

**Hematopathology Laboratory,  
Tata Memorial Centre  
Mumbai**



**Organizes**

**Third CME For Medical  
Laboratory Technologist**

**Date : 21<sup>st</sup> & 22<sup>nd</sup> December 2013**



# Foreword

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Dear all,

We wish to welcome you all to the 3<sup>rd</sup> Annual CME for Medical Laboratory Technologists held by the Hematopathology Laboratory of Tata Memorial Hospital, Mumbai.

Aim of this CME is to update the Medical Laboratory Technologists on current diagnostic and management issues. Our second CME was resoundingly successful with participation by delegates from all over India. This year once again we have an excellent academic program with renowned faculty as speakers.

This year also we have poster presentations. We have received 27 abstracts this time.

Three best posters will be suitably awarded.

We would like to thank you all for making this conference a success.

**Sheetal Gaware and the Team**  
Hematopathology Laboratory,  
Tata Memorial Hospital,  
Mumbai.

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# 1 Comparison of Turbidimetric and Nephelometric methods for Immunoglobulin Estimation

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**Introduction:** Nephelometric and turbidimetric methods are used commonly for estimation of immunoglobulins & measure amount of ag-ab complexes formed. Nephelometry measures scattered light, turbidimetry measures transmitted light. Aim of this study was to determine the correlation between the results of the two tests and assess the suitability of using them interchangeably.

**Materials and Methods:** This bicentric study was conducted at the biochemistry services, ACTREC and TMH. We analysed 50 patient samples for Immunoglobulin (IgG, IgM, IgA) estimation that had been assayed by nephelometry and turbidimetry. The results were analysed for correlation and bias. We used Siemens Dimension RxL analyser for turbidimetric and Beckman Coulter Immage for nephelometric studies. Results were analysed for correlation using Regression analysis and for bias using Bland Altman Plots.

**Results:** 'R' value between nephelometry & turbidimetry for IgG, IgM and IgA were 0.985, 0.994 & 0.994 respectively. Bias was observed between the two methods as 9.5, -23 and 9 units for IgM, IgG and IgA respectively.

**Conclusion:** The results of this investigation reinforce the findings that both nephelometry and turbidimetry are suitable techniques for the assay of immunoglobulins. The bias showed in the results of two methods should be evaluated and correlated clinically.

**Key words:** Turbidimetry, Nephelometry, Immunoglobulins, Correlation.

# 2 Unusual Co-Infection in a Patient of Ulcerative Colitis

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**Introduction:** Intestinal Infections are not uncommon in UC. Presenting a case with unusual co-infection.

**Materials and Methods:** 45 / male, k/c/o Ulcerative Colitis for past eight years, treated in the past with Mesacol and steroids. Developed increased stool frequency since 15 days and was treated with antibiotics. One day developed sudden onset explosive diarrhoea, vomiting and giddiness. He was hospitalized and found to have severe dehydration, low BP, metabolic acidosis and raised creatinine. Patient was resuscitated with IV fluids and IV antibiotics.

**Results:** Stool routine examination revealed watery, white stools, few pus cells and short, motile worms, suggestive of *Strongyloides stercoralis*. Hanging drop preparation was negative for darting motility. Ivermectin was started. Two days later, stool culture revealed *Vibrio cholera*. Patient improved over 4 days.

**Conclusion:** Both the organisms are not the usual suspects in case of diarrhoea in UC and hence a careful stool examination is necessary. A high degree of suspicion and careful search is necessary on the part of the microbiologist as well as the technologist to render an accurate diagnosis.

**Key words:** Ulcerative Colitis, *Strongyloides*, *Vibrio*

## 3 MACRO – CPK – Confusion For The Laboratory And Cardiologist

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**Introduction:** Presence of Macro CK is a common cause of discrepancy in cardiac markers.

**Materials and Methods:** Two patients presented in casualty with chest discomfort. In one case CPK value was 21 U/ml and CPK-MB was 181 U/ml . In the other patient CPK was 45 U/ml and CPK-MB 220 U/ml. Both did not have ECG changes or raised Troponins. Assay was performed on Fusion 51 FS Dry Chemistry analyser. Clinicians informed, no treatment given for cardiac injury, kept under observation. Repeat enzyme assays after 7 days revealed similar results.

**Results:** The possibility of Macro- CPK enzyme is to be considered in this cases, confirmation by electrophoresis (not done in this case). Macro CPK are of two types – I and II.

**Conclusion:** Awareness about macro forms of enzymes is essential to the laboratory technicians for interpretation of such unusual results. Clinical correlation with severity of symptoms and enzyme levels will help resolve the dilemma in emergency cases.

**Key words:** Macro CPK, cardiac injury markers

## 4 Anti A1 Antibodies In A2 Rh+ve Blood Group

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**Introduction:** Incidence of anti A1 antibodies is relatively rare. There may be post-transfusion reactions or complications in recipients, the carriers of such antibodies, who are transfused donor red blood cells containing the antigen A1.

**Materials and Methods:** 26/F, G2P1L1, with 4months amenorrhea, for routine antenatal check up. Prior blood group done outside in 2009 reported O Rh Positive. No H/o prior blood transfusions, surgeries. First pregnancy and delivery uneventful. The blood group performed during this visit was by tube method. Forward group came as A2 Rh Positive. However, in the reverse typing 1+ agglutination was noted with A cells. Further analysis was done using gel card having A1 cells. Positive agglutination was noted.

**Results:** Blood group by tube method: Forward Grouping A2 Positive. The agglutination however was delayed. Reverse typing: Positive with B cells but also showed 1+ agglutination with A cells. This prompted us to do the further evaluation using the gel card having A1 cells. The positive agglutination was seen determining the presence of Anti A1 antibodies.

**Conclusion:** This highlights the importance of carrying out blood group testing by tube method with forward grouping and reverses typing. The rare occurrence of Anti A1 antibodies in a person with A2 blood groups can only be detected by proper testing methodology.

**Key words:** Anti A1 antibodies

## 5 SWOT Analysis Of The Sample Collection Area At A Tertiary Care Cancer Hospital

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**Introduction:** Ours being a tertiary care cancer institute the sample collection area directly interacts with patients & their relatives, who come from various parts of the country and are under tremendous stress due to the nature of the disease. SWOT (Strengths Weakness Opportunities Threats) analysis provides a good tool to judge the quality of the services provided and to look for opportunities to better the services.

**Materials and Methods:** A comprehensive analysis of the sample collection area was performed to assess the following criteria. **Strengths:** characteristics of the business or project that give it an advantage over others. **Weaknesses:** characteristics that place the team at a disadvantage relative to others. **Opportunities:** elements that the project could exploit to its advantage. **Threats:** elements in the environment that could cause trouble for the business or project.

**Results:** Strengths of the area were strategic location, quality & variety of the services provided under one roof & multi-tasking nature of the area. Weakness was identified as shortage of space. Opportunity identified was need to upgrade to advanced technology. Threat was perceived as increased risk of errors due to ever-increasing work load relative to staff strength.

**Conclusion:** Based on the analysis, roadmap for the improvement of the area was prepared.

**Key words:** SWOT, sample collection area, quality, improvement.

## 6 Study Of Peripheral Blood Culture Isolates In Hematopoietic Stem Cell Transplant Patients

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**Introduction:** Septicemia remains a major cause of life-threatening complication in patients with cancer. The aim of this analysis is to describe the microbial spectrum of blood culture isolates and the antibiotic susceptibility patterns of commonly growing microorganisms in peripheral blood culture.

**Materials and Methods:** Peripheral blood samples from suspected blood stream infections which were received from Bone marrow transplant and Hemato-lymphoid oncology patients of the hospital during the period of 2011-2013 were analyzed. The microbiological profile and the antibiotic sensitivity patterns of these isolates were studied.

**Results:** Of 2248 blood cultures processed, 115 were positive for microorganisms; out of which 47 were gram positive bacteria, 68 were gram negative bacteria and one was a fungus. The most common isolates among Gram negatives were Escherichia coli (38.23%) followed by Pseudomonas spp.(22.05%). Coagulase negative staphylococcus (80.85%) was the most common Gram positive organism. ESBL positivity was 36.36%. In case of gram negative bacteria, imipenem & amikacin were the most effective with 61.76% sensitivity each. Among staphylococci no MRSA was documented.

**Conclusion:** Gram negative bacteria with high rates of antibiotic resistance were commonly isolated from peripheral blood cultures in our study. In contrast Gram positive bacteria were generally susceptible to commonly used agents.

**Key words:** Antibiotic sensitivity, bacterial isolates, resistance



# 7 Assessment Of Blast Flag In Advia 2120i By Peripheral Blood Smear Examination

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**Introduction:** Advia 2120i, a fully automated hematology analyzer by Siemens provides , in addition to routine parameters , flags for blasts and LUC %. The criteria for generating blasts flag are %blasts >1.5 & %LUC (Large unstained cells)  $\geq$  4.5 or % blasts >5 of the total WBC. It is recommended that peripheral blood smear examination should be done for such samples. The aim of this exercise was to correlate the blast flags with the presence of blasts in peripheral smear.

**Materials and Methods:** In this study we evaluated 100 samples which generated the blasts flag in Advia 2120i. Peripheral blood smears were prepared and manual differential count (MDC) was done. MDC was correlated with the LUCs & blast %.

**Results:** Seven samples showed presence of blasts. There was no correlation between LUC % & presence of blasts in peripheral smear. Blast % of >4 was seen to correlate with presence of blasts in the peripheral smear.

**Conclusion:** From our study we found that LUC% does not have any bearing on the presence of blasts in the sample. However, samples with blasts % of more than 4 should be reviewed for presence of blasts on peripheral smear.

**Key words:** Advia 2120i, LUC, Blast%, MDC, correlation.

# 8 Analysis Of External Quality Assessment Scheme (EQAS) Results Of Clinical Hematology In An Oncology Setup

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**Introduction:** Proficiency Testing is one of the important tools to determine the technical competence of the testing laboratories. Participation in EQAS provides laboratories with an objective demonstration of reliability of the data they produce. Z-score is given as assessment score for the results among the laboratories (EQA–External Quality Assessment) and within the laboratory (IQA–Internal Quality Assessment).

**Materials and Methods:** The composite laboratory (Haematology) at ACTREC, TMC participates in ISHTM-AIIMS EQAP Programme. In four years, fourteen EQAS samples-blood, peripheral and reticulocyte smears were analyzed. Corrective actions taken for Z-score >3. For assessment results 2010-2013 trend analysis was done.

**Results:** Result of May-2012 showed EQA Z-score for WBC-4.24, MCV-3.19. This sample had deteriorated hence no corrective action could be taken. In March-2010, EQA Z-score for MCV-3.04 and Reticulocyte count-4.27 and corrective action comprised calibrating analyzer for MCV and training staff for reticulocytes screening. All other results of EQAS showed satisfactory Z-score for both EQA and IQA.

**Conclusion:** Regular participation in EQAS has helped us improve our clinical services in terms of performance, patient care and overall lab quality practice. Therefore we recommend active participation in the EQAS program to ensure greater accuracy & better quality of laboratory results.

**Key words:** EQAS, Z-score, trend analysis, accuracy.

# 9

## A Review Of Emergency Laboratory Services At Tertiary Cancer Care Centre

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**Introduction:** A major problem experienced in many hospitals centers around the inappropriate ordering of tests. Objective of this study is:

- 1.To identify the test parameters most frequently requested during the emergency hours
- 2.To review the turn around time (TAT) of the emergency laboratory services
- 3.To reduce the inappropriate use of laboratory services during emergency hours

**Materials and Methods:** A retrospective analysis of all the test requests received for the month of June 2013 during the emergency hours was carried out. Areas most prone to inappropriate use of the services were detected. Following intervention a prospective analysis was done for the requests received for the next three months July-September20.13.

**Results:** A total of 784 (Biochemistry), 627 (Haematology) and 595 (Microbiology) samples were studied between June-September2013.Following the intervention, decrease in the requests observed was 27% (Biochemistry) & 30% Haematology. In microbiology, major reduction was seen in stool culture samples of 53.04% followed by blood culture samples at 11.91%.Turn around time (TAT) for all the samples was maintained as per laboratory protocol.

**Conclusion:** Our findings correlate with those of other centers around the world regarding inappropriate use of emergency laboratory facilities. Periodic analysis of the requests received, which is also one of the quality indicators, should be carried out.

**Key words:** Emergency lab services, Inappropriate ordering of tests, Tertiary care centre

# 10

## Technical Relation Between Reticulocyte Count And Anaemia

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**Introduction:** Reticulocyte count of circulating blood determines the severity of anaemia. More reticulocyte count in circulating blood more the severity of anaemia due to loss of oxygen binding capacity of red blood cells in circulating blood. A case study of 25 such severely anaemic patient has been done and presented here.

**Materials and Methods:** Whole blood or capillary blood is used for reticulocyte count. Increased reticulocyte count in circulating blood after it leaves bone marrow is considered a disorder and result in anaemia. Stain used is methylene blue or supravital stain. 25 patients study was conducted suffering from severe anaemia by using Reticulocyte production index[RPI]=%reticulocytes\* [patient hematocrit / normal hematocrit] \* [1/maturation]. RPI determines disease and severity. Reticulocyte count is 3-7% in newborn and is 0.5-1.5% in adult normally. Any deviation is considered as disorder usually anaemia.

**Results:** Out of 25 patients with severe anaemia 18 patients had increased reticulocyte count in which 15 patients had reticulocyte count of 10-15% and 3 patients had reticulocyte count of more than 20%. And 7 patients had lower or normal reticular count of 0.5-1.5%. The reason behind this decrease or increase can be maturation disorder of red blood cells or red cell aplasia.

**Conclusion:** All severe anaemia patient do not have reticulocyte count high or low but is a major contributing factor with 70% occurrence rate depending on reticulocyte count used here to determine the relation between reticulocyte count and anaemia.

**Key words:** RPI=reticulocyte production index ,supravital stain ,methylene blue, anaemia.

# 11

## Review Of External Quality Assessment Scheme (EQAS) Of Clinical Biochemistry In An Oncology Setup

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**Introduction:** Proficiency Testing is one of the important tools to determine the technical competence of the testing laboratories and one of the essential requirements for accreditation. The Biochemistry EQAS program that we participate in is conducted by BIO-RAD and is used to evaluate quality and reliability of tests performed by clinical labs. More than 250 labs participate in our peer groups depending on the equipment infrastructure and methodologies used in the labs. Corrective action was taken against the parameters that have gone out of +/- 2SDI (Standard Deviation Index), The samples were also observed for trend / bias if any.

**Materials and Methods:** We evaluated 24 EQAS samples for Siemens Dimension- Xpand analyser and 12 samples for Siemens RXL analyser for past 2 cycles (June 2011-May 2013). Test results of 18 routine parameters were analysed and documented.

**Results:** Discordant results were observed for some parameters having Standard Deviation Index (SDI) more than +/- 2 SDI and corrective actions were taken.

**Conclusion:** Regular participation in EQAS has helped us improve our clinical services in terms of performance, patient care and overall lab quality. We recommend active participation in the EQAS program to ensure greater accuracy & better quality of laboratory results.

**Key words:** EQAS, Accreditation, SDI, Accuracy

# 12

## Anti Mullerian Hormone - A Novel Ovarian Reserve Marker: Indian Data For 14080 Patients

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**Introduction:** Anti Mullerian hormone (AMH) is secreted in females by granulosa cells of ovarian follicles and during embryogenesis of foetal male by sertoli cells of testis. Serum AMH is a predictor of ovarian response and pregnancy in assisted reproductive technology cycles.

**Materials and Methods:** A retrospective study of AMH test human serum samples was done for 14080 samples over a period of 6 months (June – Nov 2013). AMH test was done by ELISA method by Beckman coulter kit 2nd generation kit. A pilot study done in house on normal population of 105 for validating the reference ranges used for stratifying female infertile patients. This patient data was analysed for gender wise, age wise and result value wise.

**Results:** Study population included 53 males, 14011 females & 16 children. 2072(14.72%) no of samples were found < 0.3 ng/mL , 5631 (40%) no of samples were found 0.3 to 2.19ng/mL , 2574 (18.29%) no of samples were found 2.19 to 4 ng/mL, 1701 (12.09%) no of samples were found 4 to 6.69 ng/mL, 2102 (14.90%) no of samples were found > 6.79 ng/mL. For Female age 20-40 yrs group (10900 = 77.42%), it was found that 1125 (8%) <0.3 ng/mL, 4422 (31.41%) 0.3 to 2.19 ng/mL , 2190 (15.56%) 2.19 to 4 ng/mL, 1434 (10.19%) 4 to 6.69 ng/mL & 1729 (12.28%) > 6.79 ng/mL. In our study 12.09% of samples were found optimal fertility, 18.29% satisfactory, 40% low fertility 14.72% very low fertility/ undetectable & 14.90% High level (?Suspicion of polycystic Ovarian Disease / Granulosa cell tumours).

**Conclusion:** AMH can be done at any day of cycle and stratifying result as per optimal fertility, satisfactory, low fertility , very low fertility/ undetectable & High level helps better interpretation.

**Key words:** AMH, ELISA, Assisted reproductive technology, Indian data.

## 13

## Cryostat- Frozen Section Technique: A Double Edged Sword. Brief Review Of Literature And Audit Of Concordance Rate For A Period Of One And A Half Years In A Tertiary Care Hospital

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**Introduction:** The frozen section technique widely practiced today is based on the description given by Wilson in the year 1905. This technique performs rapid microscopic analysis of a specimen. Cryostat was first designed in the year 1938 but it was Coons et al who described the importance of this technique in 1942. Cryostat is a device used to maintain cryogenic temperature of samples. It freezes the tissue and converts interstitial water into ice which acts as an embedding medium.

**Materials and Methods:** We describe literature regarding cryostat-frozen section technique including its components, types, principle, working, trouble shooting, recent advances, various advantages, disadvantages and limitations. We also assess the accuracy of frozen section diagnosis by analyzing concordant and discordant diagnosis rates in relation to corresponding paraffin section reports.

**Results:** Cryostat operation and maintenance is expensive and needs expertise, knowledge and power backup. Limitation includes sampling and embedding errors, freezing and drying artifacts. Out of 232 frozen section samples received in the study period, 224(97.81%) were concordant, 5(2.1%) discordant and 3 samples were inadequate and hence excluded.

**Conclusion:** Although, cryostat frozen section technique has high diagnostic accuracy for rapid diagnosis especially in oncological surgery, we recommend that laboratories should periodically assess frozen section discordance and reconcile all discordant cases in final paraffin section reports.

**Key words:** cryostat, interstitial water, chuck, freezing artifact, paraffin section, concordant

## 14

## Comparison And Utility Of Haematological Parameters In Patients Presenting With Fever In Emergency Hours With Emphasis On Dengue And Malaria

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**Introduction:** This study aims to determine and compare hematological parameters in patients presenting with fever to Bhabha Atomic Research Hospital, Mumbai in emergency hours.

**Materials and Methods:** An observational study was performed including 377 patients who presented with fever to casualty. Of these, 80 (21.2%) patients were positive for dengue on serology, 60 (15.9%) patients were diagnosed with malaria on microscopy and 33 (8.7%) were diagnosed as enteric fever. All these positive cases were studied to determine age, gender, clinical and haematological profile.

**Results:** For dengue, male to female ratio was 1.1:1 with mean age of 31.3 yrs and for malaria, 2.75:1 with mean age of 38.75. Thrombocytopenia was found in 88.75% of dengue and 95% of malaria patients. Leucopenia was seen in 52.5% of dengue and 18.3% of malaria cases. RBC parameters were normal in majority of the cases in both dengue and malaria. *P. vivax* was the predominant species in malaria (100%) while *P. falciparum* was found in 4 cases mixed with *vivax*.

**Conclusion:** Thrombocytopenia was commonest hematological abnormality in both dengue and malaria. Thrombocytopenia was more common in malaria while leucopenia was more common in dengue.

**Key words:** Dengue, Haematological parameters, Malaria, Thrombocytopenia

# 15 Rapid And Reliable Identification Of Uropathogens Using Novel Chromogenic Media

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**Objective:** To evaluate a simple chromogenic medium for rapid identification of Uropathogens.

**Principle:** URICHROM agar is a chromogenic non-selective medium that allows for the spontaneous coloration & direct detection of colonies. The precipitation of the dye in the colonies is read visually. Additionally, confirmatory tests like 'Indole' can be carried out on the plate itself.

**Materials & methods:** Nearly 172 consecutive clean-catch urine samples were set up on routine culture plates i.e. Blood Agar, MacConkey's Agar" and "URICHROM agar". Growth on URICHROM was examined and interpreted using colour characteristics which was then compared with the routine plates. Identification based on URICHROM was confirmed on Vitek 2 Compact system.

**Results:** Out of 172 urine samples tested, 75 showed No bacterial growth. Out of the 84 samples that showed significant growth ( $>10^5$ CFU/ml), 47 samples (27%) showed growth of E.coli. Klebsiella pneumonia, Proteus spp, Pseudomonas aeruginosa were the other pathogens identified.

**Discussion:** Identification of colonies is visual and hence very simple. Few add on reagents added on the plate help in rapid direct identification of major uropathogens on the same day.

**Limitations:** Colonies that show their natural pigment & do not react with the chromogenic substrates must be further differentiated with appropriate biochemicals or identification system.

# 16 Preanalytics In Histopathology

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**Introduction:** Pathological and cytological reports are unique, because the results are not numerical, and they may become the final diagnosis, having a direct bearing on further therapeutic decisions. Recently, the numbers of medical incidents/accidents, in which pathology technologists and/or pathologists have been involved, have been increasing. It is of concern that a lack of proper procedures in pathology practice may give rise to serious accidents affecting patients' lives. Hence it is important to analyse preanalytical error percentages in histopathological examination of biopsy and resection specimens

**Materials and Methods:** Statistical analysis of preanalytical errors which were subgrouped (as Incorrect history, Labelling Errors, Fixation Errors, Errors in number of samples sent) was performed.

**Results:** Out of 500 specimens received, 300/500 (60%) had incorrect patient history, Labeling errors were present in 20/500 cases (10%) and 10 cases each (5%each) of Fixation errors and errors in number of samples sent for HPE were seen.

**Key words:** Incorrect history, Labeling Errors, Fixation Errors, Errors in number of samples sent

# 17

## Multidrug Resistant Bacterial Isolates From Wound Infections In Post Operative Head And Neck Cancer Patients On Radiotherapy

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**Background:** The term 'Multidrug resistant (MDR)' applies to a bacterium that is simultaneously resistant to number of antimicrobials, and is an increasing cause for concern particularly in immune-compromised cancer patients. The aim of this study was to determine the incidence of MDR bacterial isolates that caused wound infection in post operative Head and Neck cancer patients on Radiotherapy (RT).

**Methods:** This study was conducted on wound swabs and frank pus received from 114 post operative Head & Neck cancer patients on RT for the period April to September 2013. Bacteriological culture and sensitivity was performed following standard microbiological techniques. The data was analyzed for descriptive statistics.

**Result:** The number of culture positive specimens was 96, out of which 63 were Gram negatives & 33 Gram positives. The commonest Gram negative organism isolated was *Pseudomonas aeruginosa* (29%), followed by *E. coli* (19%) and *Klebsiella pneumoniae* (16%). ESBL production was 19% and carbapenem resistance was 29%. Staphylococci were the commonest Gram positive organisms and the MRSA rate was 27%.

**Conclusion:** Majority of bacterial isolates showed wide spread resistance against different microbial classes. Increasing levels of resistance to higher antibiotics like carbapenems is alarming, and a cause for concern.

# 18

## Effect Of High Hematocrit On Routine Tests of Coagulation - A Case Report

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**Introduction:** In today's era, there is rising incidence of diagnostic errors, mainly due to preanalytical factors. It may prove hazardous especially in tests of haemostasis. We are discussing a case report depicting: effect of high hematocrit on Prothrombin time (PT) and activated partial thromboplastin time (aPTT) with method of correction and possible mechanism of this effect.

**Case History:** 70 years man gave blood sample for Complete blood counts (CBC),PT and aPTT. Samples were run in Coulter750 & Stago Compacta.

**Results:** CBC findings suggested diagnosis of Polycythemia vera with haematocrit(72%)and abnormally elevated PT(35sec) and APTT(50sec). Following Latest CLSI (Clinical and Laboratory Standard Institute) guidelines on coagulation testing,we applied the correction formula, recollected the sample with adjusted citrate volume in optimal blood to anticoagulant ratio. The test result of PT & APTT-which were abnormally elevated in non-adjusted sample came to normal in citrate volume adjusted sample.

**Conclusion:** The present case emphasizes the significance of adjusted citrate to blood volume for routine tests of coagulation in patients with hematocrit above 55% & recommends strict following of CLSI guidelines for coagulation by all laboratories to have quality results hence avoiding erroneous diagnosis and treatment.

**Key words:** haematocrit, anticoagunt, citrate.

# 19

## An Approach To The Screening Of Thalassemia Trait

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**Introduction:** To provide recommendations for screening of patients for thalassemia trait

**Materials and Methods:** The Mentzer's Index, Srivatsava Formula and Shine and Lal formula were used to screen suspected patients for beta thalassemia and confirmation achieved by subjecting patient's sample to hemoglobin electrophoresis .

**Results:** A 63 year old female presented with fatigue hair fall and irritability. The consultant requested for the CBC which arose a suspicion of thalassemia. Hemoglobin electrophoresis was performed. The chromatogram revealed a diagnosis of beta thalassemia trait in the patient.

**Key words:** Thalassemia, Hb Electrophoresis

# 20

## “ CV versus SIGMA”: The Quality Tool For Next Generation – A Comparative Study

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**Introduction:** Quality Controls are materials designed to detect, reduce, and correct deficiencies in a laboratory's internal analytical process, in order to improve the quality of the results reported by the laboratory. These materials are run prior to the processing of patient samples and the values are plotted on Levey Jennings chart, analysed using Westgard rules and interpreted using various statistical tools. There are a few statistical tools available among which CV (coefficient of variation) is most commonly used for interpretation. But taking to the fact that CV measures only precision but not accuracy, a standard quality planning tool is the need of the hour. In our present study, we have tried to explore the role of SIGMA as a new standard Quality planning tool, since it measures both precision and accuracy.

**Objectives:** To calculate and compare CV and SIGMA of 12 biochemical analytes .

**Materials and Methods:** We conducted this pilot study for duration of 3 months (JULY –SEPTEMBER 2013). Twelve biochemical analytes were included in our study, for which we used BIORAD controls and the data were entered into Quality Assessment software (unity real time) and We utilized the software for calculation of CV(%), while SIGMA was calculated using the formula:

$$SIGMA = \frac{TeA (total allowable error)- Bias (\%)}{CV (\%)} \text{ Wherein, TeA was based on BV (biological variation)}$$

guidelines by Carmon Ricos et al; & Bias (%) was calculated based on EQAS values of these analytes.

**Results & Discussion:** The analysis of performance of analytes based on CV showed that all 12 analytes had acceptable CV (< 10%). But when the analysis was done based on SIGMA grading system, it was found that 4 out of 12 analytes showed world class performance (sigma > 6), 3 analytes showed good performance (sigma > 4), 3 of them showed acceptable performance (sigma > 3), while 2 analytes showed poor performance (sigma < 3). We found out that creatinine and urea which had acceptable CV (< 5%), showed a poor performance on SIGMA scale, due to poor accuracy of these analytes. On the other hand, bilirubin direct which had a higher CV (5.91%), when compared to urea and creatinine, showed world class SIGMA, due to the fact that it had good accuracy.

**Conclusion:** Our study shows that SIGMA is a better analytical quality indicator than CV. But a definitive conclusion could be arrived at, by extending this study to longer duration, including a larger number of analytes.

# 21

## Nocardia Asteroides Cerebral Abscess In Immunocompetent Host: Case Report

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**Introduction:** Nocardial brain abscesses are a rare central nervous system infection with high morbidity and mortality. Localized and multisystem nocardiosis is an opportunistic disease that occurs commonly in immunocompromised patients. Rarely, it is also seen in immunocompetent individuals. We reported a case of 68 years old woman who is a known case of myelofibrosis for more than 5 years. Based on the finding, the most probable diagnosis of cerebral abscess was suggested. Patient underwent craniotomy with aspiration of abscess.

**Materials and Methods:** Gram staining of aspiration showed Gram-positive branching filaments, Kinyon's staining showed acid-fast branching filaments, and culture showed colonies of Nocardia Asteroides.

**Results:** Diagnosis of nocardiosis depends on the demonstration of the bacterium by direct microscopy and culture. Surgical management for cerebral nocardia infection is often based upon the patient's clinical and immune status and the number and size of the lesions. In immunocompromised patients and those with multiple abscesses, a more aggressive surgical approach, such as craniotomy and excision, is indicated to maximize likelihood of survival,

**Conclusion:** Nocardia is a gram positive aerobic filamentous bacteria found in soil and water. Despite their low incidence, we need to consider nocardial infection in the differential diagnosis of a cerebral lesion in order to obtain an early diagnosis and start treatment as soon as possible.

**Key words:** Nocardia asteroides, Cerebral Abscess, Opportunistic Disease.

# 22

## Cytochemical Myeloperoxidase Staining Using 4-Chloro-1-Naphthol Is A Safe, Sensitive And Reproducible Alternative To Benzidine Dihydrochloride

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**Introduction:** Cytochemical detection of myeloperoxidase (MPO) is a strong indicator of myeloid differentiation. It is routinely used to differentiate between myeloid and lymphoid blasts. Benzidine-dihydrochloride is the main reagent used for cytochemical MPO staining; however, it is known to have potential toxic and carcinogenic effects and has been banned in many countries. 4-chloro 1-Naphthol (4C1N) is a known but poorly studied alternative for benzidine. In this study, we standardized MPO staining using 4C1N and compared the results with benzidine-based MPO staining.

**Materials and Methods:** Air dried bone marrow and peripheral blood smears from 20 cases of acute leukemia [10 lymphoblastic, 10 AMLs (FAB - 3 M1, 3 M2, 2 M3 & 2 M4)] and 20 normal individuals were evaluated for MPO activity using benzidine-based and 4C1N-based methods. Minimum 200 cells (granulocytes in normal individuals & or blasts in leukemia cases) were examined. Cut off of  $\geq 3\%$  positivity is used. Results were validated with flow cytometric immunophenotyping in leukemia cases. Additional cases will be done.

**Results:** In 20 normal individuals, 100% neutrophils showed myeloperoxidase activity by both methods. Blasts from lymphoblastic leukemia showed no myeloperoxidase activity. Of the 10 cases, abnormal-promyelocytes showed extremely strong MPO-reactivity and blasts from remaining AMLs showed weak to moderate MPO-reactivity. Results of both methods revealed a very good correlation.

**Conclusion:** 4-Chloro-1-Naphthol based MPO staining method is a safe, sensitive and reproducible alternative for determining cytochemical myeloperoxidase activity. It can easily replace the benzidine-based method.



# 23

## Classical Case Of SLE Undiagnosed For 2 Years: A Case Report

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**Introduction:** SLE is a disease of unknown etiology in which tissues are damaged by auto antibodies

**Materials and Methods:** A 33year old female presented with H/O chest pain & weakness for 3 Months, difficulty in standing up from squatting position, joint pains, facial & pedal edema for last 6 months. She had bad obstetric history & history of repeated blood transfusions for anemia.

**Results:** Patient had pancytopenia, hypocomplementemia, raised ESR & CRP, increased APTT, presence of lupus anticoagulant, positive ANA & anti dsDNA antibodies by Immunofluorescence. ANA profile revealed antibodies for nucleolar, ds DNA, histone & Sm antibodies.

**Conclusion:** Putting together clinical & lab data, it was a classical case of SLE

**Key words:** SLE, ANA, dsDNA, ANA profile, complement, lupus anticoagulant

# 24

## Comparison Of CBC Parameters In Venous And Capillary Blood In Oncology Patients

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**Introduction:** Venous blood is normally collected for CBC test. In chemotherapy patients collection of finger-prick blood may be easier. The comparison between CBC from capillary and venous blood would enable us to explore the possibility of using the two methods interchangeably.

**Materials and Methods:** Venous and capillary blood collected from 200 adult cancer patients were analyzed in 5-part differential HMX-BC hematology analyzer and smears were screened. The results were compared for WBC, RBC & platelets.

**Results:** WBC, Neutrophil, Monocytes, Hemoglobin, RBC & PCV showed no significant mean difference (i.e. bias) also regression analysis reveals no significant slope coefficient. Whereas significant slope coefficient (1.653) was observed for Monocytes. Platelets showed significant mean difference of +15.1 (less platelets in FP). Regression analysis showed good correlation  $R=0.9267$  for platelet counts  $<210 \times 10^9/L$ . Whereas correlation decreased for platelets  $>210 \times 10^9/L$ .

**Conclusion:** CBC parameters like WBC, Neutrophil, Hemoglobin, RBC & PCV except Monocytes are comparable. Platelets count  $<210 \times 10^9/L$  showed good correlation. Platelet counts  $>210 \times 10^9/L$  although showed less correlation does not make much clinical significance. Hence if Monocytes is considered, both finger-prick and vein-puncture can be used as a suitable technique for blood collection for CBC test.

**Key words:** Finger-prick, Vein-puncture, CBC parameters, Comparison, Oncology adult patients

# 25

## Blast Crisis Of CML Simulating Acute Myeloid Leukemia

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**Introduction:** Chronic Myeloproliferative Disorders are a group of stem cell disorders characterized by overproduction of mature white cells, red cell or platelets suggesting that the maturation of neoplastic cell line is relatively normal.

**Materials and Methods:** A 25 year old male presented with history of abdominal pain & weakness for 6 months. On examination patient had massive splenomegaly. Peripheral smear, bone marrow aspirate & biopsy & ancillary techniques were performed.

**Results:** Peripheral smear revealed marked leucocytosis with 38% blasts with eosinophilia & basophilia, hypolaobated megakaryocytes. Ancillary techniques were performed. Cytogenetics showed presence of Philadelphia chromosome. Immunophenotyping revealed blasts positive for CD13, CD33, CD 117, HLA- DR & CD-34. Assay for bcr-abl p210 was positive.

**Conclusion:** Despite the initial suspicion of AML, a diagnosis of CML in blast crisis was made due to presence of eosinophilia, basophilia, abnormal megakaryocytes, Philadelphia chromosome & bcr-abl (p210)

**Key words:** CMPD, CML, AML, Blast crisis, ancillary techniques

# 26

## Evaluation of International Society for Thrombosis and Haemostasis scoring system for DIC in acute leukemia patients: A retrospective study

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**Introduction:** Disseminated intravascular coagulation (DIC) is one of the important complications to develop in patients with acute leukemia (AL). International Society for Thrombosis and Haemostasis (ISTH) has provided a laboratory-based objective scoring system for evaluation of DIC. In this study, we retrospectively evaluated ISTH scoring system in AL patients with suspicion of DIC and its predictive value for the requirement of fresh frozen plasma (FFP) and platelet transfusion

**Materials and Methods:** We retrospectively analyzed results of PT, PTT, INR, Fibrinogen, D-dimer assays from 145 cases of clinically suspected DIC in AL patients (58 B-ALL, 16 T-ALL, 40 APML & 33 AML) and ISTH DIC-scores were calculated. Cases with score  $\geq 5$  were defined as "Overt-DIC" and with  $< 5$  were defined as "Non-overt-DIC". In 48 cases, scores were correlated with history of FFP and platelet transfusion.

**Results:** Out of 145 cases, 60% cases had "Over DIC" and 40% had "Non-overt" DIC. Individually, in 62.5% of B ALL, 50% of T ALL, 52.5 of APML and 70% of AML had "Overt-DIC". For FFP transfusion, ISTH score  $\geq 5$  revealed positive predictive value (PPV) of 59.26% & negative predictive value (NPV) of 42.86%. For platelet transfusion, ISTH score  $\geq 5$  revealed PPV of 46.67% & NPV of 22.22%. Additional evaluation will be presented in meeting.

**Conclusion:** The frequency of "Overt-DIC" is higher in non-APML type of AMLs is higher than APML and ALL. Cut-off of ISTH score  $\geq 5$  is not a good predictor for requirement of FFP and platelet transfusion for treating DIC in AL.

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**Introduction:** Nearly 20 million people suffer from sickle cell anemia in India. The sickle cell gene in India was first described among tribal groups in South India(2) but is now recognized to be widespread, especially in Central India, where the prevalence in different castes and communities varies between 9.4-22.2%(3). Our hospital caters to tribal population (Garasia tribe) of Southern Rajasthan (Sirohi district). We conducted this study to assess the prevalence and pattern of sickle cell disease in this community.

**Materials and Methods:** The study is a prospective descriptive hospital based study, taking the following parameters as its study variables: Age, sex, sickling test, CBC, Hb electrophoresis, splenomegaly and morbidities. All tribal patients who attended the OPD and mobile clinic of J Watumull Global Hospital and Research Centre, Mount Abu between Oct 2006 to Oct 2009 were screened for sickle cell anemia.

**Results:** Out of 5741 patients, 506 were found to be sickling positive (8.8%). Of these 0.81% had HbSS whereas 8.0% had HbAS.

**Conclusion:** We can screen tribal patients for sickle cell anemia and associated haemoglobinopathies, as we have observed sickle cell anemia with thalassemia, Hb C and Hb D in some of our patients. To screen for associated hemoglobinopathies quantitative estimation of Hb F (by alkali denaturation method) and Hb A2 (by column chromatography method) is required. Effects of preventive measures like penicillin prophylaxis, hematinics, vaccination and health education advised to 39 patients who were under regular follow-up were encouraging .

**Key words:** India, Prevalance, Sickle Cell Disease, Sickle Cell Trait, Grasia, Sirohi.

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