancy's first and second trimesters.

Duchene muscular dystrophy is an X-linked recessive disorder affecting approximately 1 in 3,500 live male births worldwide. DMD gene (ID 1756) is located on the short arm of chromosome X at cytoband Xp21. It is the largest known protein-coding gene in the human genome. DMD gene consists of 79 exons and spans a region of 2.2 million base pairs. DMD gene was discovered in 1986 by researchers using the positional cloning approach, which in the mutated form leads to the development of Duchene Muscular Dystrophy disorder. DMD gene provides instructions for forming a protein called dystrophin, a large 427 kDa rod shape protein that resides in the cytoplasm. Primarily this protein is found in the cardiac and skeletal muscles. In addition, a small amount of dystrophin protein is also identified in neuronal cells of the brain.

Pathogenesis and Clinical Features of DMD

Patients with muscular dystrophy have little or no dystrophin. It has been suggested that this results in the disruption of muscle membranes, which alters calcium channel activity, thereby strongly increasing intracellular calcium concentration. Dystrophin absence leads to harmful effects, such as muscles becoming weak and prone to damage, initiating progressive loss of muscle tissue and function. Fibrous tissue begins to form in the muscle, and inflammation sets in starting in early childhood. Affected patients have a massively reduced life expectancy and a poor functional prognosis. In most cases, DMD is due to frame-shift mutations in the DMD gene, leading to a complete absence or low dystrophin protein levels (no more than three percent normal levels).

Molecular Diagnostic approach to Screen for DMD

Molecular testing is a valuable tool for confirming the clinical diagnosis of DMD in affected children. In addition, through prenatal screening, one can determine whether a developing fetus carries specific genetic abnormalities. For this purpose, ultrasonography, blood tests, amniocentesis, CVS, and cell-free DNA analysis are more frequently done in the first or second trimester. Screening tests further enable physicians to advise required diagnostic tests to rule out genetic abnormalities. DMD patients who are suspected of dystrophinopathy based on clinical signs and an elevated serum creatine kinase (CK) level are tested for mutations in the DMD gene, which also obviates the need for a muscle biopsy. Identifying the causative mutation in an affected individual and recommendations for genetic counseling of family members allows the carrier and prenatal testing to be performed as appropriate. According to the scientific literature, in approximately 65 percent of cases of DMD (and 85 percent of cases of BMD), deletion of one or more exons is observed. Furthermore, duplication of one or more exons is reported in six–ten percent of cases of both DMD and BMD. The remaining cases are primarily due to point mutations, small insertions/deletions, or splice site changes. Since whole exon deletions are the predominant type of mutation in the DMD gene (65 percent), an initial screen that detects most deletions should be

the minimum diagnostic test offered in such cases. Currently, MLPA is used in many clinical laboratories worldwide for the genetic diagnosis of DMD.

Diagnostic Test for Prenatal DMD by MLPA (Multiplex Ligation-Dependent Probe Amplification)

Prenatal testing for DMD is generally carried out on amniotic fluid and chorionic villus biopsy specimens. Chorionic villous sampling is typically accomplished between 10 and 12 weeks of pregnancy through an ultrasound-guided procedure. Figure 1 shows Chorionic villous Sample.

Figure 1: Chorionic villous sample

In contrast, amniocentesis for collecting the amniotic fluid sample is performed between 15 and 20 weeks of gestation. Subsequently, amniotic fluid samples are processed for fetal cell culture in tissue culture flasks. After obtaining confluency, cells are harvested, and DNA is extracted for mutation analysis. In the case of chorionic villus biopsy, villi are separated from maternal tissue under a dissecting microscope. Cleaned villi are used for DNA extraction and further processing for mutation analysis. Figure 2 shows diagram of Maternal cell contamination check MCC which is performed through STR analysis once MCC results are obtained clear test is proceed further.

MLPA is one of the molecular techniques that can

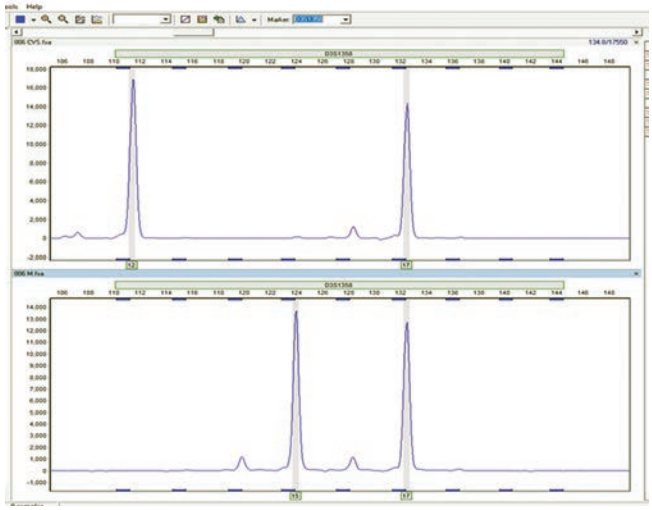


Figure 2: Maternal cell contamination check MCC

quickly and reliably identify small deletions and duplications below the resolution of karyotyping and FISH techniques. MLPA is based on fluorescently labeled probes that target the region of interest of the genome. Each probe set consists of two oligonucleotide DNA fragments that are first hybridized to their specific targets in the DNA, ligated using ligase enzyme, and subsequently, PCR amplified. The amplification products are separated by capillary electrophoresis, and the relative quantity of each fragment is determined. MLPA testing kits manufacturer provides software for prompt analysis of MLPA data. Figure 2 shows a typical MLPA reaction. Following DNA denaturation, a mix of MLPA probes is added to the sample tube for ligation. Each probe in the MLPA probe mix has a unique amplicon length, typically ranging between 130 and 500 nucleotides. All ligated probes are amplified simultaneously using the same PCR primer pair. One of the two PCR primers is fluorescently labeled, enabling the amplification products to be visualized on a capillary electrophoresis instrument and yielding an electropherogram.

The relative height of each probe peak, compared to the relative peak height in various reference DNA samples, reflects the relative copy number of the corresponding target sequence in the sample. A deletion of one or more target sequences thus becomes apparent as a relative decrease in peak height while an increase in relative peak height reflects amplification. Below figure shows an electropherogram of a test sample (bottom)

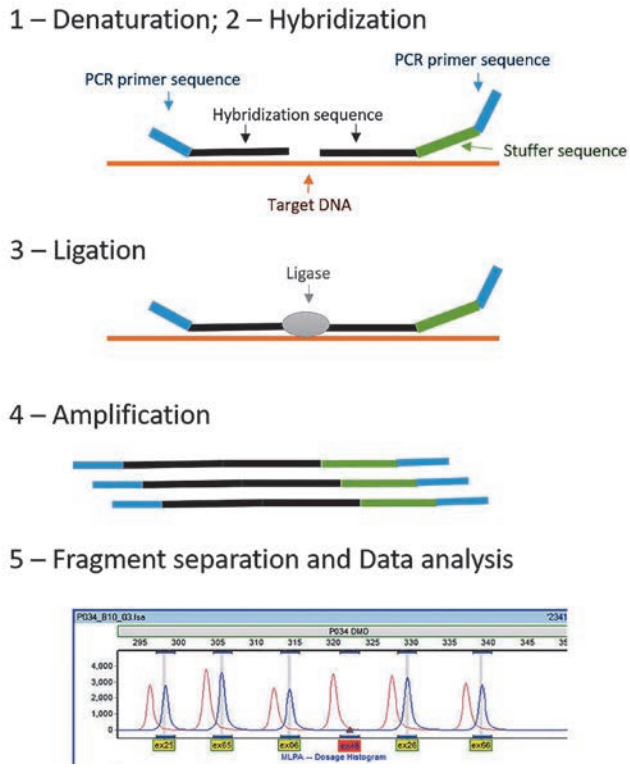


Figure 2: Multiplex Ligation-Dependent Probe Amplification

compared to a reference sample (top), showing a relative decrease of three probes in the test sample.

In summary, Duchenne/Becker muscular dystrophies can be caused by deletions, duplications, or point mutations in the DMD gene that encodes the protein dystrophin. However, to what extent DMD manifests depends on whether the translational reading frame is lost or maintained. Partial gene deletions or duplications in the DMD gene account for as much as ~65% of cases of these dystrophies. This extremely high percentage may be due to the nature of the protein and the gene's extreme length. The MLPA-based DMD probe mix contains probes for each of the exons of the DMD gene (79 exons) on Xp21.2 chromosomes. Performing MLPA reactions is thus sufficient to investigate the copy number of all exons. In final assessment, MLPA offers a more extensive screening than many other methods used in DMD analysis as it makes it possible to find deletions/duplications that were previously overlooked.

TORCH – Why, When, and How?

Dr Ali Raza Nasir, Dr Sobia Khan, Dr Riffat Saleem and Muhammad Arsalan
Clinical Microbiology

TORCH is an acronym used to refer to infections that are associated with congenital defects in a newborn. These infections include toxoplasmosis, rubella, cytomegalovirus (CMV) and herpes simplex virus (HSV). Certain literature uses a modified acronym, TORCHeS, that includes syphilis in the profile. Knowing which profile, a laboratory uses as a policy to perform the panel testing for congenital infections is important for the clinician. Other congenital infections also associated with congenital defects include viral hepatitis, HIV, measles, mumps, parvovirus B19 and varicella zoster virus, and while these are not a routine part of the TORCH or TORCHeS profile in most laboratories, their investigations can be requested separately. The causative organisms are likely to transmit to a developing fetus or newborn either trans-placentally, during passage from the birth canal, or after birth. There is a variation in the presentation of each of these infections in the baby, but certain features are commonly encountered, such as fever, microcephaly, low birth weight, lethargy, cataracts, hearing loss and congenital heart disease. Signs and symptoms may also manifest later in the form of intellectual disability, deafness, or vision impairment. Early recognition of these infections in pregnancy is important in treating the disease, fetal monitoring, and counselling of the patient regarding adverse fetal outcomes.

WHY?

Being a developing country, Pakistan faces a significant burden of adverse pregnancy outcomes.

One of the factors leading to this concerning situation is the lack of screening for feto-maternal infections and timely management. In Pakistan, the incidence of symptomatic CMV infections in neonates in 2016 was reported to be around 21 percent. For rubella, studies have shown that a considerable proportion of pregnant women in rural areas turn out to be positive for IgG and IgM antibodies (16 percent IgG, 2.5 percent IgM). Similarly, alarming figures for seroprevalence of toxoplasma gondii in pregnant women have also been reported from Pakistan (34.5 percent). This evidence puts forth a strong argument warranting the screening of pregnant females for TORCH infections.

WHEN?

Current practices do not involve routine screening for TORCH in pregnant females on antenatal visits. TORCH profiles are usually requested if there is suspicion on clinical grounds. Certain guidelines have been published that provide useful knowledge indicating when to screen for TORCH infections, such as the 'TORCH testing in Obstetrics and Neonatology' guidelines in Ireland (Tables 1 and 2) [4]. Abnormal findings on fetal ultrasound, neonatal clinical abnormalities and clinical symptoms of the mother such as rash or hepatitis, warrant the screening of TORCH infections.

HOW?

The test involves sending a serum sample to the laboratory where it is tested for the presence of antibodies against the concerned pathogens. The serum sample is stable for 48 hours after collection if kept at 2-8oC, and for 6 months if kept at -20oC. A common method of analyzing the presence of antibodies involves an enzyme-labeled chemiluminescent sequential immunometric assay, where antigen coated beads are made to react with the patient's serum, followed by addition of anti-human IgG antibody bound to an enzyme. A substrate for this enzyme is added that gets broken down resulting in generation of light. The presence of antibodies is determined quantitatively by measuring the amount of light generated. Slightly different methods are used for each of the microorganisms to be tested.

Samples with indeterminate results require retesting. Similar results on repeat testing require testing by another method or collecting a second sample after a reasonable time (e.g., one week). IgM antibodies are raised when there's a current or recent infection. The presence of IgG antibodies in a pregnant woman usually indicates a past infection or immunity. If there is a question of an active infection, a second blood test is performed a few weeks later so the IgG levels can be compared. If levels increase, it can mean the infection was recent or is ongoing. Samples that are hemolyzed, lipemic, icteric, have hyperproteinemia or hypergammaglobulinemia can interfere with the results.

New commercially available rapid tests are also available that qualitatively detect IgG and IgM antibodies against toxoplasma, rubella, CMV and herpes simplex one and two. These are based on an immunochromatographic principle (much like the malarial ICT) and provide results within 15 minutes. A positive result by these methods should be confirmed by alternative methods.

In conclusion, considering the burden of feto-maternal infections in Pakistan, it becomes important to educate clinicians regarding when to screen pregnant women for TORCH, the knowledge of tests to order and the interpretation of their results. With the introduction of rapid tests, clinicians in rural areas can effectively screen patients preliminarily if they do not have the facility of established laboratories in the vicinity. Those that screen positive may be referred to urban centers for proper evaluation.

KEY POINTS

- **TORCH vs TORCHeS: know what your lab offers**
- **Screen patients for investigation of TORCH based on:**
  - **Fetal ultrasound findings**
  - **Clinical symptoms of baby**
  - **Clinical symptoms of mother**
- **IgG vs IgM: know how to interpret results; ongoing infection vs past infection/immunization**
- **Rapid tests are available, but should be confirmed with alternative methods**

Tables and Figures:

Table 1: Adapted with modifications from 'TORCH testing in Obstetrics and Neonatology' guidelines by Cillian et al

Recommended investigations for the neonate with clinical/laboratory abnormalities at birth				
	Toxoplasma	**Rubella**	**CMV**	**HSV**
Hepatitis/Jaundice/Hepatosplenomegaly	x		x	x
Rash			x	x
Thrombocytopenia			x	
Anemia			x	x
IUGR		x	x	
Microcephaly	x	x	x	
Hydrocephalus	x		x	
Failed newborn hearing test			x	
Patent Ductus Arteriosus (at term)		x		
Intracranial calcification	x	x	x	
Congenital cataracts or microphthalmia		x		
Hydrops				
Culture negative sepsis not responding to antibiotics in the first month of life				x

Table 2: Adapted with modifications from 'TORCH testing in Obstetrics and Neonatology' guidelines by Cillian et al

Recommended investigations for the pregnant woman with abnormalities detected on fetal ultrasound			
	Toxoplasma	**Rubella**	**CMV**
Micro/macrocephaly	x	x	x
IUGR	x	x	x
Intracranial calcification	x	x	x
Echogenic bowel			x
Ventriculomegaly	x		x
Structural heart defects		x	

Figure 1: A commercially available rapid test kit for simultaneous detection of Toxoplasma, Rubella, CMV and HSV IgG and IgM antibodies. Picture credits: CTK Biotech

Twin to Twin Transfusion Syndrome (TTTS)- Radiology Approach

Dr. Shayan Anwar
Radiology

Twin to twin transfusion syndrome (TTTS) is one of the complications that can occur in a monochorionic monoamniotic or monochorionic diamniotic twin pregnancy. It can occur in 1:2000 of all pregnancies. Most striking clinical feature is abdominal distension. The pathophysiology behind TTTS is imbalanced supply to both twins because of arteriovenous and arterioarterial anastomoses in the placental circulation; one twin receiving more blood supply than the other (Figure 1).

The imbalance between blood supply leads to reduced perfusion and blood pressure in one twin (donor) and increased perfusion and blood pressure in another twin (recipient). This phenomenon activates the renin-angiotensin system which subsequently leads to chronic tubulopathy and oliguria in the hypovolemic (donor) twin with consequent oligohydramnios, and polyuria and consequent polyhydramnios in the hypervolemic (recipient) twin.

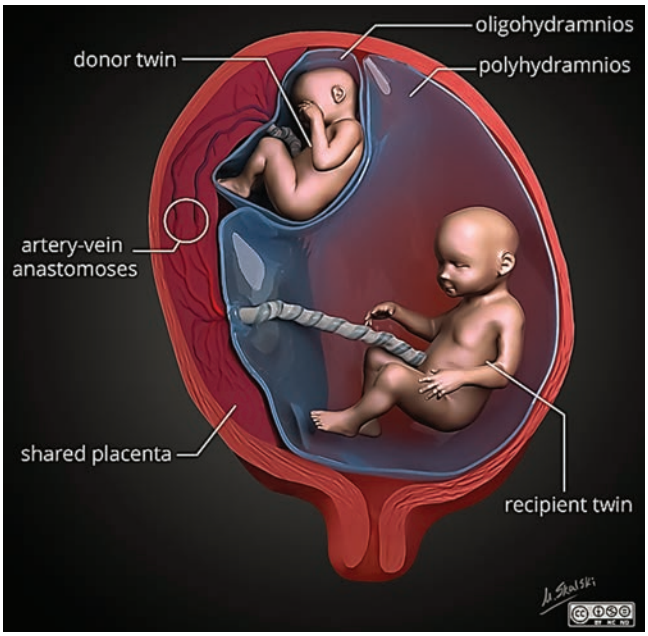


Figure 1: Illustration showing the placental anastomotic connection within a shared placenta that leads to TTTS:

Case courtesy of Dr Matt Skalski, Radiopaedia.org, rID: 68846

Staging
Staging of this syndrome according to severity is proposed by Quintero et al. is as given below:

stage I:	• oligohydramnios/polyhydramnios
stage II:	• bladder not visible in donor twin
stage III:	• abnormal Dopplers in either twin
stage IV:	• hydrops fetalis in either twin*
stage V:	• in-utero demise of either twin

***Almost always in the recipient; rarely in the donor if there is coexistent Twin anemia-polycythemia sequence TAPS.**

Radiographic features
On antenatal ultrasound, a) monochorionicity is a key criterion at any stage of gestation, b) growth discordance is often but not invariably present and does not contribute as diagnostic indicator.

First trimester
Increased risk of developing TTTS has been associated with the following findings on a first-trimester ultrasound of a monochorionic pregnancy, although predictive value remains low:

- Nuchal Translucency discordance: 20 percent or more
- Crown Rump Length discordance: 10 percent or more
- Ductus venosus reversed a-wave
- Disparity in amniotic fluid volumes (amniotic fluid discordance) leads to folding of the inter-twin membrane as an early sign.

Second and third trimesters
Features that may be noted individually in each twin include:

Recipient Twin	Donor Twin
Polyhydramnios (deepest vertical pocket (DVP) >8 cm) (Fig 2)	Oligohydramnios (DVP <2 cm), which may result in the twin appearing pinned to the side of the gestational sac: "stuck twin" (Fig 2)
Large or constantly cycling urinary bladder	Small or non-visualized urinary bladder
Features of cardiac overload	Evidence of fetal anemia
Fetal hydrops	
Fetal cardiomegaly	
In few cases, fetal echocardiography may also show Atrioventricular valve incompetence	
Doppler abnormalities - predominantly venous	Doppler abnormalities - predominantly umbilical artery +/- venous

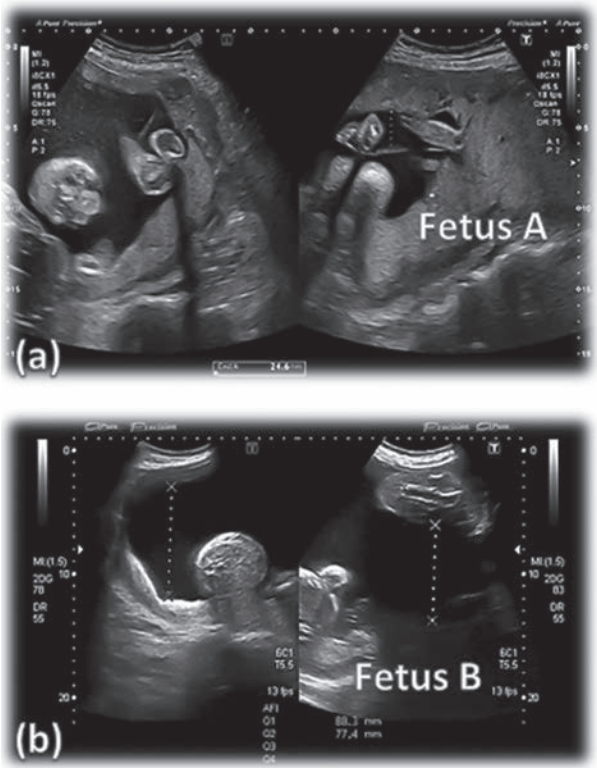


Figure 2: 24 week gestation, monochorionic diamniotic alive twin pregnancy, Anomaly scan: No structural anomaly identified in either twin. (a) DVP around fetus A measures 26mm (donor), (b) DVP around fetus B measures 88mm (recipient).

Findings are suggestive of twin to twin transfusion syndrome.

Doppler

Umbilical artery Doppler: Absent or reversed diastolic flow is an indication of worsening twin-to-twin transfusion syndrome.

Ductus venosus Doppler: Cardiac diastolic dysfunction is suggested by abnormal waveform pattern.

Treatment and prognosis

Successive sonographic monitoring is standard practice. Screening for TTTS should start from 16 weeks' gestation, in an uncomplicated monochorionic twin pregnancy, with evaluation of fetal growth, deep vertical pockets (DVPs) and umbilical arterial pulsatility index (UA-PI) carried out fortnightly. Detailed anomaly scan ultrasound is common practice at 18-22 weeks. Additional fetal middle cerebral arterial-peak systolic velocity (MCA-PSV) is advocated from 22 weeks onwards.

Up to 90 percent perinatal mortality is observed in untreated TTTS with a poor prognosis. The treatment of choice is **laser coagulation of the chorionic plate** as it remarkably improves the prognosis for both twins. Other treatment options would include conservative management with surveillance for Quintero stage one TTTS and serial amnioreduction, where laser treatment is not available.

Differential diagnosis

Oligohydramnios and/or polyhydramnios are related to fetal structural anomalies.

Isolated placental insufficiency in one of the twins- will not produce polyhydramnios in opposite twin.

Best of the Recent Past

Radiologist #Womenimaging #Followtheirlead

Interview Recorded by Dr Shayan Anwar
Interviewee: Associate Prof Shaista Afzal

1. **Please briefly share your initial phase of journey i.e., from medical graduate to consultant.**

   I graduated from Dow Medical College, after which I completed my internship at Civil Hospital. I had never thought of becoming a radiologist. As time passed, I found radiology to be an emerging field and partook in it as a challenge. I began my residency in Radiology at Civil Hospital and after graduation, I started working at Liaqat National Hospital. Very soon I got the opportunity to work at AKU where I joined as an instructor.

2. **Considering your entire time as a women imaging radiologist at your organization, can you recall a time (any AHAA moment) when you felt most alive or most excited about your involvement in the organization?**

   A distinct time is when our department set up the section of Women Imaging. Aside from that, when we get the opportunity to apply the best possible practices in the field and are able to train and guide others both at AKU and other institutions in the country, I feel elated that we are able to make such a positive contribution.

3. **Let's consider for a moment the things you value deeply. Specifically, the things you value about yourself and the nature of your work, what is the single most important thing your work has contributed to your life?**

   Work has added meaningfully to my life. Some of the useful qualities that I have learned through work are collegiality, discipline and perseverance.

4. **As a senior women imaging radiologist of the country, please share your experience of development of women imaging practices in Pakistan and its future in next 10 years.**

   There were and are many hurdles particularly related to the provision of equipment, training of faculty and staff and awareness in our population. Women commonly do not come for their breast screening. When they do the tumors have grown significantly. We have to increase awareness among women about their well-being through all channels such as media and health authorities. In the years to come, I do see some improvements but only after we get support from both the public and private sectors.

5. **Any advice for Junior Radiologist?**

   Over time radiology has been referred to as clinical imaging. Hence it is important to have all the information about your patient before you read any scan or perform any intervention. A holistic picture of the patient is vital for correct interpretation and better patient outcomes. I would also encourage junior radiologists to actively master the usage of technology and artificial intelligence as opposed to being hesitant in using them.

 # Sonography # Followtheirlead

Interview Recorded by Dr Shayan Anwar
Interviewee: Ms. Munawar Jabeen, Senior Charge Radiographer

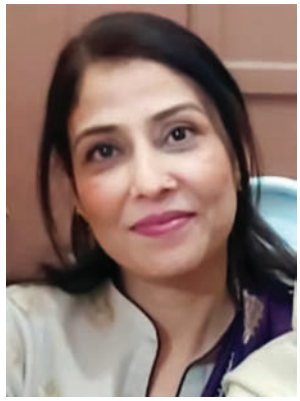
1. **Considering your entire time as charge radiographer at your organization, can you recall a time (any AHAA moment) when you felt most alive or most excited about your involvement in the organization?**

   In 2001, when I passed my test and interview for charge sonographer, that phase was one of the most exciting ones. I knew the opportunities that were ahead of me, and I was excited to learn and grow in my field. Even though throughout my journey, there have been moments when I felt the most alive but since this training of charge sonographer was my first step towards actual professional growth, I was eager to learn and explore the world beyond.

2. **Please briefly share your initial phase of journey i.e., from radiographer to charge radiographer.**

   The first five years as a radiographer were quite challenging for me. I completed my intermediate and bachelors with this job, simultaneously. After five years of struggles, being a radiographer, I got promoted to staff radiographer. Then in 2000, I got married and my responsibilities skyrocketed. It was tough balancing personal and professional life, but I still managed to keep a balance between both. From staff radiographer, I got promoted to senior 1 and after some time to senior 2. During this time, I became a mother to two girls, but I ensured my work life balance does not get disrupted. Being a wife or a mother never stopped me from getting ahead, I was always enthusiastic about being a part of training sessions and attempting to get ahead in my career. After completing these training sessions, I became a sonographer and eventually, charge sonographer. Promotion comes with added responsibilities, but I always set my work ethic high. And hence, with my determination and hard work, I stand on the position of 'charge radiographer' today.

3. **Let's consider for a moment the things you value deeply. Specifically, the things you value about yourself and the nature of your work, what is the single most important thing your work has contributed to your life?**



   Accomplishments cannot be bagged without being honest, showing dedication and striking a balance in life. My work keeps me proactive, executing my daily activities with devotion. I have learnt that your faith might waver but keep pushing and staying committed is the way to go.

4. **As a senior charge radiographer of your department, please share your experience of development of Medical Imaging Technology (MIT) in Pakistan and its future in next 10 years.**

   I am extremely glad to be a representative of such an organization that ensures quality over anything, I have witnessed breakthrough advancements while working here. When I first started working in radiology, conventional methods were employed for radiography which made the process quite time-consuming. I have seen the changes from conventional radiography to computed radiography which is now replaced by digital radiography. Radiology underwent several stages of advancements that helped boost its accuracy and efficiency. With progress and development in some ground-breaking technologies, I have experienced some massive improvements in Radiology. And, in the next 10 years, I believe there will be further developments in radiology that will continue to evolve.

5. **Any advice for Junior Radiographers?**

A few advice I would like to give to junior radiographers would be to respect your work and colleagues, both. Respect goes a long way. It's simple, give respect and earn respect in return. Secondly, time management - it is an art of using your time productively without stressing yourself out and getting things done before the deadline. Plan, organize and then implement. You will see a huge change. Last and the most important thing is not to treat your work as a burden. Instead, try to enjoy it and treat it as a learning experience. Take constructive criticism in an effective way and use it as a tool to make positive improvements. Moreover, always be honest with your work. It will help you to stay consistent and develop a sense of trust between your work and colleagues.

#Technologist #Accreditation #CAP

Interview Recorded by Ms. Iffat Arman

Accreditation of clinical laboratories according to an internationally accepted framework leads to improvement in quality practices of a laboratory, generation of accurate test results, reduction in errors and overall improved efficiency. Accreditations by College of American Pathologists (CAP) is a very rigorous practice. The CAP has more than 3500 standards that should be met. The Aga Khan Clinical Laboratory was accredited by CAP in 2017 and since then have been reaccredited thrice.

The main stakeholders and the persons doing all the work to achieve this daunting task are the faculty and staff of the clinical laboratory. However, the role each section of our clinical laboratory plays in clinical care are too many as well as very diverse, so we requested them to focus on two major areas only. We interviewed the Quality control coordinators of the sections to understand challenges faced by each section in achieving accreditation and how it has impacted their practices.

Interviewee: Mr. Abdul Mateen, Quality Control Coordinator, Chemical Pathology

Q.1. What were the challenges you faced while preparing your section for the CAP accreditation and how did you resolve these challenges?

Talking about the challenges being faced some of them were tricky, firstly 2022 was the year for document revision and with a test menu of around 250 tests it was a huge task to achieve. Reviewing every single SOP, equipment work instruction, sectional policy, QM plan, sectional patient procedures and numerous other documents complaining CAP standards and in accordance with new hospital based templates. Challenging was the fact that these would go directly for document control to be reviewed by inspector and responsibility would be cater by sectional QC coordinator as directed by Director laboratory CAP.

Q.2. How has CAP accreditation improved your clinical laboratory practices, quality assurance and patient safety in your sections?

Quality control and error detection practices, document control, practices and procedures of lab improved, operations became lean and accurate, results reporting improved, staff efficiency increased, staff continued educational programs were developed, competency assessment of staff initiated. Compliance with the CAP standards lead to overall improvement of the test result quality and hence patient safety, raising the standards of care. Nevertheless, it was a team effort who spend hours and hours of working to achieve reaccreditation status unaccompanied by deficiencies.

Interviewee: Ms. Ayman Jalali, Quality Control Coordinator, Blood Bank

Q.1. What were the challenges you faced while preparing your section for the CAP accreditation and how did you resolve these challenges?

Preparation for CAP accreditation is always challenging. Some of the major issues which we faced are:

- Training and awareness of staff in the mid of pandemic: Due to Covid, it was very hard for us to conduct training and awareness sessions as we had to follow the strict Covid guidelines. Online sessions were arranged so that maximum number of participants can be trained at a time.
- Revision of SOPs according to changes in checklist and its implementation: As revision in some of the standards demanded changes in current SOPs, so modifying the work flow and training the staff according to it was quite challenging. We conducted some pilot projects to see which solution suits us as we had to manage all these changes in current number of staff and limited space. Although it was very tough, but with team work and support of our respectable section heads and physicians, we made it possible.

Q.2. How has CAP accreditation improved your clinical laboratory practices, quality assurance and patient safety.

It has helped us in many ways. In blood bank, the introduction of Bedside ABO has helped in preventing wrong transfusions. Also, the introduction of Antibody screening before red cell transfusion has also contributed towards patient safety as it ensures safe transfusion. Quality standards of CAP which include continuous monitoring of QC and use of only CE/ FDA approved reagents and instruments have let us improve our overall laboratory practices and quality assurance.

Interviewee: Ms. Fauzia Naureen, Quality Control Coordinator, Hematology

Q.1. What were the challenges you faced while preparing your section for the CAP accreditation and how did you resolve these challenges?

The biggest challenge was announcement of dates just few days before the actual inspection. However, we were able to easily overcome this concern as CAP standards are now part of our daily work and extra preparations in this regard were not required. Our trust in each other as a team further boosted our confidence. Review of revised SOPs and Policies had to be done in very short time frame but our cooperation amongst us helped us achieve this task on time as well.

Q.2. How has CAP accreditation improved your clinical laboratory practices, quality assurance and patient safety in your sections?

CAP accreditation has brought remarkable improvement in our section. Our knowledge of quality assurance and staff practices regarding maintaining quality assurance have improved significantly. They now better understand the significance of fulfilling all desired QC steps. Implementation of advanced QC tools such as delta check, moving averages, validation of tests before implementation and participation in proficiency testing surveys on unknown samples are few examples that has contributed to culture of ensuring patient safety.

Interviewee: Ms. Samina Ghani, Quality Control Coordinator, Molecular Pathology

Q.1. What were the challenges you faced while preparing your section for the CAP accreditation and how did you resolve these challenges?

Molecular pathology is one of the most diverse section of clinical laboratory and its continuing evolving with new and recent technologies in research and diagnosis. This time Covid 19 is a great challenge for our laboratory and initially its very tough to maintain high volumes of covid 19 in limited resources with CAP parameters. But later on with support and encouragement of our management we introduced High Throughput automated testing and by the continuous struggle and hard work of our team we achieve the goal.

Q.2. How has CAP accreditation improved your clinical laboratory practices, quality assurance and patient safety in your sections?

CAP standards are very effective for highly complex molecular testing along with Quality assurance. It has great impact on validation of new tests. It improves testing efficiency, customer service and reduce rates of laboratory errors. CAP accreditation also improve our patient care by aiding the timeliness and accuracy of medical decision. our section also Participation in Proficiency testing(PT) which is a key component of accreditation, leads to more accurate test results.

Charge Technologist #Accreditation #CAP
Interviewee: Ms. Najma Shaheen, Quality Control Coordinator, Microbiology

Q1. What were the challenges you faced while preparing your section for the CAP accreditation and how did you resolve these challenges?

Due to the pandemic scenario this audit was conducted after gap of 4 years. The major challenge was the scarcity of human workforce to continue the routine laboratory operations but also handling CAP related issues and preparation. To limit the COVID exposure, we had not only implemented the hospital policy very vigilantly but also restrict our laboratory staff. Initially we were unable to do onsite meetings because of COVID related restrictions; however, when things look black, there's always a silver lining. We started to use online meeting tools i.e., ZOOM and MS team and everyone get skilled with these methods within short span of time.

Q.2. How has CAP accreditation improved your clinical laboratory practices, quality assurance and patient safety in your sections?

1. We have updated our laboratory processes and diagnostic technology in view of CAP standards and regular proficiency testing.
2. Quality projects is an important tool that has helped us to identify on our gaps and get the solutions with better outcome for patients.
3. Our staff is now trained for laboratory diagnosis and safety according to the international recommendations and guidelines.

Interviewee: Ms. Somiayya Sohail, Quality Control Coordinator, Histopathology

Q1. What were the challenges you faced while preparing your section for the CAP accreditation and how did you resolve these challenges?

Preparing for the CAP inspection was a laborious task, but at the same time offered great learning experience. We had to cover almost 300 standards mentioned in the checklist, that has been provided by the College of American pathologists. These standards are pertinent to the areas of Anatomic pathology, Cytology, flowcytometry and semen analysis. Since there is heavy service load in our lab, accomplishment of this task has been challenging. A few exceptionally demanding tasks encountered during preparation included daily updating of equipment binders. Since service took most of our time during the morning hours, this part was done during the night duty hours. Another tedious task was to formulate a policy for the new standard that had been recently introduced by CAP. This was related to the fixation time of tissue. Since it was a new edition, we had to ensure that this policy was followed by consultants across the board.

Q.2. How has CAP accreditation improved your clinical laboratory practices, quality assurance and patient safety in your sections?

Implementing the CAP standards strictly reduces the chances of errors and improves accuracy of tests. This is related to the stringent quality assurance measures as laid down by CAP.

In short, accreditation establishes safe practices that are necessary for accurate, reliable, and timely reporting of specimens. These measures are not only beneficial from the patient perspective, but also provide a safe working environment for the laboratory personnel.

HAPPENINGS IN PATHOLOGY
CAP Accreditation Journey- Excellence in Total Quality Management

Dr Sibtain Ahmed
Clinical Chemistry

From healthcare perspective, accreditation is defined as a robust process involving an official review of an organization to ensure that it meets official regulatory requirements and standards. College of American Pathologists (CAP), laboratory accreditation program was initiated in 1961. This accreditation is regarded as the epitome of laboratory excellence in total quality management. The process entails a continuous quality improvement journey subjected to a stringent review of the entire operations by an external team of laboratory professionals (trained CAP auditors) using more than 3000 standards. Compared to other standards and quality management programs CAP accreditation holds a distinct edge, based on the detailed set of factors and areas it inspects focused upon performance of clinical laboratories. The standards are broad-based statements that specify the requirements for the director of the laboratory, physical facilities and safety, quality management, administrative requirements of the program, performance improvement, and inspection. In a nutshell, CAP accreditation ensures that the laboratories demonstrate continuous steps to identify and correct deficient areas and improve performance, in compliance with the Clinical Laboratory Improvement Amendments of 1988 regulatory requirements.

AKUH Clinical Laboratories is the largest laboratory network in Pakistan. Housing the hub at

the main clinical laboratory in Karachi, the network comprises of more than 291 collection units and 13 regional stat laboratories, across the country. With a highly trained team of professionals, and state-of-the-art automated equipment, the laboratories offer over 500 different types of testing mounting to approximately 10 million tests annually. The AKUH clinical laboratory achieved CAP accreditation in 2016, then successfully passed the biennial review in 2018. In the wake of COVID-19, the subsequent re-accreditation audit took place from 10-11th March 2022. In this comprehensive audit a team of eight international CAP trained auditors physically inspected the facility. The two days inspection involved a thorough and stringent review of Clinical Chemistry, Haematology & Transfusion medicine, Microbiology, Molecular Pathology, HLA and Cytogenetics, Immunology, Point of care testing, Coagulation and Flow Cytometry, Histopathology and Cytopathology, laboratory general and safety. Subsequently, on 2nd June 2022, we received our 3rd CAP re-accreditation with ZERO deficiency for the main lab Karachi, which is a testament of the highest standards of total quality management. The CAP inspection team leader quoted that "AKU lab is one of the best labs in the region and are well on their way to become one of the best labs in the world". Collection of glimpses from the recent audit is presented here:

Audit of Clinical Chemistry

Histopathology faculty with the auditor

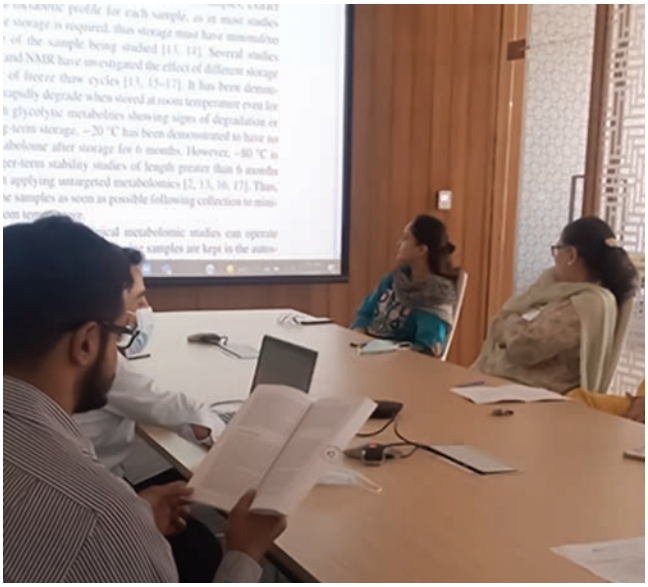
Team POCT with CAP auditor

Microbiology faculty with auditor during inspection

Metabolomics Book Club-A New Teaching Learning Strategy in Chemical Pathology

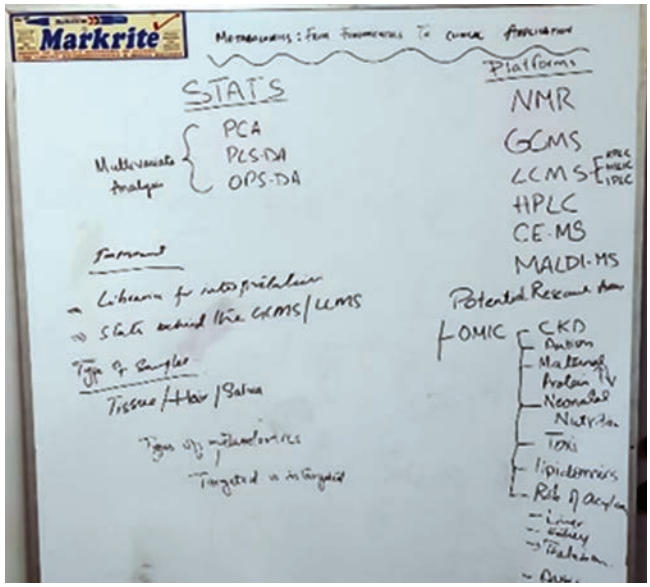
Dr Arsala Jameel Farooqui and Dr Lena Jafri
Chemical Pathology

Developments in the techniques of analytical clinical chemistry and metabolomics are the source of the rapid evolution of a new omics era. The introduction of metabolomics in the Section of Chemical Pathology at Aga Khan University, Karachi Pakistan pertains to important platforms: analytical techniques like GCMS, FTIR, HPLC and LCMSMS. With the introduction of mass spectrometers and HPLC in the section almost eight-nine years ago metabolic profiling of patients suspected of having inherited metabolic disorders



can be done. We have screened > 22000 high risk children and adults in past eight years and have identified around 40 disorders using these cutting-edge technologies. The Biochemical Genetics Laboratory in the section has established some targeted metabolomics panels for quantitation of selected groups of metabolites using mass spectrometry specifically for inherited metabolic disorders and new-born screening (LCMSMS).
To facilitate understanding of metabolomics and to enhance the science of metabolomics a monthly activity of 'Metabolomics Book Club' was initiated in the section of Chemical Pathology, Department of Pathology and Laboratory Medicine at Aga Khan University. The goal was that the participants facilitate the discussion and are at the same level of understanding by the end of the one-hour long monthly session.

In the beginning the activity director introduced the structure of the book club and the cover of the book. The participants were seated in a small room and read a pre-selected book on metabolomics in a circle. New knowledge gained was discussed between readings. The first chapter "Introduction to Metabolomics" of the book titled "Metabolomics: From Fundamentals to Clinical Applications"



was read aloud in circle and discussed in depth. There were nearly nine to ten people in the room, including faculty, residents, technologists, and research associates. Discussion on various techniques and methods of metabolomics and their sensitivities and specificities was done. The group identified potential research areas on metabolomics between readings.

The second session emulated the energy of the initial session, the readers engaged in perusing the second chapter of the same book: Collection and Preparation of Clinical Samples for Metabolomics. Eight to ten readers indulged in an hour-long discussion on the fundamentals of metabolomics related to non-specific samples received in the

clinical laboratory. Types of samples and testing techniques were discussed by the consultants, residents, and biogenetics lab scientists in attendance, who took turns to read aloud the pages and actively discussed the important aspects of metabolomics related to sampling in real-time.

At the end of each session the group of readers summarized reading, shared their experiences, made predictions, and clarified difficult and new concepts of metabolomics. Novel information of metabolomics was disseminated to all on the same level. All participants enjoyed this novel approach of teaching and besides discussion on science it promoted a love for books and a positive attitude towards reading.

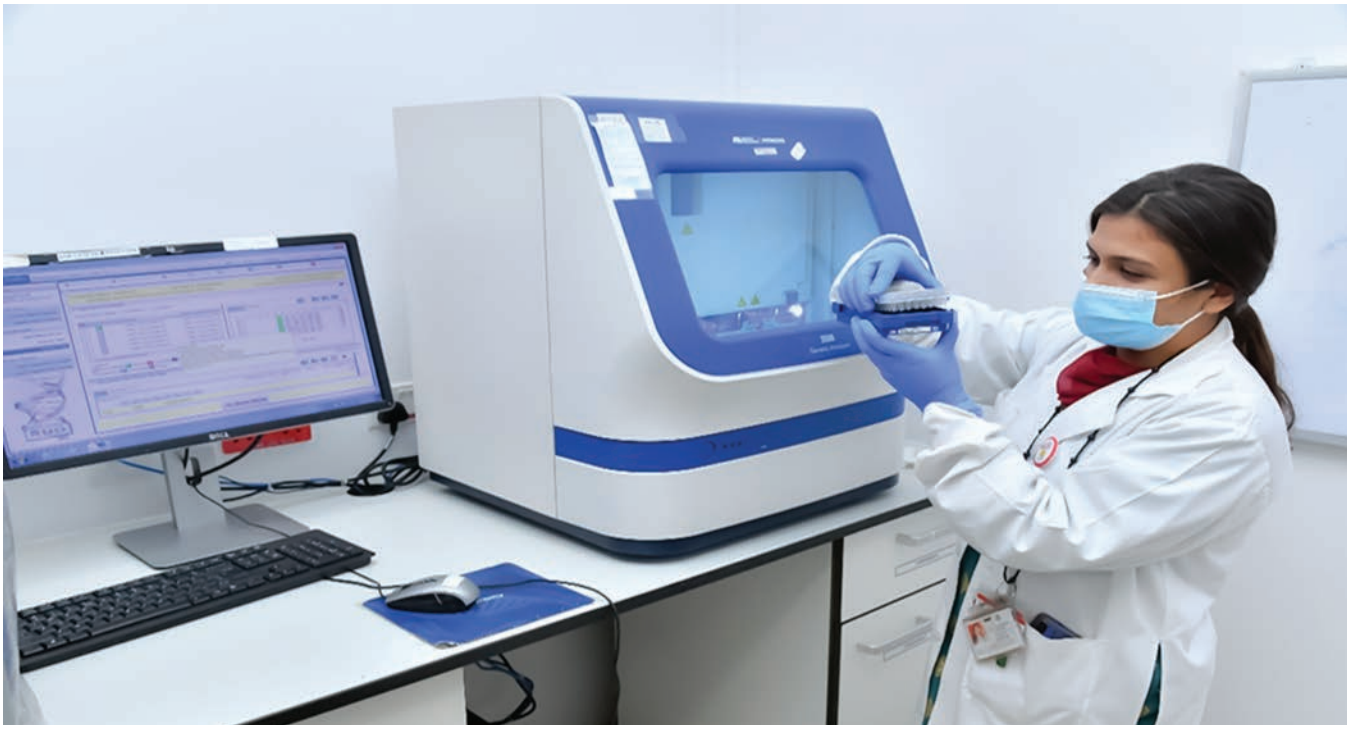
The metabolomics book club has allowed participants to have a deeper understanding of the book and metabolomics' application to service, education and research.

Polaroid

Laboratory team member processing Amniotic fluid samples for fetal cell culture in tissue culture flasks. In the Section of Molecular Pathology Karyotyping, FISH aneuvysion, Thalassemia, DMD and SMA tests are performed in an amniotic fluid specimen.

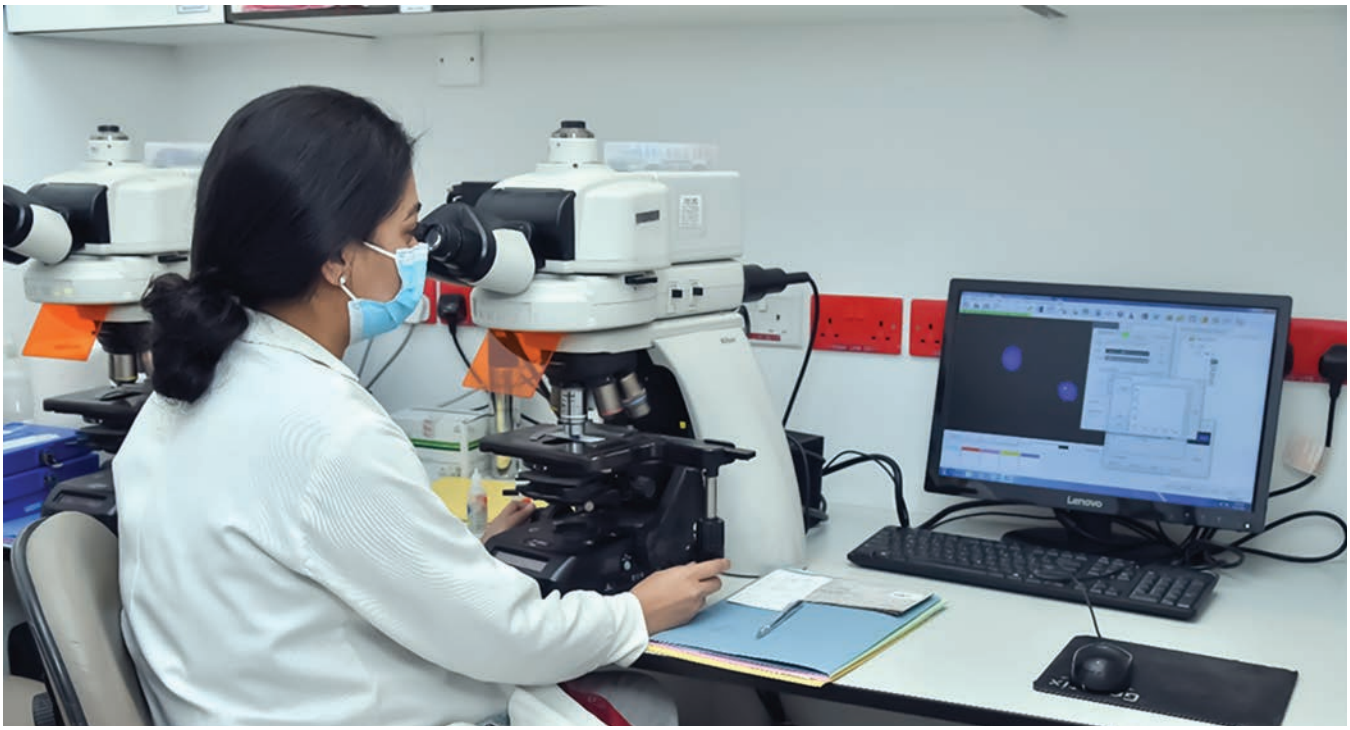
Polaroid

Molecular pathology team members involved in Feto-Maternal Diagnostics testing. Feta Maternal Diagnostic tests performed at the Section of Molecular Pathology include Karyotyping, FISH aneuvysion, Thalassemia, DMD and SMA.



A senior technologist at the Section of molecular Pathology preparing patient's sample for molecular analysis of Alpha Thalassemia by MLPA technique.

Polaroid



Visualization of Amniotic fluid slides prepared using Fluorescence in situ hybridization (FISH) technique for Trisomy 18.

Figure: Beta Hemolytic colonies of Group B Streptococcus

Polaroid

Performance of automated red cell antibody screening & identification in Hematology Lab.

Technologist performing renal stone analysis of a pediatric renal stone using Fourier Transform Infrared Spectroscopy. The most common component reported in pediatric age group is ammonium hydrate.

Polaroid

Technologists performing Kleihauer Betke test for evaluation of feto-maternal hemorrhage

Pathologist reporting the triple test results for evaluation of Down syndrome and neural tube defects.

Polaroid

Technologist performing the Thyroid Receptor antibodies (TRAb) using Electrochemiluminescence Immunoassay methodology on the Cobas e411 analyzer (Roche Diagnostics, US)

Gynecology tumor board group. Left to right: Dr. Taha Nafees (PGY-I), Dr. Rabia Qureshi (PGY-III), Dr. Naila Kayani and Dr. Romana Idress

The Tests for Congenital Endocrine and Inherited Metabolic Disorders

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Clinical Chemistry

Name	Indication	Sample Type	Patient Prerequisite	Reporting Schedule	Interpretation
DBS Acylcarnitine and Amino Acids	Screening for inherited metabolic disorders (Organic Acidemias, Aminoacidopathies, Urea cycle disorders, Fatty acid oxidation defects)	Dry Blood Spot (Filter paper)	Sample collected from heel prick from 0-30 days of birth	Performed on 2nd and 4th Thursday of month and report on following Wednesday	Interpretation based on quantity and pattern recognition
Neonatal biotinides deficiency (NBTD)	Early detection of curable congenital diseases as a part of NBS	Dry Blood Spot (Filter paper)	Sample collected from heel prick from 0-30 days of birth	Performed on 2nd Wednesday of month and report on following Friday	BTD is termed as screen positive if levels are <60nmol/min/dl
17-Hydroxy Progesterone (17OHP)	Newborn Screening for Congenital Adrenal Hyperplasia	Dry Blood Spot (Filter paper)	Sample collected from heel prick after 24-72 hours of birth	Performed on every Tuesday and Friday and reported on the following Thursday and Monday	Interprétation base on gestational age and birth weight based cutoffs
Neonatal Thyroid Stimulating Hormone (NTSH)	Newborn Screening for Congenital Hypothyroidism	Dry Blood Spot (Filter paper)	Sample collected from heel prick after 24 hours of birth (ideally 48-72 hours of birth)	Performed daily from Monday to Saturday and reported after 2 working days	Screen Positive if levels are >10mIU/L
Urine Organic Acid (UOA)	Diagnose inherited metabolic disorders (Organic Acidemias, Aminoacidopathies, Urea cycle disorders etc.)	Urine Sample	Random urine sample without preservative (preferably early Morning sample)	Performed daily from Monday to Friday Reporting after 10 working days of sample collection	Interpretation based on pattern recognition
Plasma Amino Acid (PAA)	Diagnose inherited metabolic disorders (Organic Acidemias, Aminoacidopathies, Urea cycle disorders etc.)	Plasma sample (EDTA tube)	Fasting at least 2 hours in infants, 4 hours in children and 8 hours in adults.	Performed daily from Monday to Friday and reported after 13 working days of sample collection	Interpretation based on quantity and pattern recognition
Succinyl Acetone	Diagnose Tyrosinemia Type 1	Urine	Early Morning random urine sample without preservative	Performed on every Wednesday and reported on the following Wednesday	Interpretation based on quantity of succinylacetone (SUAC)
Maple syrup urine disease (MSUD Profile)	Diagnose and monitor patients with MSUD	Plasma Sample (Lithium Heparin Tube)	Fasting at least 2 hours in infants, 4 hours in children and 8 hours in adults	Performed on every Monday and reported on the following Wednesday	Quantitative results for the branched chain amino acids
Homocysteine Profile	Diagnose and monitor the homocystinuria (cystathionine β-synthase deficiency, methylenetetrahydrofolate reductase deficiency and intracellular cobalamin defects etc.)	Plasma Sample (Lithium Heparin Tube)	Fasting at least 2 hours in infants, 4 hours in children and 8 hours in adults	Performed once in a week (Tuesday) Reporting same day	Quantitative results for the Homocysteine and methionine
Methyl malonic acid (MMA)	To diagnose and monitor patients with Vitamin B12 Deficiency and Methylmalonic Aciduria	Plasma Sample (Lithium Heparin)	Random sample	Performed on 1st and 3rd Monday and reported on the following Wednesday	Quantitative results for the MMA

Orotic Acid	Diagnose Urea cycle disorders	Urine sample	2 to 5 ml of spot or Random Urine Sample	Performed on Thursday and reported on Monday	Quantitative results for the Orotic Acid
Sugar chromatography	Detects Sugars (glucose, Fructose, lactose, galactose, sucrose, xylose) using thin layer chromatography	Urine	Random urine sample.	Perform every Tuesday and report on the following Thursday	Presence/absence of different reducing sugars

New Tests in Clinical Laboratory

Renal Indices

The Section of Chemical Pathology, Department of Pathology & Laboratory Medicine, is pleased to announce the introduction of six new tests/ biochemical indices. Decisions based on these biochemical indices using several urinary and serum markers may provide aid in the evaluation of electrolyte disorders, tubular function, metabolic bone disorders and differentiating between prerenal, renal and post-renal etiologies. These tests will support clinical decision making in many specialties.

S#	Test Description & Mnemonic	Sample requirement	Reporting Turnaround Time	Clinical Utility
1	Fractional Excretion of Potassium, FEK	Random urine and 3-5 cc blood sample	Monday to Saturday, reporting next day	Is useful for determining the cause for hyper- or hypokalemia. Renal losses of K+ may occur during the diuretic (recovery) phase of acute tubular necrosis, during administration of non-potassium sparing diuretic therapy, and during states of excess mineralocorticoid or glucocorticoid
2	Trans-Tubular Potassium Gradient, TTKG	Random urine and 3-5 cc blood sample	Monday to Saturday, reporting next day	It is an index reflecting the conservation of potassium in the cortical collecting ducts of the kidneys. It is useful in diagnosing the causes of hyperkaliemia or hypokalemia
3	Urine Anion Gap, UAG	Random urine sample	Monday to Saturday, reporting next day	It represents an indirect index of urinary ammonium excretion in patients with hyperchloremic metabolic acidosis
4	Spot Urine Calcium to Creatinine Ratio, SUCCR	Random urine sample	Monday to Saturday, reporting next day	It is used to screen for hypercalciuria. Increased urinary excretion of calcium accompanies hyperparathyroidism, vitamin D intoxication, metastasis from prostatic cancer, and following calcium supplementation
5	Tubular Phosphate Reabsorption to the Glomerular Filtration Rate, TmP/GFR	Random urine and 3-5 cc blood sample	Monday to Saturday, reporting next day	Is used to evaluate renal phosphate transport. It is useful in assessing renal reabsorption of phosphate in a variety of pathological conditions associated with hypophosphatemia
6	Fractional Excretion of Calcium, FECA	24hrs urine & 3-5 cc blood sample	Monday to Saturday, reporting next day	Widely used as a measure for differentiating patients with familial hypercalcemic hypocalciuria from those with Primary hyperparathyroidism

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