12-2012

LABRAD : Vol 38, Issue 2 - December 2012

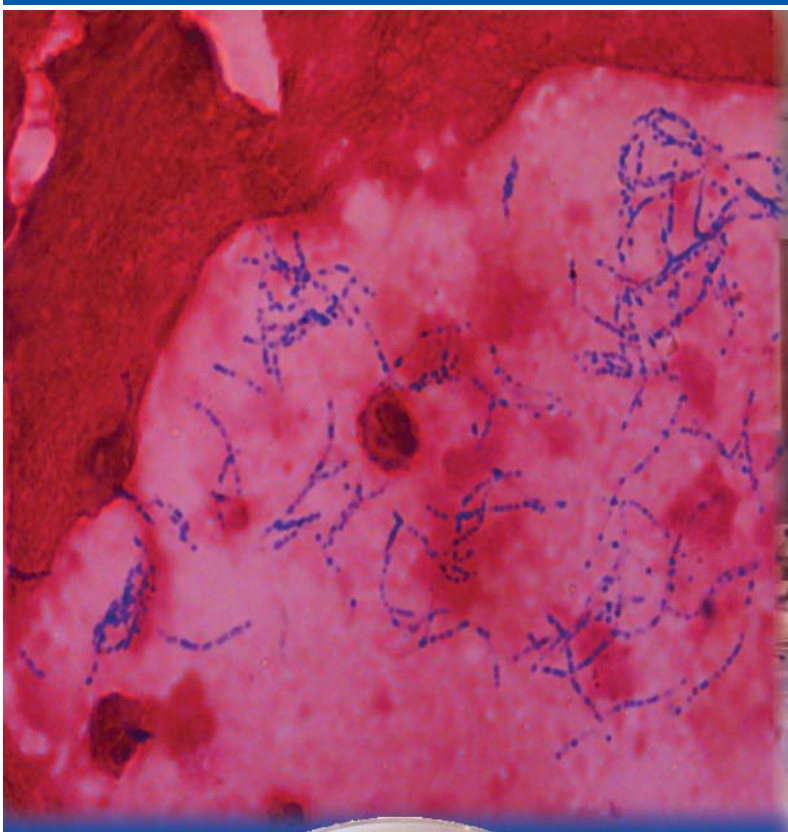
Aga Khan University Hospital, Karachi

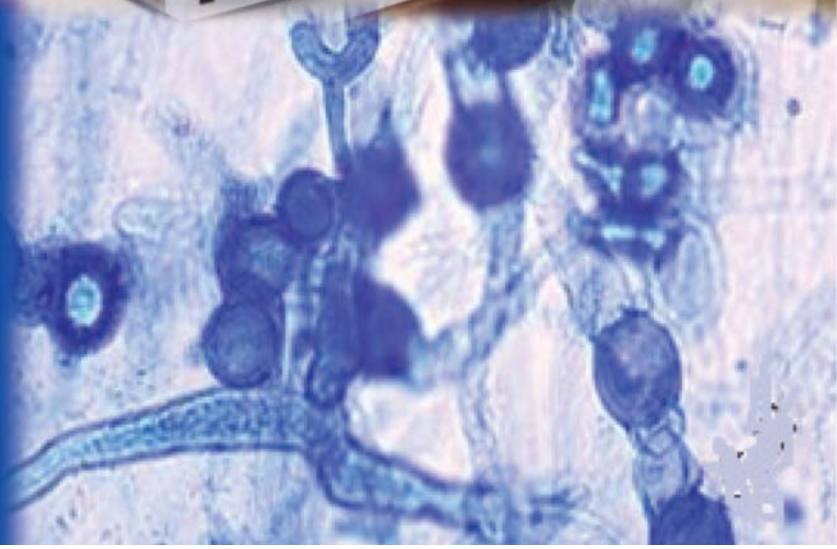
NEWSLETTER OF THE DEPARTMENTS OF PATHOLOGY, MICROBIOLOGY AND RADIOLOGY

LABRAD

DECEMBER 2012

VOL. 38, ISSUE 2





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The Aga Khan University Hospital, Karachi

LABRAD

A Publication of the Departments of Pathology, Microbiology and Radiology

December 2012
Volume 38, Issue II

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From the Editor's Desk

Advances in Microbiology

The discipline of microbiology could be said to have begun over 300 years ago when Anton van Leuwenhoek described the first bacteria seen through the microscope. At the turn of the 19th Century Pasteur, Koch and many others led a knowledge expansion leading to microbial classification as well as establishment of procedures for isolating and identification of organisms. In recent years clinical microbiology has entered a new phase with the introduction of automation and use of rapid techniques for diagnosis of infectious diseases.

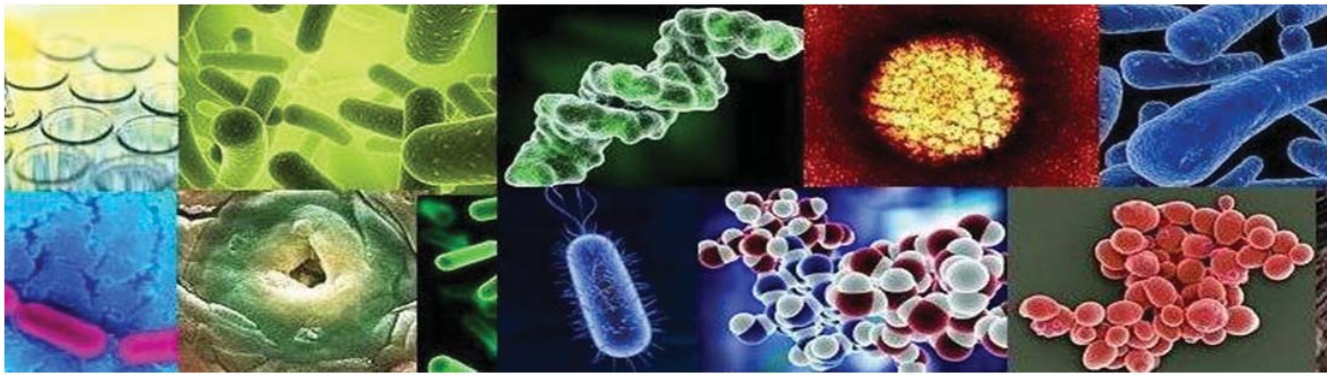
Today, automation is an integral part of many clinical laboratories and used for microbial detection, identification and susceptibility testing, detection of positive blood cultures, screening urine samples for potential pathogens, as well as for assaying levels of antimicrobial agents in body fluids. This issue discusses automation introduced at AKUH's Clinical Microbiology Laboratory to improve quality as well as reducing turn-around time.

Rapid diagnosis is essential for effective treatment. In particular major developments in diagnosis of tuberculosis and malaria have greatly reduced time to diagnosis. Use of Line Probe and GeneXpert for diagnosis of tuberculosis and of drug resistant strains is discussed in this issue. Additionally the role of rapid tests in diagnosis of malaria is evaluated.

Over the past couple of years there is increasing concern about meningitis caused by Naeglaria fowleri: a fresh water amoeba that enters the central nervous system through the nose resulting in meningitis. The infection is associated with extremely mortality and few treatment options, as such prevention is essential. This edition of LABRAD includes guidelines to prevent Naegleria fowleri (Amoebic) Meningitis so that the disease risk can be minimized. Another parasitic infection common in Pakistan is Leishmania and diagnosis of this infection is addressed in the latter part of this issue.

The importance of safe laboratory practices cannot be emphasized enough. These are essential to ensure safety of laboratory personnel and health care workers. To highlight importance of this aspect of clinical laboratories the issue concludes with a quiz on biosafety.

Professor Rumina Hasan
Department of Pathology Microbiology



Source: http://www.ccleanenergy.com/Techmicrobiology.html

Automation in Microbiology Specimen Processing and Inoculation

Dr Kauser Jabeen
Microbiology

Increasing incidences of infectious diseases in developing countries are posing a burden on clinical microbiology laboratories. Many laboratories are receiving increased number of culture samples. Specimen inoculation for culture is a tedious and time consuming process that requires expertise and trained manpower. Human errors and poor reproducibility could also be an issue. Automation in specimen inoculation will reduce the time consumed by a trained technologist on this procedure leading to more emphasis on quality improvement, research, teaching and training.

Recently third generation instruments have been introduced and include the WASP (Copan), Previ-Isola (BioMerieux), Innova (Becton-Dickinson) and Inoqula (KIESTRA). These systems have been introduced in clinical microbiological laboratories worldwide leading to more accurate, rapid, and cost-effective management of clinical specimens. The purpose of automated inoculation is easy and quick reading of plates. The overall benefit is a reproducible inoculation process and to obtain a larger number of isolated colonies than manual.

One of these, Previ Isola system (BioMérieux) is about to be introduced in the Aga Khan University Clinical Laboratory, Department of Pathology and Microbiology. It uses a circular applicator; a standard quantity of inoculum is streaked on agar plates (Fig. 1). Around 180 plates can be streaked in an hour.

Fig 1. Isolated colonies obtained on different culture media using Previ Isola system (BioMérieux)
Source: http://www.ccleanenergy.com/Techmicrobiology.html

It can be used for both fluids and swabs with improved diagnosis of aerobes, anaerobes, fastidious bacteria, and fungi. The choice of the appropriate system depends on specimen types, variety and volumes in a given laboratory. To provide correct and rapid identification of pathogens, automation in clinical laboratories is ongoing to improve and accelerate detection of infectious agents.

Use of Automated Urine Analyzer for Urinanalysis

Dr Imran Ahmad
Microbiology

Urinanalysis is a most frequently requested test in a Microbiology laboratory. This test has been shifted recently on automation in the section of Microbiology, Aga Khan University Clinical Laboratory, Department of Pathology and Microbiology. The system that has been adopted is LabUMat (urine chemical analyzer) and UriSed (urine microscopic analyzer) (Fig. 1). Results of both analyzers are stored in one database, making it possible to access each patient's data easily.

Fig. 1. LabUMat (urine chemical analyzer) system
Source: AKU/Faisal Malik

Barcode labelled tubes filled with patients' urine samples are loaded onto LabUMat platform where urine chemical analysis strips robotically receive each Patient's sample and the results are read. Once

chemical analysis is complete, sample is passed on to UriSed. Just prior to microscopic examination urine sample is mixed by the machine so that a homogenous sample is taken which is then centrifuged at 2000 RPM and sediment is analyzed for the presence of various particles which may be present in urine. Sharp, high power field microscopic images are taken by UriSed's inbuilt microscope which can be viewed on attached computer screen to verify the results if needed.

The combined system is able to evaluate urine sample with an 80 sample/hour throughput. Microscope view is available on screen with an opportunity to edit the results if needed. Whole image stored for up to 1000 samples and it does not need any expensive reagent for its operation.

Automation in Blood Culture

Dr Joveria Farooqi
Microbiology

Rapid reporting of blood cultures is essential for the management of patients with sepsis. Conventional blood culture methods are slow and more technically demanding. This has led to the development of automated blood culture systems. Automation of the "blood culture" process can help provide earlier results and more timely management of patients. Automated blood culture systems have been in use since the 1980s. Earliest systems with radioactive CO_2-based (BACTEC Radiometric) were devised followed by fluorescence-based and colorimetric systems which are commonly in use today. Other systems monitoring pressure changes are also in use. The most popular systems are BacT/Alert and BACTEC 9000 series. These devices use a continuous monitoring system and once it indicates a positive results the blood culture bottle is pulled out from the system and is processed (Fig 1).

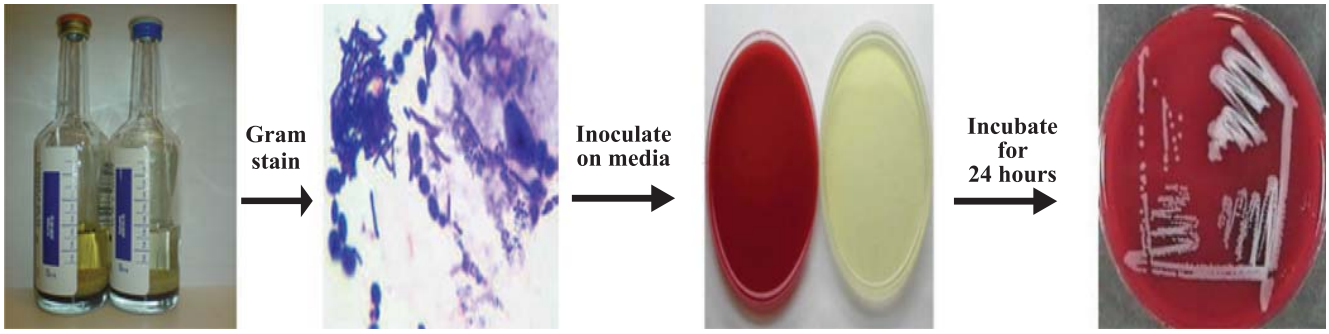


Fig 1: Blood culture processing after a positive result from the automated blood culture system

Principles

Semi-automated BACTEC Radiometric: Presence of C^{14}-labeled substrates in the broth results in production of radioactive CO_2 detected by the instrument.
Fluorescence-based: These are continuous -monitoring systems.

BACTEC 9000 series detects increase in fluorescence with increase in CO_2 production by viable micro-organisms. Vital blood culture system detects fluorescence in response to changes in CO_2, pH or redox potential.

Colorimetric: BacT/Alert is based on colorimetric changes in response to CO_2 production. This is also a continuous-monitoring system.

Pressure-monitoring systems: Another continuous-monitoring system, DIFCO Extra Sensing Power System detects changes in pressure due to gas production or consumption by micro-organisms.
Once the vial signals positive and gram stain from the broth has been made, identification and sensitivities of the organism can be performed biochemically or genotypically.

Biochemical activity-based systems:
The broth can be used to inoculate identification and susceptibility testing cards or 96-well plates for gram positive or gram negative bacteria in automated systems available in the market. Some of these are Vitek 2, Microscan, Phoenix and Biolog, Liquid Gas Chromatography. These are read at 18-

24 hours of incubation for the bacteria's 'metabolic fingerprint' or growth in the presence of certain substrates or their fermentation. This pattern of biochemical activity is then used to identify the micro-organism using photospectrometry/ colorimetry.

Genotypic tests:
These tests are based on 16S rRNA identification of bacteria directly from positive blood culture vials. The methodologies involved may be line probe assays, real time PCR, flowcytometric analysis, et cetera for detection of pathogens and their resistance genes.

Use of an Automated System for Antimicrobial Susceptibility Testing

Dr Naima Fasih
Microbiology

Infectious diseases are an important cause of morbidity and mortality in Pakistan. Guided antibiotic therapy helps in the early treatment of infected patients. The goal of susceptibility testing should be to report accurate and timely results. Recently several automated systems for antimicrobial susceptibility testing have been developed that provide commercially prepared and formatted microdilution panels for the convenience and requirement of the user. These systems are furthermore supplemented with instrumentation and automated reading of plates. However one of the major limitations of these systems is high cost in initial purchase, operation and maintenance of the machinery. Some of the most commonly used systems are Vitek System, Walk-Away System, Sensititre ARIS and Phoenix.

The Vitek 2 System recently introduced in Microbiology section of our laboratory is a highly automated system and uses very compact plastic reagent cards (credit card size) that contain microliter quantities of antibiotics and test media in a 64-well format. The susceptibility cards allow testing of common, rapidly growing gram-positive, and gram negative aerobic bacteria in a period of 4–10 hours. This system employs repetitive turbidimetric monitoring of bacterial growth during an abbreviated incubation period. The instrument can be configured to accommodate 30–240 simultaneous tests.

Principle

The principle of the Antimicrobial Susceptibility Testing (AST) cards is based on the microdilution Minimum Inhibitory Concentration (MIC) technique. This AST card is essentially a miniaturized, abbreviated and automated version of the doubling dilution technique.

Method

Each AST card contains 64 wells with a control well that is without antibiotic and wells containing premeasured portions of a specific antibiotic. The bacterial isolate to be tested is diluted to a standardized concentration with 0.45-0.5 per cent saline before being used to rehydrate the antimicrobial medium within the card. The System automatically fills seals and places the card into the incubator and monitors the growth of each well in the card over a defined period of time (up to 18 hours). At the completion of the incubation cycle, a report is generated that contains the MIC value along with the interpretive category result for each antibiotic contained on the card. (Fig.1)

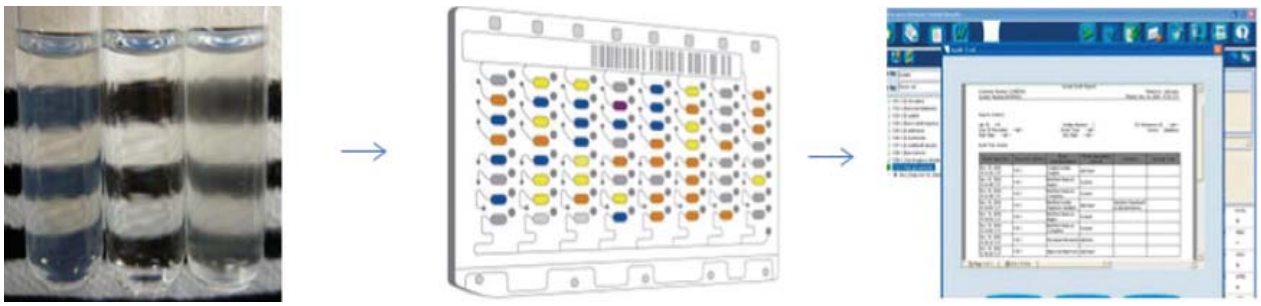


Fig 1: Reporting of antimicrobial susceptibility results using an automated system. Modified from Source www. biomerieux-diagnostics.com

TB Diagnosis-a New Horizon

Gene Xpert MTB/RIF: a Fully Automated Molecular Test for TB Case Detection and Drug Resistance

Dr Imran Ahmad
Microbiology

Rapid diagnosis of tuberculosis and resistance profile is central to its control. Failure to quickly recognize and treat affected patients leads to increased mortality, secondary resistance (including extensively drug-resistant tuberculosis), and ongoing transmission.

A fully automated molecular test for TB case detection and drug resistance (rifampicin resistance) has been recently developed by the name of Xpert MTB/RIF which integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection.

Processing involves three simple steps

1. Sputum liquefaction and inactivation with sample reagent (15 minutes)
2. Transfer of material (from step 1) into test cartridge
3. Insertion of cartridge into machine (Xpert MTB/RIF). Subsequent processing is automated, and the results are provided in 1 hour and 45 minutes (total time from sputum processing to result: 2 hours). The results are provided in a printable version e.g. MTB detected; RIF resistance not detected.

Case detection:
Sensitivity: 99.8 per cent in smear and culture positive and 90.2 per cent in smear negative and culture positive (overall 97.6 per cent)
Specificity: 99.2 per cent overall
Rifampin resistance detection:
Sensitivity: 99.1 per cent
Specificity: 100 per cent

Advantages

- Point-of-treatment use, results available in the clinic and decision regarding therapy could be taken
- No requirement of a biological safety cabinet
- Minimum training required for test performer (2-3 days v/s 2 weeks for microscopy)
- Simple to perform, minimum hands-on-time of 15 minutes
- Unambiguous result readout

Disadvantages

- Cost of the equipment
- Needs a reliable electricity supply and temperature maintenance
- Requirement of annual calibration

Fig. 1.Xpert® MTB/RIF technology for MDR-TB detection
(Source: http://www.finddiagnostics.org/programs/tb/find_activities/automated_naat.html)

Line Probe Assay for Detection of Mycobacterium Tuberculosis-Complex and its Resistance to Rifampin and Isoniazid

Dr Joveria Farooqi
Microbiology

Tuberculosis is one of the leading causes of death worldwide. Pakistan ranks eighth in highest burden countries for tuberculosis. Increase in drug resistance in *Mycobacterium tuberculosis* (MTB) is further complicating its treatment and control. It has become even more important to detect drug-resistant TB early for timely initiation of treatment which will ensure administration of appropriate treatment and thus limit its spread.

In 2008, WHO issued a statement approving two commercial line probe assays for detection of MTB-complex and mutations conferring resistance to rifampin and isoniazid. These assays could be performed directly on Acid Fast Bacilli (AFB) smear positive pulmonary specimens and thus reduce turn-around time for detection of strains resistant to the two key first-line agents. Thus Multi-Drug Resistant TB (MDR-TB), defined as resistant to both isoniazid and rifampin, could be detected within one day as opposed to six weeks by conventional culture based drug susceptibility testing. In 2010 WHO approved another test, GeneXpert MTB/RIF, for rapid detection of rifampin resistance as a marker for MDR-TB, but this test may miss isoniazid mono-resistance in rifampin sensitive strains.

One of the line probe assays, Genotype MTBDR® from Hain Lifescience, Germany, was introduced in 2005 and since then improvements in the test have increased its sensitivity in detecting resistance to isoniazid and an even lower number of MTB bacilli in clinical samples. The current version, Genotype MTBDR*plus*® 2.0, can detect MTB loads as low as 102 bacilli per milliliter. It can therefore be used in AFB smear negative pulmonary specimens as well. The sensitivity and specificity of the assay in identifying MTB-complex in AFB smear positive samples is 100 per cent compared to culture. However in smear negative pulmonary specimens, sensitivity decreases to 70-79 per cent, while specificity remains 99 per cent. Detection of rifampicin resistance based on *rpo*B gene mutation is 95.9-99.1 per cent sensitive and 97.3-99.4 per cent specific, while for isoniazid resistance based on *inh*A and *kat*G gene mutation sensitivity is 76-90 per cent and specificity 97.5-100 per cent.

The assay is performed on specimens decontaminated by 1-2 per cent NaOH-NALC and centrifuged as recommended for concentrated AFB smear and culture processing. These pellets are then heat inactivated, sonicated in a water-bath to release DNA from the bacteria and supernatant used as template for PCR. DNA extraction may also be performed using commercially available kits for manual (GenoLyse®) or automated (GenoXtract®) methods for improved sensitivity. Ready-to-use amplification mix already containing the Taq polymerase is provided with each kit.The PCR protocol and primer design was modified in the later version to enhance amplification of specific DNA sequences present in the template. Finally hybridization to oligonucleotide probe-labeled strip can be performed automatically using GTBlot 48 device (Hain Lifescience) to reduce the workload but there is also an option to perform this step manually. Interpretation of the results may be manual or automated.

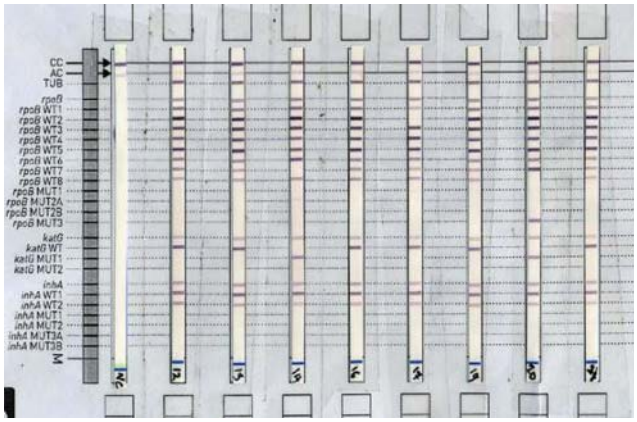


Fig 1: Line probe assay for rapid detection of drug resistance in Mycobacterium tuberculosis
Source: AKU/Joveria Farooqi

The Microbiology section of Aga Khan Clinical Laboratory, Department of Pathology and Microbiology has recently introduced this test. Specimens will be collected throughout the week, tests will be run every Thursday and reports issued on Friday. Introduction of a rapid diagnostic test for detection of isoniazid and rifampin resistant tuberculosis in Pakistan is expected to revolutionize TB diagnostics and management significantly in this era of drug resistance.

Serological and Molecular Diagnosis of Malaria

Dr Najia K Ghanchi and Dr M Asim Beg
Microbiology

Malaria is one of leading causes of morbidity and mortality in Pakistan with approximately 50,000 deaths including neonates each year. Accessibility of effective, affordable diagnostics and drug resistance to mainstay antimalarial are major problems, which hinder, malaria control strategies in Pakistan. According to WHO recommendations, malaria diagnosis should be based on parasite detection in all cases. However, in various situations where diagnostic facilities are not available diagnosis is based on signs and symptoms. Laboratory confirmation of clinical diagnosis of malaria can prevent misdiagnosis or unnecessary treatment due to sign and symptom, which may overlap with other infections. By applying specific diagnostic methods an estimated 450 million unnecessary treatments could be prevented annually.

Microscopy

Conventional light microscopy of blood smear is a low cost most widely utilized technique for detection of malaria parasites. Various staining methods such as Field, Leishmann and Giemsa stains are available to stain blood films. Giemsa stained thin blood film for species identification and thick films for quantification of parasite is the gold standard. However, microscopic examination of thin film is less sensitive (1/10) as compared to thick films for quantification of parasites. Poor slide preparation and staining errors greatly affect the outcomes of microscopy.

Microscopic examination of stained blood films is highly sensitive and specific with detection limit of 5-10 parasite/µl. Detection limits vary with experience of the microscopist and the average microscopist can detect 50-100 parasite /µl in field conditions. Despite being a widely implemented method, microscopy remains labour intensive, time consuming and needs considerable expertise.

Rapid Diagnostic Tests

Rapid Diagnostic Test (RDTs) was introduced in early 1990s to provide simple and rapid diagnosis of malaria. RDTs are based on detection of parasite product released into the blood such as Histidine rich protein 2 (HRP2), P.falciparum lactate dehydrogenase (PfLDH), Pan lactate dehydrogenase (PLDH) and Pan aldolases (common to all malaria species). These tests are available in card and cassette format for ease of use and storage. The detection threshold of RDTs is 100 parasite /µl. Forney et al reported 100% sensitivity at parasitemia greater the 500 parasites/µl which reduces to 86 per cent at <500parasites/µl.

RDTs are reliable, simple field applicable diagnostic test. However, number of issues still need to be addressed. With context to Pakistan where *P.vivax* coexists with *P.falciparum*, the lower sensitivity and specificity in detection of mixed infection is a point of concern. The performances of RDTs were reportedly affected by number of factors such as temperature variations, lot-to-lot variability, storage conditions and methodology.

Polymerase chain reaction

Recent advances in molecular techniques have generated number of diagnostic modalities based on identification of DNA (Fig. 1). Methods such as multiplex, nested, semi-nested PCR and real time PCR have been introduced. Of these tests, nested PCR is the most sensitive and considered the gold standard. PCR methodology targets small-subunit

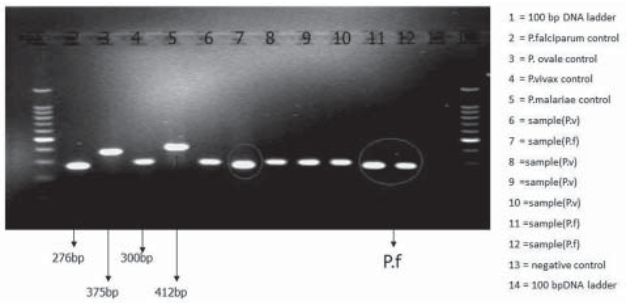


Fig. 1. Agarose Gel Electrophoresis-Species differentiation of Plasmodium

18S rRNA and circumsporozoite proteins genes and is a sensitive and specific method for malaria diagnosis. PCR can detect as low as five parasite/µl and has shown greater sensitivity and specificity over other diagnostic methods. The ability of PCR to detect low parasitemia, which can be missed in the absence of expert microscopy makes it a reliable method of diagnosis in non-endemic areas.

In recent years, numbers of other molecular methods are introduced to improve malaria diagnosis such as Loop-mediated isothermal amplification (LAMP) (Fig. 2). It is a relatively simple and less expensive

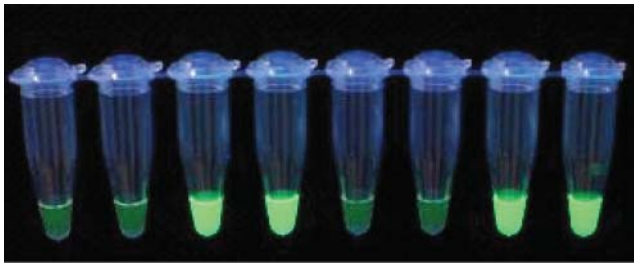


Fig. 2. Detection of positives samples (shown as bright green)

method. LAMP is a novel molecular technology which is highly sensitive and specific, faster, requiring minimal processing and instrumentation, and allowing result detection with the naked eye. It is a recently introduced rapid sensitive diagnostic method which provides results in real time (<1 hr). Various studies have reported 100 per cent specificity and 94 per cent sensitivity against the gold standard. Recently Lucchi et al introduced RealAmp method for malaria diagnosis using simple portable device that can perform amplification and detection of fluorescence by LAMP on the same platform. Furthermore, improved methods can provide better tools for field applicability.

Guidelines to Prevent Naegleria fowleri (Amoebic) Meningitis

Professor Afia Zafar
Microbiology

Background

Amoebic meningitis is an infection of the brain and its lining membrane (known as meninges). This infection is also known as primary amoebic meningoencephalitis (PAM). Meningitis is of sudden onset and is caused by an environmental single celled parasite known as Naegleria fowleri (Fig. 1).

Naegleria is commonly found in freshwater such as lakes, ponds, rivers, hot springs and soil. Its resistant form (cyst) survives in soil for a long time and transforms into infectious form trophozoite and flagellated form, when it contaminates fresh water at an appropriate temperature. Infection occurs primarily after

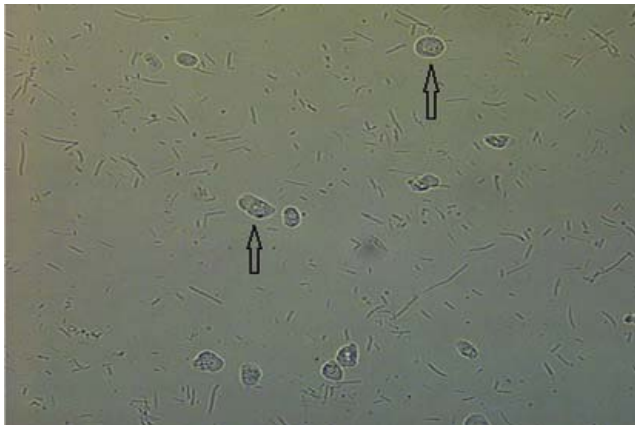


Fig. 1. Wet preparation of CSF sample showing trophozoites of Naegleria fowleri
Source: AKU/Imran Haq

flagellated forms of the amoebae come in contact with the nasal passage. It enters the brain through the roof of the nasal cavity where it destroys the brain tissue and causes death. This typically occurs after exposure to warm, untreated water such as while swimming or diving into lakes, ponds and dams.

It is a heat-loving (thermophilic) microbe which multiplies well at between from 28°C to 40°C. It tolerates temperature of up-to 450°C. It is less likely to be found in the water as the temperature declines. Naegleria fowleri is not found in salty water, such as sea water.

Mode of Transmission

disease does not spread from one person to another or a person cannot be infected with Naegleria fowleri by drinking contaminated water. This infection may occur when unchlorinated or inadequately chlorinated fresh water (domestic tap water, swimming pool water, bore water) enters the nose when such as when people submerge their heads or cleanse their nose during religious practices, or and when people irrigate their nose using contaminated tap water.

In the developed world, where domestic water supply is safe, most victims are children as they expose themselves to unclean water in lakes and rivers during water related recreational activities. Unfortunately in Karachi, due to compromised chlorination and water quality of domestic water supply, the disease is not limited to children. mostly Young healthy male adults aged 16-42 years are contracting the disease.

Sign and Symptoms

Onset of symptoms occurs 1-7 days after exposure. Sign and symptoms of infections are similar to those of bacterial or viral meningitis and include, headache, fever, stiff neck, anorexia, vomiting, altered mental status, seizures, coma and death. The mean time from symptoms onset to death is typically five days.

Prevention

Prevention requires both prevention to exposure (Table 1) and decontamination of water supply.

Table 1. Prevention to exposure

Prevention to Exposure
• Chlorine concentration of tap water should be maintained at 0.5ppm (mg/liter) • Use chlorinated or boiled water for cleansing and ablution • Don't use tap water or untreated fresh water, when irrigating, flushing, or rinsing the nasal passage. • Instead use sterile distill water or previously boiled water to make the irrigation solution • Don't allow water to enter the nose during bathing, showering and washing of face • Overhead and underground water tanks must be cleaned twice a year and filled with chlorinated water • Only swim in well maintained and properly chlorinated swimming pools • Don't allow children to play with hoses or sprinklers as they may accidentally squirt water up their nose • Don't jump or duck dive into warm fresh water without closing the nose as it damages the mucosal layer of nose. Hold the nose shut or use nose clip • Keep wading pools clean by emptying, scrubbing and allowing them to dry in the sun after each use • One can swim in sea water as the amoeba can't survive in water containing more than two per cent salt

Decontamination of Water at Domestic Level

Naegleria fowleri (cysts, trophozoites and flagellated form) will be killed if the residual chlorine concentration of tap water is kept at 0.5 ppm. In water with higher organic load and pollution (water appears turbid), the residual chlorine levels may have to be increased to 1 ppm.

Chlorine is safe and cost effective. In humans and animals exposed to chlorine in drinking water, specific adverse treatment related effects have not been observed. The taste and odor thresholds for chlorine in water are 5 and 2 PPM respectively Chlorine is available in various forms:
Domestic bleach solution (5 per cent)

This is equivalent to 50,000 ppm. For the preparation of a 0.5 ppm water solution, add 1ml of this liquid to 100 liter of water.

OR
Add 8 drops or 1/8th of a teaspoon of chlorine in 1 gallon (4 liters of water), in turbid water 24 drops and wait for an hour

Bleach powder is available in various strengths

For the preparation of a 0.5 ppm water solution, one should calculate the amount according to its strength

Chlorine tablets are available in various strength. Use them as per manufacturers instruction.

Diagnosis of Leishmaniasis

Dr Naima Fasih
Microbiology

Leishmaniasis is a vector-borne disease that is transmitted by sand flies and results in both local and systemic infections.

Laboratory Diagnosis

Microscopy

Examination of Giemsa stained slides of the relevant tissue is the most commonly used technique to detect this obligate intracellular parasite. Specimens that are usually received in laboratory are skin scrapping, tissue biopsy, lymph node, bone marrow aspirates and blood. Amastigotes of *Leishmania* spp. are present intracellularly in macrophages of the host and can be found throughout the body. These amastigotes commonly known as Leishmania donovani (LD) bodies are spherical to ovoid in shape and measure 1-5 µm long by 1-2 µm wide (Fig. 1). They possess

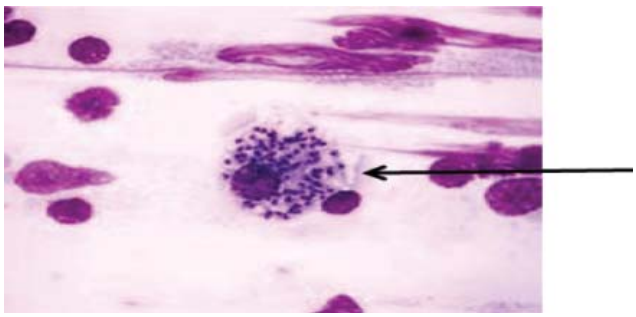


Fig 1: Giemsa stain of bone marrow aspirate from a patient with visceral leishmaniasis showing histiocyte loaded with amastigotes of Leishmania donovani (Courtesy: Dr. Sarwar Ali, Hematology, Aga Khan University Laboratory)
Source: AKU/Sarwar Ali

a large nucleus, a prominent kinetoplast visible by light microscopy. The sensitivity of microscopy is 40-60 per cent and specificity is 80-90 per cent. This test is available at the Aga Khan University Laboratory.

Culture

Isolation of the organism in culture (using for example the diphasic NNN medium) is another method of the diagnosis. Its sensitivity is 30-40 per cent and specificity is70-80 per cent.

Both microscopy and culture although are effective tests for the diagnosis of cutaneous leishmaniasis; however their main limitation in the diagnosis of systemic infections is requirement of invasive specimens.

Serology

Antibody detection although is useful for diagnosis of visceral leishmaniasis but is of limited value in cutaneous disease, where most patients do not develop a significant antibody response. Rapid immunochromatographic test for the qualitative detection of anti-*Leishmania* circulating antibodies in serum is a relatively new test. This test utilise antigen-impregnated nitrocellulose paper strips and is highly sensitive and specific (>95 per cent) and has been reported to be comparable with PCR. It detects leishmanial recombinant antigen K39 (rK39), which is the product of a gene cloned from *Leishmania chagasi* and contains a 39-amino-acid repeat conserved among viscerotropic *Leishmania* species (*Leishmania donovani, L. infantum,* and *L. chagasi*). This test is available at the Aga Khan University Clinical Laboratory, Department of Pathology and Microbiology.

PCR

The PCR for *Leishmania* spp. has high sensitivity and specificity but is currently not available in many clinical laboratories.

Biosafety quiz: What is Wrong in These Pictures?

Ms Maqboola Dojki and Professor Afia Zafar
Microbiology

Fig. 1. Source: AKU/Maqboola Dojki

Fig. 2. Source: AKU/Maqboola Dojki

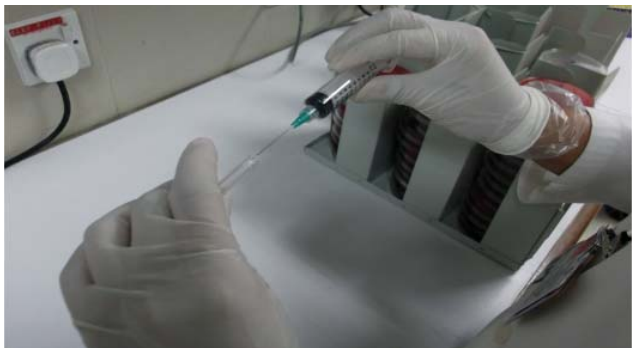


Fig. 3. Source: AKU/Faisal Malik

Fig. 4. Source: AKU/Maqboola Dojki

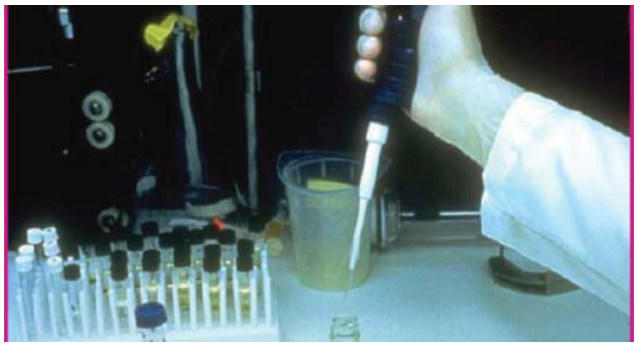


Fig. 5. Source: AKU/Maqboola Dojki

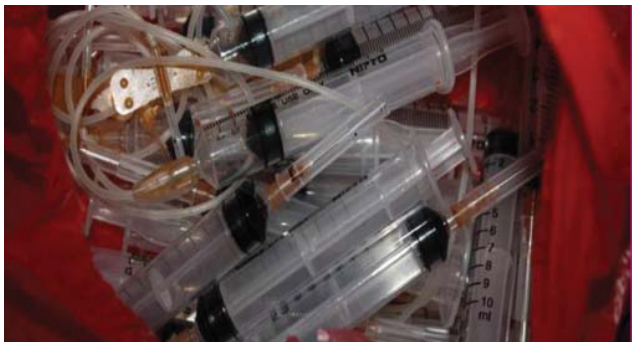


Fig. 6. Source: AKU/Afia Zafar

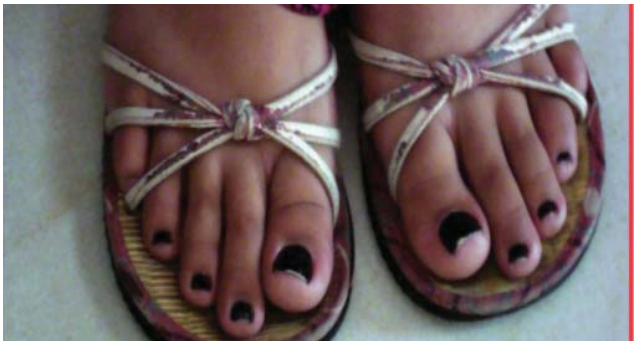


Fig. 7. Source: AKU/Afia Zafar

Fig. 8. Source: AKU/Faisal Malik

Rapid Detection of Dengue NS1 Antigen by ELISA in Suspected Dengue Patients

Dr Binish Arif
Microbiology

Dengue is an endemic disease in Pakistan, and its severity could range from dengue fever to life threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Serological tests for IgM and IgG are routinely used in clinical laboratories for rapid diagnosis of dengue and can differentiate between primary and secondary infections. However, this test may be negative if performed very early in the course of the disease.

Dengue virus non-structural protein one (NS1) has been identified as an early marker for acute dengue, and typically presents itself between days 1–10 post-onset of illness. NS1 detection can help to diagnose an active dengue infection from the very first day of when the symptoms appear as the dengue NS1 antigen can be detected in serum from the first day that a fever appears (Fig. 1). Detection of NS1 can also provide

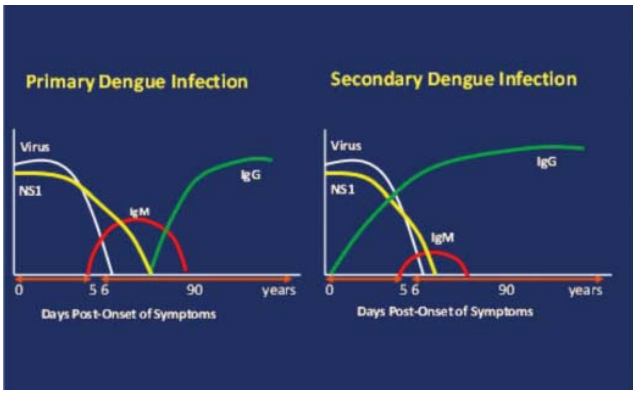


Fig. 1. NS1 antigen positivity during Dengue infection
Source: http://www.cdc.gov/dengue/clinicallab/laboratory.html

a specific diagnosis of dengue infection, especially valuable in areas where flavivirus serological cross reactivity could confound a diagnosis. However, NS1 antigen may not be detectable in patients presenting late after the development of symptoms and other dengue serological tests should be used in these patients. It also does not differentiate between primary and secondary infection.

The Panbio Dengue Early Rapid is a dengue NS1 antigen immunochromatographic assay (Fig. 2). It is

Fig. 2. Panbio Dengue Early Rapid, an immunochromatographic assay for detection of NS1 antigen
Source: AKU/Binish Arif

for the qualitative detection of NS1 antigen in serum, used as an aid in the clinical laboratory diagnosis of patients with clinical symptoms consistent with dengue fever. This test is available in the microbiology section of the Aga Khan University Laboratory.

Earlier diagnosis of dengue allows earlier implementation of supportive therapy and monitoring. This reduces the risk of complications such as DHF or DSS, especially in countries where dengue is endemic.

Saline Infusion Hysterosonography to Evaluate Abnormal Uterine Bleeding

Dr Shaista Afzal, Dr Imrana Masroor, Dr Gulnaz Shafqat
Radiology Department

Introduction

Abnormal uterine bleeding includes menorrhagia or poly menorrhea, which are explained as a long duration of menstrual flow, excessive, heavy or profuse bleeding, frequent periods, short cycles, spotting or inter menstrual bleeding. The common causes of abnormal uterine bleeding excluding the hormonal and obstetrical reasons are uterine polyps, fibroids, atrophy, hyperplasia and carcinoma of the endometrium.

It is important to evaluate and treat abnormal uterine bleeding because it may have serious medical consequences and because excessive or prolonged bleeding may cause undue disruption of women's daily activities. Approximately, 70 per cent of peri and post menopausal gynecologic consultations are related to abnormal uterine bleeding.

Though, Dilation and Curettage (D & C) used to be the procedure of choice, outpatient endometrial biopsy has been demonstrated as an efficient and effective replacement for D & C.[ii] Although, it is convenient and safe, it is a blind sampling procedure and there is concern about the non-representative nature which may be related to the small proportion of the endometrial surface sampled and the non-sampling of focal intra uterine lesion.

It has been suggested that failure to diagnose intracavitory lesions could be reduced or even eliminated by complementing endometrial sampling with outpatient hysteroscopy which is now considered the gold standard and is recommended in women with abnormal uterine bleeding by evidence based guidelines. However, it is expensive and invasive as compared to ultrasound based tools and in 50 per cent of women it is in retrospect unnecessary on account of normal uterine cavity.

Transvaginal sonography is a valuable diagnostic tool in evaluating women with abnormal uterine bleeding as it can detect anatomic lesions that were not evident on physical examination, endometrial thickness and the ovulatory and hormonal status. Presence of thickened endometrium suggests possible masses However, it is not useful in determining the exact location of the masses. Saline infusion sonohysterography (SIS) is a simple examination that yields additional information of the uterus over TVS. By virtue of distention of uterine cavity it allows detailed visualization of the endometrial lining and possible intracavitory masses.

SIS is more cost effective, less invasive, less time consuming and more emotionally tolerable for the patient. No hospital stay is needed as the procedure is completed in less than an hour. Abnormalities such as endometrial polyps, endometrial hyperplasia, sub mucosal leiomyomas and endometrial carcinomas can therefore be detected with precision.

The reported sensitivity and specificity of SIS are 0.95 and 0.88 respectively with post test probabilities of 0.91 and 0.07. The sensitivity of hysteroscopy is 0.84-0.97 and specificity is 0.88-0.93. TVS and SISare cost effective as compared to diagnostic hysteroscopy. Evaluation of the clinical impact of SIS revealed that it added certainty to the diagnosis in 88 per cent of the patient studied and changed the patient's treatment in 80 per cent of cases. Normal SIS findings markedly increased diagnostic confidence by 86 per cent.

Procedure

The procedure is explained to the patients in detail and informed consent obtained by the radiologist performing the procedure.

Patients examined in lithotomy position after voiding. A preliminary TVS is done; uterus examined longitudinally and axially. Sonohysterography will be performed at the same time; a 5-7 French sonohysterography catheter will be used and advanced until the balloon has crossed the internal os. (Fig. 1) The balloon inflated with 1-3cc of normal saline depending upon the tolerance of patients and os size. 10-15 cc of saline will be infused into the uterine cavity which will act as a negative contrast media. Uterus will be scanned in longitudinal and axial plane. (Fig. 2) Patient will be allowed to go home if she has no complaints.

Conclusion :

SIS is a reliable and effective method for the diagnosis and assessment of uterine lesions without adverse effects and compliments the findings of TVS and helps to distinguish different types of endometrial lesions to reduce unnecessary surgeries and invasive diagnostic investigations.

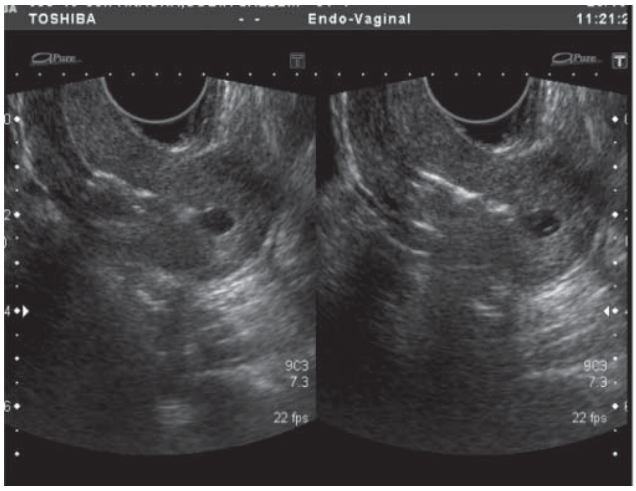


Fig. 1. Catheter within endometrial cavity

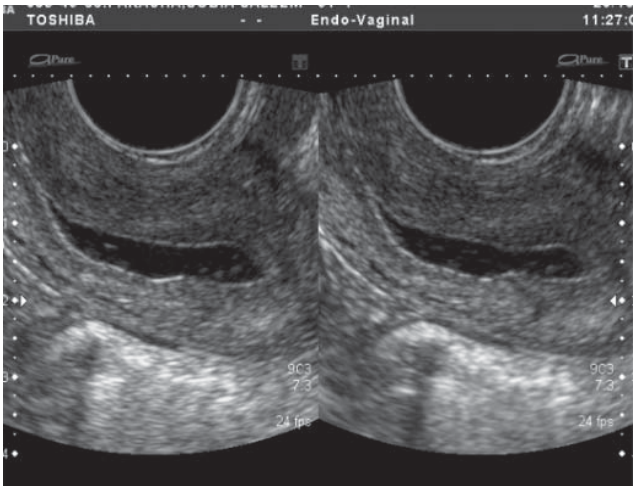


Fig. 2. Fluid within endometrial cavity showing smooth outline of endometrium without any abnormal thickness or focal lesion. Normal SIS Endometrial cavity clearly outlined by saline.

Answers to Biosafety Quiz

Fig. 1: Working without gloves, dupatta outside lab coat, lab coat sleeve not covering street clothes

Fig. 2: Front grill of Biosafety cabinet blocked, working outside of Biosafety cabinet, loose hair

Fig. 3: needle being recapped

Fig. 4: Mouth pipetting

Fig. 5: Pipetting from height

Fig. 6: Syringe with needle discarded in red waste bag instead of sharp container

Fig. 7: Open toe shoe not to be worn in lab

Fig. 8: Vortexing tube without cap, generating aerosols

Microbiology Art

Microorganisms produce beautiful colours and pigments when grown in laboratory. These images have been produced by Microbiologists using colourful fungi and bacteria. They highlight the beauty of nature even evident in microscopic creatures.

Source: http://blogs.jcvi.org/2010/12/holiday-art/

Source: http://www.elon.edu/e-net/Note.aspx?id=956820
Source: http://www.newscientist.com/gallery/microbe-art/10

Workshop on "Diagnosis of Fungal Infections in Clinical Laboratory"

Ms Raunaq Mehboob and Dr Muhammad Rawala
Microbiology

Participants of the workshop
Source: AKU/Faisal Malik

A Workshop on "Diagnosis of Fungal Infections in Clinical Laboratory" was organized by Clinical Laboratory, Department of Pathology and Microbiology, Aga Khan University Hospital, Karachi from 3rd to 6th September 2012. The venue of this workshop was Microscopy Laboratory, Aga Khan University Hospital, Karachi. This workshop was supported through grants from the Higher Education Commission and United States Agency for International Development (HEC/ USAID). The workshop director was Professor Afia Zafar and workshop coordinators were Dr. Joveria Farooqi, and Dr. Kauser Jabeen from Section of Microbiology, Department of Pathology and Microbiology, Aga Khan University Hospital, Karachi.

A total of 37 participants were invited from throughout the country and the audience included consultant microbiologists, trainee residents and technical staff from different teaching hospitals and laboratories from various cities of Pakistan including Karachi, Quetta, Peshawar, Lahore, Rawalpindi and Islamabad. The facilitators included faculty from Microbiology and Histopathology sections, Medicine, Aga Khan University and Dr. Mary Brandt, Chief of Mycotic Diseases Branch at Centers of Disease Control and Prevention, Atlanta.

It was a four day hands-on workshop with an objective to increased awareness amongst consultants, residents and technical staff about proper handling and processing of fungal samples in order to diagnose fungal infections in clinical laboratory. During the course of the workshop, various lectures and practicals were demonstrated as well as performed by the participants in order to achieve the goal of the workshop. Participants were evaluated by pre and post workshop single best answer test.

Participants during the practical sessions
Source: AKU/Faisal Malik

Clinical Laboratory, Soparivala Building, Aga Khan University Hospital, Karachi

hospitals.aku.edu/Karachi/pathologymicrobiology