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PRESIDENT'S MESSAGE

Dear Members,

Wish you all a happy 2023. We as clinical microbiologists need to intensify our efforts towards effective patient care by actively implementing infection control measures.

With rapidly evolving multi drug resistant organisms and non-availability of new antibiotics the onus is on the current generation of clinical microbiologists to prevent the pandemic of AMR.

My best wishes to all the contributing authors of this newsletter.
Hearty congratulations and best wishes to Dr Ranganathan Iyer and team for the forthcoming XXVI IAMM TAPC chapter conference.

Dr. I. Jyothi Padmaja,
Professor of Microbiology & Principal,
GITAM Institute of Medical Sciences and Research,
Visakhapatnam

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SECRETARY'S MESSAGE

Dear Members,

I, Dr Mohammed Khaleel welcome you to the 26th Annual IAMM TAPC State Conference, Hyderabad City and thank all the Life members of IAMM TAPC State for placing your trust in me and bestowing upon me the honour and privilege of serving the Association as the Honorary Secretary General.

Registration of Association this year, an important milestone of gloriously completing 26 years. It is undoubtedly a remarkable occasion that calls upon us all to appreciate and rejoice at the sublime deeds done by our predecessors in building the organisation to its present stature. It is also an occasion that calls for a celebration of the past, present, and work towards inspiring the future. I am fortunate to serve this association in this milestone year and I hope we can continue the legacy of our predecessors and ensure that IAMM TAPC scales further heights.

Past members – 357 before our executive taking as charge (September 2021)

By the GIMSR Conference was held – Life Members - 517 (Sept. 10th 2022) Pro-Life PG Memberships - 41

Post 25th GIMSR Conference Life Membership State -73; Pro -life PG Memberships - 38

Presently State Life members – 590; Pro -life PG Memberships- 79 (As on February 2023)

E-Certificate has been shared with all life members. Planning to issue with Photo on Certificate from here.

Regular ANNOUNCEMENTS are being shared by Executive committee through GROUP email or WhatsApp groups concern academic programme

Inclusion of Mock PG Exam session in Pre-Conference Workshop has been implemented as promised.

Roll down of UG QUIZ in Both States nominating a coordinator under the banner of our Association

Certificate for Dr Rajalakshmi Oration made and sponsored by association as Promised.

Inauguration function has been done one day prior in evening of the Conference to encourage and justify the time for presentation of paper/poster and other academic events

General body meeting conducted in Pre-lunch session as promised, to encourage participation of all life members for important decision making and involvement.

Association members shall read the Biodata of oration and prize papers

Newsletter will be uploaded on website post Conference. Soft copy will be circulated through Group mail.

Panel discussion: Inclusion of Implementation of CBME for UG and PG on Challenges and Solutions with Expert Panellist has been an innovative at this Conference.

It's my honour and privilege to work with Senior faculty (Dr Nagamani madam, Dr Jyothi Padmaja madam, Dr Ranganathan Iyer Sir) and our committed EC members (Joint Secretary – Dr Vijender/Executive members- Dr Lakshmi Jyothi, Dr Sudha Madhuri, Dr Uma Rani, Dr Shabnum).

The presence of Dr Animireddy Kishore, Honorary Treasurer, has an added advantage to ensure smoother and efficient functioning of the Association.

I wish to acknowledge the achievements of our EC whose steady and pragmatic leadership has helped our glorious association sail through and take the responsibility for PG Quiz, PG Write up, Pg Pedagogue, News Bulletin articles etc

IAMM TAPC, EC invites the active participation and involvement of all members of combined State.

IAMM National body, President Dr Arti Kapil, AIIMS New Delhi and Secretary Dr Sonal Saxena, MAMC, New Delhi has insisted that PG Master Class Series, initiated and well organized monthly, shall be attended by Post Graduates of both States including Faculty.

Request to all from both States to enrol for National Life membership

Last, but not the least, I express my gratitude towards the academic events conducted across both states initiated by our esteem faculty

Dr Gayathri. V- CME on sepsis and Anti-microbial resistance on 13th August 2022 at Vizag;

Dr Wajid & Dr Shazia Naaz, ESI, Sanathnagar, Hyderabad- Post Graduate Quiz on Acute Undifferentiated Viral Febrile Illness- Navigating the diagnostic Challenges Online platform on 27th August 2022

Dr Shazia, Bhaskar Medical college under the guidance of Dr Rama Rao Sir conducted Essay writing Competition for Post Graduates on International Microorganisms Day 17th September 2022

Dr Abhijit Choudhury- IATP Combined State Conference at Tirupati on 11th & 12th November 2022.

Dr Manic Dass, AIMSR, Hyderabad - Undergraduate Quiz on occasion of World AIDS Day 1st December 2022

Dr Sumit Rai, AIIMS, Mangalagiri - conducted 1st National Advanced Hands-on workshop on Antibiotic stewardship Practices for faculty and Post Graduates on 22nd- 23rd Feb 2023.

Let us remember with love and respect all our members we lost recently. I am thankful to Senior faculty who have been super annuated and who have been Promoted at their respective workplace. I, express my Gratitude to Organising Committee under the leadership of Dr Ranganathan Iyer Sir for his optimistic thoughtfulness and conducting grand 26th IAMM TAPC State Chapter Annual Conference at Hyderabad!

Best regards,

Dr Mohammed Khaleel,
Professor & HOD, Microbiology,
Mahavir Institute of Medical Sciences, Vikarabad,
Secretary General, IAMM- TAPC Chapter.

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CLINICAL MICROBIOLOGY LABORATORY IN THE ERA OF POINT OF CARE & INSTRUMENT FREE DIAGNOSTIC ASSAYS

Dr. V. Lakshmi, **Professor, Department of Microbiology, Kamineni Academy of Medical Sciences & Research Centre, Hyderabad, lakshmi57vemu@gmail.com**

Introduction:

Health care policies are heterogeneous worldwide, but a global effort is striving to achieve a higher value of care delivery. Towards this end, Clinicians and the patients are demanding rapid, accurate and quality assured results.

With the everlasting threats by emerging and re-emerging infectious diseases, a justified need for sample-in-result-out, on site analysis (within a clinically useful time frame of less than an hour) for immediate clinical decision, has never been so demanding unlike in earlier times. This rising need for rapid diagnostics in the healthcare sector has led to advanced research and development of numerous rapid and simple diagnostic technologies and devices.

Need for Rapid assays & POCTs:

Despite the global advancements in laboratory-based clinical diagnostics technologies, developing countries, such as India, are facing problems in obtaining high-end, automated and expensive instruments, skilled professionals, appropriate infra structure, all which add up to the overall cost of diagnosis. The inaccessibility to advanced medical technologies is ultimately burdening the end-user i.e., the patient.

There are several ways to accelerate microbiology results by rapid, real time and random access. These include

- Direct detection by Automated systems,
- Culture free Real time Molecular assays
- Rapid & simple bench side Point of Care tests (POCTs).

Definition of a POCT:

Although there are numerous definitions, an insight into POC testing defines it as a diagnostic aid that will result in a clear and actionable management decision, such as when to start treatment or to require a confirmatory test, within the same clinical encounter.

Innovations in biotechnology, molecular biology, micro-fabrication (Cartridge based automated Real Time PCR) and bioinformatics, have paved a way for the development of ideal Next Generation POCTs that are generally instrument free, rapid bench side and highly performing assays with least limit of detection and easy to interpret.

The POCTs have enhanced the diagnostic capacity for several important diseases and disorders. In contrast to the traditional remote laboratory-based tests that can take days for a result, the POCTs assure the key objective of an immediate diagnostic feedback.

Also, they are simple to perform, have an insignificant risk of delivering erroneous results and require reduced regulatory oversight.

They offer a great opportunity for testing outside the physical facilities of the clinical laboratories. And hence have a wide utility in intensive care units to outpatient clinics to personal / home care.

- Bedside testing,
- Near-patient testing,
- Physician office-based testing,
- Decentralized testing,
- Off-site testing,
- Ancillary/alternative site testing,
- Testing performed by non-laboratory trained personnel.

To bring in standardization in the general framework for POCT development, the WHO proposed the "ASSURED" criteria, for every POCT:

- Affordable, Sensitive, Specific, User friendly, Rapid and Robust, Equipment-free, and Deliverable to end users.

Since it is essential to provide a greater understanding and flexibility on how POC testing should be applied in nontraditional laboratory environments, the working construct of POCTs should address the "test target profiles" (TTPs) of a POCT. These define the user, the location and test complexity. Each variable covers a spectrum, from simple to sophisticated, layperson to hospital and laboratory staff, and home to in-patient hospitalized patient. (Figure 1).

Types of POCTs:

POCT devices come in both handheld and benchtop formats based on the technology and the environment where the device would be used.

1. The 1st Generation POC tests (Rapid Diagnostic tests) were based on immuno-chromatographic or agglutination technologies.

2. With the miniaturization and full automation of molecular methods, quicker real-time PCR-based 2nd generation POCTs, mainly for the rapid detection of pathogens, have been developed. Integrated NAATs for detection of disease-causing bacteria and viral agents along with growing emphasis on management of epidemics, have dominated the NAAT market, in recent times.

Two essential components offered by integrated NAAT tools are:

1) efficient nucleic acid extraction technologies for diverse & complex sample types.

2) robust and sensitive Isothermal NA amplification & detection technologies. allowing a rapid and simple sample-in to result-out single platform devices.

In the years 2019 – 2020, the NAAT segment accounted for a large share in the Global market. The Isothermal Nucleic Acid Amplification Technology (INAAT) market is estimated to surpass US$3,622 Million, with a Compound Annual Growth Rate (CAGR) of 8.3% by 2027.

3. Advancements in biosensors, microfluidics, bioanalytical platforms, assay formats and lab-on-a-chip technologies have triggered the development of the 3rd Generation POCTs, available as Hand-held devices using technologies that are beyond genotypic assays.

Generally, a POCT result interpretation is either a visual readout or carried out by automatic multiplex analytical tools without the need for an expert technician. Integrating with appropriate software, the results can be stored in the medical database and shared with healthcare workers through a cloud server, which benefits the patient as well as the clinicians.

Syndromic POCTs with a specific menu of deadly pathogens along with the most frequent and highly contagious pathogens, contained in an appropriate format along with prelabeled sampling tubes and containers, are now available commercially (e.g. BIOFIRE). The summarized sampling instructions provided, are user-friendly for even an untrained technician.

Developments in POC diagnostic devices

Recent advancements in the field of biosensing, microfluidic and paper-based technology have improved the quality and efficiency of diagnostics, applicable in remote and resource-limited areas.

Potential improvements that are being explored in the context of improving multiplexing capabilities and include

- simplifying the microfluidic design and fabricating using a lower-cost material, such as paper and
- using POC-compatible detection technologies (colorimetric, turbidity, fluorescent, electrochemical) are being

Advancements in the area of paper-based diagnostic devices have led to the development of simple POCT platforms, wherein the multistep fluidic processes can be carried out by means of wicking activity of an absorbent paper material. POCTs, with all the 4 major steps for a

nucleic acid amplification test (NAAT), such as extraction, purification, amplification and detection of the NAs integrated and performed on a single paper platform, have been developed. These promising technologies are being translated and commercialized for diagnostic purposes.

Recently, POC detection methods have been trending towards colorimetric assays, often dependent upon changes in pH or oxidation, with an advantage of increased reaction rate. Such systems are particularly well suited for resource-limited areas due to their low cost, short assay time and visual readout.

But the real breakthrough is the application of smartphone-based biosensors to POC devices. With an ability to harness the embedded CMOS optical sensors, Bluetooth connectivity and capacity for networking, optical signal acquisition and data-processing and an app-based user interface, the capability of the mobile phone is being extended to design sample-to-answer NAAT and Lab-on-a chip assays. For e.g., as in the Cepheid's Gene expert system, a Bluetooth-enabled cartridge-processing unit facilitates complete process integration from sample lysate, isothermal amplification to detection by optical imaging using optics, connected to the device. The data is acquired and analyzed digitally.

Clinical Microbiology laboratory & POCTs

It is clear that, with the advent of POCTs and instrument free molecular assays, a radical change has been made in Clinical and diagnostic microbiology over the 21st century.

The question & Concern:

Is the Clinical Microbiologist threatened by automation & advanced technologies? Will POCTs replace conventional Clinical Laboratories?

The discipline of Clinical microbiology is currently in transition and standard-of- testing is now a hybrid of old & new methodologies. With substantial changes and the ongoing technological revolution, a Clinical Microbiology laboratory has moved on from Pasteur to PCR and to a Lab in a tube platform. The continuously changing spectrum of assays has rapidly transformed research, diagnostic and therapeutic tools.

Development & deployment of new tests is a part of overall effort to improve
- ***Public health services* to *facilitate control of infectious diseases***

Present day and budding clinical microbiologists should adapt to POCTs, because these are the assays of the future.

The goal of a clinical microbiologist should be to assist the clinicians in significantly shortening the process from empirical to evidence-based treatments.

- The present-day Clinical Microbiologists need to look into the crystal ball & speculate the challenges of the future.
- They should develop & master technological advances and find niches for their skills to deliver a patient-focused service

In the era of advanced technologies, the responsibility of Clinical Microbiologists is also to verify & monitor analytical performances, such as Quality Control and method development & comparisons. **The vison, mission and goal should be towards an integrated & cohesive, quality assured Microbiology service.**

The combination of a repertoire of geographic infections, the syndromic approach, and the versatility of microorganism testing and remote validation are the steps in this clinical microbiological revolution.

There will always be a need for highly equipped laboratories to identify the repertoire of microorganisms as well as their sensitivity to antimicrobial agents. However, it is likely that these sites will be limited in number and will provide regional poles of reference and infectious agent repertoire monitoring.

And once the repertoire has been established and detection tools are in place, very simple POC molecular tools may be sufficient to allow medical staff to answer simple questions, such as the rapid detection of antimicrobial resistances.

Mobile Microbiology Laboratory & the Next generation Microbiologists:

The evolving changes in test services & methods along with a shift from
- centralized to decentralized POC facilities and
- single-analyte testing to multiplex testing

will enforce the Next generation Microbiologists to grow & keep pace with advanced technologies and move into a mobile microbiology laboratory or a Diagnostic Box.

This pocket-sized yet fully automated Microbiology laboratory, shall be able to inform the clinicians, within minutes, whether the patient had contracted a life-threatening infection.

Are You not fortunate to be a part of that future!

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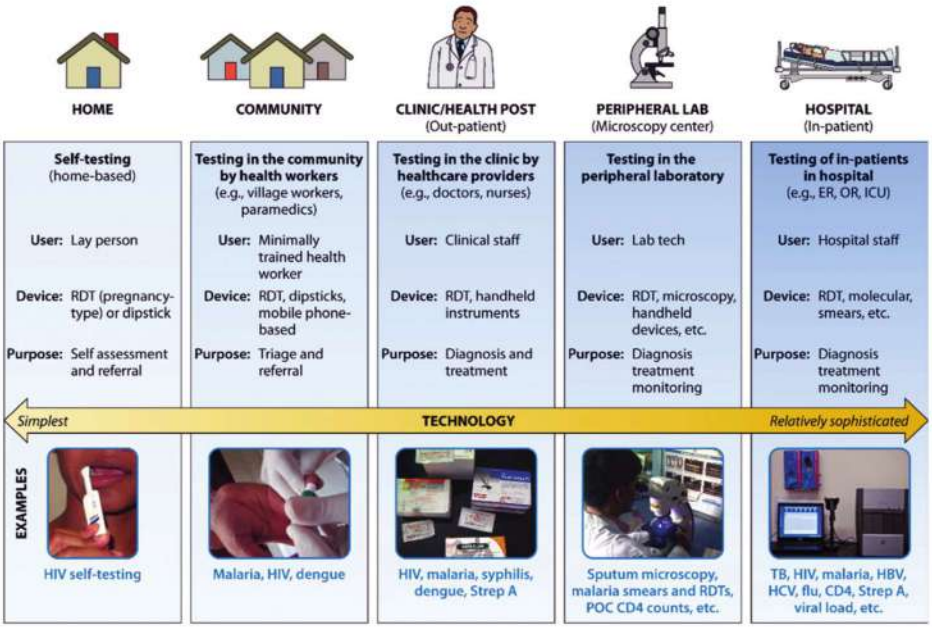
The spectrum of point-of-care testing



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IMMUNOLOGY OF HELMINTH INFECTIONS

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Introduction

More than 1.5 billion people, or 24% of the world's population, are infected with soil-transmitted helminth infections. Similarly lymphatic filariasis affects 90 million people in 73 countries and Schistosomiasis causes more than 200 000 deaths/yr while food borne trematodes causes 200000 illnesses and more than 7000 deaths annually.

The mammalian immune system has evolved to cope with immense microbial presence. The helminths have coevolved with their host for a long time. This has led to a strict adaptation which enables them to settle and persist in the host by modulating the host immunity so as to ensure their survival with minimum harm to the mammalian host. This is done by immune evasion, mimicry, and induction of host immunoregulatory pathways.

Immunity to Helminth Infection: The Rejection Phenomenon

A. Role of Innate Immunity: The innate cell populations act as the initiator of immunity strategy. Following infection, epithelial cells start producing 'alarmin' cytokines (IL-25, IL-33 and Thymic stromal lymphopoietin or TSLP) which stimulates innate lymphoid cells (ILCs). ILC-2 start producing type 2 cytokines: IL-4, IL-5 and IL-13. Expansion of basophils is also linked with IL-4 production.

TSLP is an IL-7-like cytokine and a critical factor linking responses at interfaces between the body and environment (skin, airway, gut, ocular tissues, and so on) to Th2 responses. It is highly expressed in the epidermis (epithelial cells and epidermal keratinocytes). Various other cell types: mast cells, airway smooth muscle cells, fibroblasts, dendritic cells also express TSLP.

As a result of the alarmin response, granulocytes are rapidly activated and recruited to sites of infection where they are important producers of Th2 cytokines such as IL-4 and IL-13.

Granulocytes can attack helminths through antibody- dependent cell mediated cytotoxicity (ADCC). The killing of antibody coated parasites is affected via the release of cytotoxic granules like major basic protein (MBP), eosinophil peroxidase (EPO), and eosinophil cationic protein (ECP), all of which are potent helminth toxins.

B. Role of Adaptive Immunity: The canonical immune response to lymphatic filariasis is that of Th2 response coupled with the production of key cytokines. The innate immune response creates an environment that favours the induction of Th2-type responses. The adaptive Th2 response also requires classical MHC class-II-mediated antigen presentation by dendritic cells. $CD4^+$ Th2 cells then drive a suite of type 2 anti-parasite mechanisms: Class-switched antibodies, activated leukocytes and innate defence molecules. The important cytokines involved include IL-4, IL-5, IL-9, IL-10, and IL-13 while the antibody response consists mainly of IgG1, IgG4 and IgE types. Most of the IgE produced is polyclonal type indicating a non- antigen specific induction of IgE producing B cells. IgE production is absolutely dependent on IL-4 or IL-13. IgG4 antibodies compete with IgE for binding sites and thus obstruct the protective activity of IgE. Elevated levels of antigen specific IgG4 is directly linked with parasite survival. This clearly suggests that the ratio of IgG4: IgE immunoglobulin is crucial to infection phenotype.

The ADCC also plays a crucial role in adaptive response and it is dependent on eosinophils, neutrophils, macrophages, or platelets as effector cells and IgE, IgG or IgA antibodies. The parasitic structures get covered by antibodies and are destroyed by cells and it also leads to immobilization of nematode larval stages in gut.

Nitric oxide (NO) is also toxic to the worm and is released by the macrophages classically activated by IFN-γ and TNF-α. This mechanism has been described mainly against trematodes (*Schistosoma sp., Fasciola sp.*) during acute infection and is up-regulated by Th1-type cytokines and down-regulated by IL-10 produced by T regulatory cells.

Intestinal anaphylaxis, with IgE-induced mast cells degranulation is responsible for changes in the intestine physiology, architecture, and chemistry of the gut epithelium. This includes stimulation of fluid, electrolyte and mucus secretion, smooth muscle contractility, increased vascular and epithelial permeability, and recruitment of immune cells such as eosinophil or mast cells. This can lead to rapid elimination of the larvae in GIT, before they reach their tissue niche, and also to expulsion of the adult. Furthermore, IgA on the surface of the gut mucosa helps to neutralize the metabolic enzymes released by helminths and interfere with the worms' ability to feed.

C. Mechanism of elimination: Resistance to infection and immune clearance of helminths depend on the orchestration of multiple pathways. Despite their essential role in driving protective immunity to helminths, T cells cannot directly damage the parasites – they remain armchair generals conducting attacks through remote means. Many of the effector pathways are ancient innate defence mechanisms. These mechanisms achieve three effects: DISABLE, DEGRADE, DISLODGE.

Disable: It restricts parasites' growth and motility, and reduce their overall fitness and ability to reproduce. It is mediated through antibodies which neutralise key physiological functions, together with innate defensin-like molecules. Amino acid deprivation through macrophage-expressed arginase may also retard larval development.

Degrade: This causes cumulative damage to parasite integrity. Attack by the granulocytes, which may be guided by specific antibodies and/or amplified by complement components and other serum factors cause extensive damage to the worm. Elevated mast cell or eosinophil responses are also able to exert strong anti-parasite effects, in addition to nitric oxide from macrophages and neutrophils which can harm tissue larvae.

Dislodge: It is mainly applicable to Intestinal parasites. Dislodgement is primarily achieved by making the chosen niche unsuitable for the worm. IL-25 and IL-13 play a critical role here by promoting changes in intestinal function and more rapid cell turnover (the 'epithelial escalator'). IL-13 also induces a switch in intestinal mucins from the dominant Muc2 to a form not normally expressed in the intestine, Muc5ac, which is required for normal expulsion. In addition, there is increase in epithelial permeability with increased fluid transfer. Furthermore,

IL-4Rα-mediated signalling activates smooth muscle cells, leading to hyper-contractibility during infection.

Immunomodulation (Down Regulation) and Chronic Infection:

Helminths appear to act as successful xenotransplants into the mammalian body, neutralizing immune pathways that would otherwise expel them and resetting the thresholds of immune reactivity. The main mechanisms of immune evasion include immunosuppression, immunological tolerance and modification of stereotypical Th2 responses. The establishment of a stable chronic infection confers a survival fitness to the parasite. As a result, the infected subjects display a state of immune hypo-responsiveness that can be considered a form of immunologic tolerance.

In the immune system, homeostatic tolerance to self-antigens and harmless environmental antigens is primarily maintained by an immunosuppressive T-cell subset, the regulatory T (Treg) cell. A strong link has emerged between long-term helminth infection and Treg cell activity, particularly in the asymptomatic or hyporesponsive state. The causal links between helminth infections and Treg cells have now been established in both directions. First, certain helminths directly drive Treg cell responses from the host or do so indirectly by inducing host cells to produce TGF-β, which is a key cytokine that promotes regulatory cell function. Secondly, the Treg cells are essential for parasites to survive in the immunocompetent host because their depletion results in clearance of the infection. In effect, Treg cells establish hypo-responsiveness in the effector population

Based on the expression of the transcription factor Foxp3, two Foxp3+ subsets have been identified: thymus-derived (tTregs) and the peripheral (pTregs). In addition to the Foxp3+ Tregs, two other subsets that do not express Foxp3 have been described based on the regulatory cytokines expressed by those cells. These include the type1 regulatory T cells (Tr1) that express mainly IL-10; and the TGF-β expressing Th3 regulatory T cells. Although IL-10 and TGF-β were originally thought to be produced by Th2 cells and can be produced by various cell types including regulatory T cells, it has been shown that the major sources of IL-10 and TGF- β are Tr1 and Th3 respectively.

The IL-10 and TGF- β directly suppress immune responses and indirectly regulate not only the antibody response to helminth antigens but also the function of antigen presenting cells . The activity of Treg cells and production of IL-10 correlate closely with an isotype switch from the pro-allergic/inflammatory IgE to the non-inflammatory IgG4. Thus, in the modified Th2 response, antibody isotype switching to the non-inflammatory isotype IgG4 and induction of alternatively activated macrophages occurs.

Macrophages in patients with helminth infection are profoundly altered in their profile, adopting an alternatively activated phenotype (also termed AAM or M2). These macrophages adopt a pattern of gene expression, metabolism, and function markedly different from that of classically activated (M1) macrophages. AAM are activated by the Th2-type cytokines IL-4 and IL-13 and they drive CD4+ Th2 responses, deviating the immune system from inducing a pro-inflammatory Th1 response that could be detrimental to parasite survival. These M2 cells also cause enhancement of Treg cell differentiation and suppress the immune response against the parasite, promoting anergy and/or tolerance.

The immunomodulation induced in the host can act as a double-edged sword. The host can benefit from suppression of collateral damage during parasite infection and from reduced allergic, autoimmune, and inflammatory reactions. However, it can also be detrimental to the host in reducing vaccine responses, increasing susceptibility to co-infection and potentially reducing tumour immune-surveillance.

Conclusion.

- **a.** Natural immunity or elimination of the parasite is mainly affected by granulocytes and innate lymphoid cells. Th2 adaptive response includes IgE, ADCC, and cytokines like IL-4. IL-13. Th2 response also re-inforces innate immunity.
- **b.** The Immunomodulation of infection is marked by immune evasion and immunosuppression. There is modified Th2 response, IgG4 expression increases and IL10 and TGF-β production also rises, Alternatively Activated Macrophages and T Regulatory cells (Tr1, Th3) play important role in immunomodulation.

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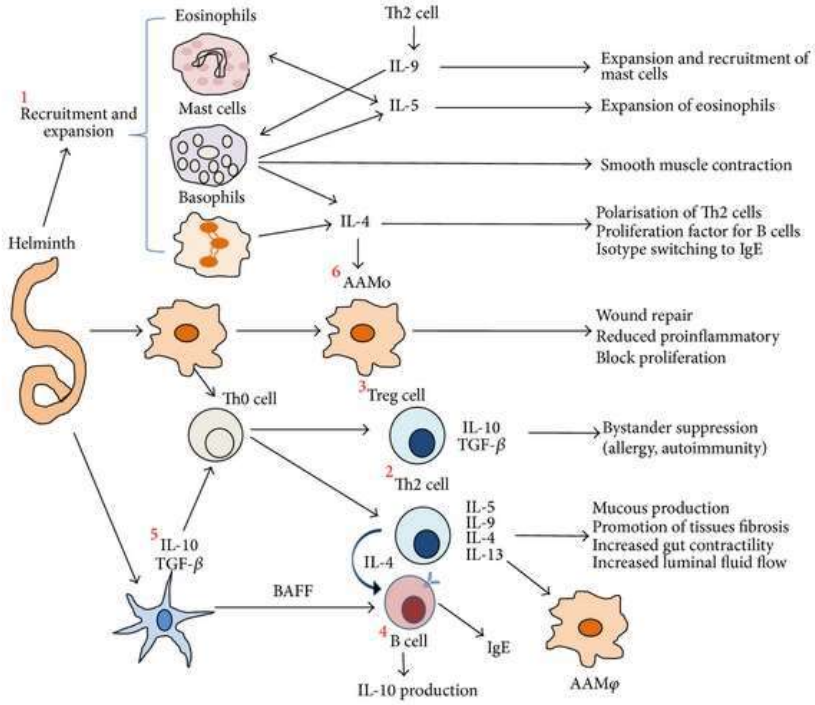

Helminth infections are strong inducers of a Th2-type immune response. These infections are characterized by the expansion and activation of eosinophils, basophils, and mast cells (1). Their upregulation due to high levels of Immunoglobulin E (IgE) and the proliferation of T cells that secrete IL-4, IL-5, IL-9, and IL-13 are part of the host immune response against the parasite (2). However, helminth infections tend to be long-lived and largely asymptomatic because helminth infections are sustained through a parasite-induced immunomodulatory network, in particular through activation of regulatory T cells (3) and systemically elevated levels of IL-10 produced by B regulatory cells (4). They are additionally affected by the expression of the regulatory cytokines IL-10 and TGF-*β*, produced by regulatory dendritic cells (5) and alternatively activated M (AAM) (6). *(From: Salazar-Castañon VH, Legorreta-Herrera M, Rodriguez-Sosa M. Helminth parasites alter protection against Plasmodium infection. Biomed Res Int. 2014; 2014:913696. doi:10.1155/2014/913696*)

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IMPORTANCE OF MICROSCOPY IN DIAGNOSTIC MICROBIOLOGY

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In spite of many advanced techniques at molecular level, microscopy gives very valuable information in diagnostic microbiology. Microscopy alone helped in the discovery of many parasites and remained as milestones in microbiology. If we recollect the history of discovery of plasmodium parasite as causative agent of malaria by Ronald Ross and demonstration of parasite by Laveran, it is by microscopy alone. Many such examples in the history make us proud to be a microbiologist. In every day practice in laboratory to see the direct smears, wet mount preparations and interpret the reports makes us proud microbiologists.

It may not be so exciting always when you see some artifacts and contamination in cultures. But when we see some anaerobic bacteria or fastidious bacteria which does not grow in routinely used culture media, it will give lot of information. It will allow us to check the quality of sample.

Some diagnostic dilemmas which are usually faced:

Gram variable organisms:

1. Gram positive organisms may lose their ability to retain crystal violet and stain Gram negatively for the following reasons:
    a. Cell wall damage due to antibiotic therapy or excessive heat-fixation of the smear.
    b. Over-decolorization of the smear.
    c. Use of an iodine solution which is too old, i.e., yellow instead of brown in colour (always store in a brown glass or other light opaque container).
    d. Smear has been prepared from an old culture.
2. Gram negative organisms may not be fully decolorized and appear as Gram positive when a smear is too thick.

Note: Always reagents and stains should be checked for quality whenever new batch is prepared.

Acid fast staining:

1. Saprophytic mycobacteria also are acid fast. Here morphology of mycobacterium tuberculosis will help us to differentiate it from saprophytic mycobacteria. Acid alcohol fast stain also can be used for differentiating *Mycobacterium tuberculosis* from other mycobacteria.

2. Modified acid fast staining: Fat globules in stool sample are also acid fast and mimic cryptosporidium oocysts. Morphology of oocysts helps us to differentiate. Size of the fat globules are not uniform whereas size of oocysts is uniform

Artifacts usually seen in Urine wet mount:

Crystals: These have a characteristic refractile appearance. Normal urine contains many chemicals from which crystals can form, and therefore the finding of most crystals has little importance. Crystals should be looked for in fresh urine when calculi (stones) in the urinary tract are suspected. Crystals which may be found in rare disorders include:

Cystine crystals, which are recognized by their six-sides They are soluble in 30% v/v hydrochloric acid (unlike uric acid crystals which they may resemble). They can be found in cystinuria, a rare congenital metabolic disorder in which cystine is excreted in the urine. Cholesterol crystals look like rectangles with cut-out corners. They are insoluble in acids and alkalis but soluble in ether, ethanol, and chloroform. They are rarely found except in severe kidney disease or when a lymphatic vessel has ruptured into the renal pelvis. Tyrosine crystals, which are yellow or dark colored and look like needles massed together. They are insoluble in ethanol, ether, and acetone. They are occasionally found in severe liver disease.

Other crystals found in urine

Occasionally sulphonamide crystals are found in the urine of patients being treated with sulphonamides. When deposited in the urinary tract they can cause haematuria and other complications. Triple phosphate crystals are occasionally found in alkaline urine. They have little or no clinical significance. Calcium oxalate crystals are frequently seen. When found in freshly passed urine they may indicate calculi in the urinary tract. Uric acid crystals are yellow or pink-brown. They can sometimes be found with calculi.

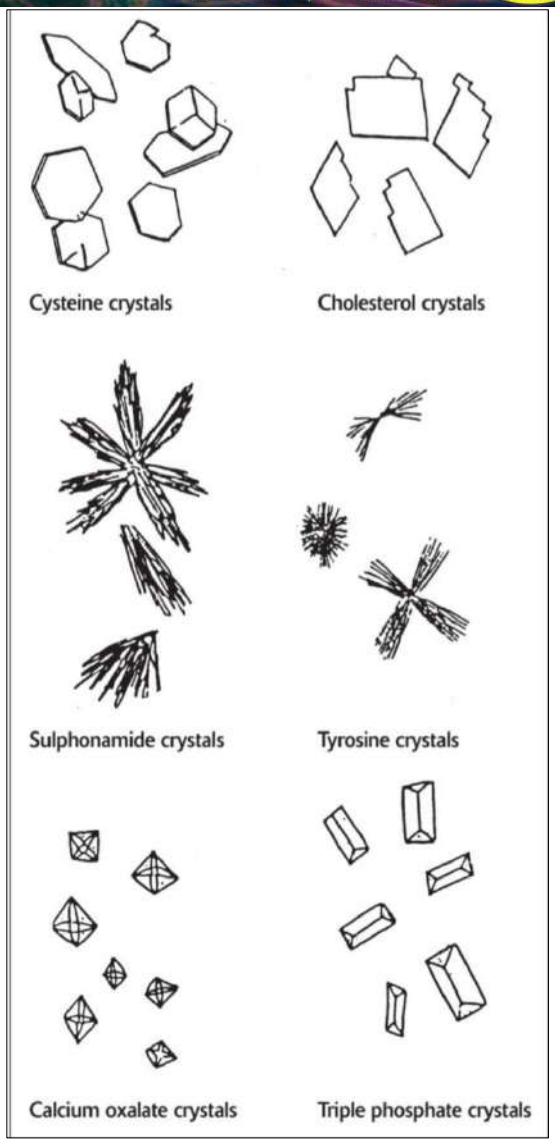
Spermatozoa: Occasionally found in the urine of men, they can be easily recognized by their head and long thread-like tail. They may be motile in fresh urine.

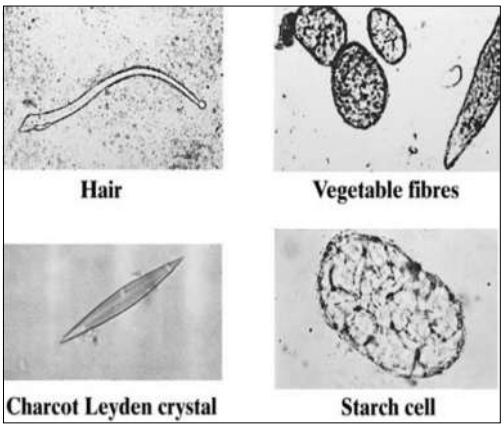
Contaminants which can be found in urine

These include cotton fibres, starch granules, oil droplets, pollen grains, moulds, single-celled plants (diatoms) and debris from dirty slides or containers.

Stool wet mount for parasites:

There are many normal structures which are found in stool examination which should not be reported as parasites. Starch cells, muscle fibres, vegetable fibres, pollen grains, fatty acid crystals, air bubbles, spores, hair are some examples. It is very important to observe the size and shape of the structure and report with caution. Red blood cell size (7 μ) can be taken as control for measuring the approximate size of parasitic forms.


Cysteine crystals
Cholesterol crystals
Sulphonamide crystals
Tyrosine crystals
Calcium oxalate crystals
Triple phosphate crystals


Hair
Vegetable fibres
Charcot Leyden crystal
Starch cell

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TEACHING BETTER, LEARNING TOGETHER: EFFECTIVENESS OF GAMES -BASED LEARNING IN MICROBIOLOGY FOR MEDICAL GRADUATES

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Background: Medical undergraduate students must go through a vast syllabus and learn various concepts in a short time period. Passive lectures deliver basic theoretical knowledge and is one-way communication thus providing the lowest knowledge retention rate[1] Thus, it's the need of the hour to discover and indoctrinate new creative ideas and innovations in our education system to complement the traditional lectures and augment learning by utilizing active strategies like game-based methods like Pictionary, Crossword puzzle, Specimen analysis game, Sketchy Microbiology, Case based, storytelling [2, 3]

Students' interest and retention of knowledge in the subject may be increased by educational games [4] The benefits of active learning strategies help in evolvement of different skills like attitude, concept building, cognition, communication, critical thinking, learning, and motivation among students [5]

Using game-based techniques during didactic lectures/practical not only breaks the monotony but also helps in active involvement of students thus improving critical thinking and boosting their cognitive skills.[6, 7] Small group discussion among students promotes team work and also complements the traditional method of teaching.[8] thus improving their critical thinking and problem- solving skills.[9]

Incorporation of active-learning methods into classroom allows students to be motivated and enhances their learning experience. Games and team-based learning are found to be an interesting educational tool for teaching medical students as it evokes interest, motivates, enhances their critical thinking, allows a better understanding of concepts, and helps in reinforcing the material acquired during lectures delivered in Microbiology.

Aims and Objectives:

1. To analyse the perceptions of students regarding traditional teaching.
2. To analyse the perceptions of students regarding Innovative teaching techniques.
3. Comparative assessment of both the methodology applied
4.Feedback and Evaluation.

Materials & Methods: Cross-sectional Observational study was conducted among Second year medical students who were randomly selected over a period of 6 months from July to December 2022.Various games with team-based activities such as Pictionary, Crossword puzzle, Specimen analysis game, Sketchy Microbiology, Image based, Case based, storytelling etc each on different topics in Microbiology were implemented after ensuring content validity.

Approval to conduct study was obtained from the Institutional Research and Ethical committee. Informed consent from the students for participation was taken. After the lecture session during Practical's, Pre-test questionnaire was given to both batches. Small groups were made in Batch A and allowed group discussion to solve game-based puzzles prepared by faculty. Batch B was control with routine session. For the next session the methodology was interchanged among both batches. Later the post-intervention assessment was done in both groups with the same set of MCQ and students' feedback on games and team-based usefulness was also obtained.

Results: A total of 120 students out of 150 enrolled in the class of batch 2020 evaluated the game-based activity. An anonymous questionnaire survey was developed to assess the student's perceptions of game-based activity as a tool to enhance their learning in the classroom. The average MCQ test score in Group A improved significantly from 6.65±3.4 pre-intervention to 11.26±2.5 post-intervention ($p < 0.05$). The average test score in Group B also improved significantly from 5.7±2.9 pre-intervention to 9.59±2.5 post-intervention ($p < 0.05$). But the post-intervention MCQ scores in group A (games and team-based activity) was higher and statistically significant ($P < 0.05$) in comparison to group B

Conclusion: Game based activity promotes active self-learning and develops critical thinking among medical students as involvement is there. It could be used as supplementary educational tool in Microbiology to enhance problem-solving skills along with the information provided through traditional teaching lectures. The biggest challenge for teachers is to provide education which inspires students. The teaching learning methodology should be engaging the student which makes it a two-way learning. The perceptions from our study indicate the usefulness of game-based techniques to endorse positive learning in an interactive environment and advocates their use as an adjunct to lecture thereby allowing them to recall the lecture material.

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AMINOGLYCOSIDES AND TIGECYCLINE: TWO MISINTERPRETED CHOICES FOR TYPHOID FEVER

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There have been some recent and past publications in journals of high repute, where antimicrobial susceptibility of blood isolates of *Salmonella* Typhi has been performed for Aminoglycosides (Streptomycin, Gentamicin, Amikacin and Kanamycin) and Tigecycline. A publication was found as a reference, in relation to ceftriaxone resistant *bla*$_{\text{CTX-M15}}$ gene positive *Salmonella* Typhi strains which we isolated two from culture positive cases of Typhoid fever, which were treated with meropenem and one surveillance strain traceable to a family member of one of the patients (published in Indian J Med Microbiol).

Data citing in vitro susceptibility profiles for Aminoglycosides and Tigecycline have been published in the past thereby posing a false impression especially among clinicians that these antimicrobials can be used as potential treatment options for Typhoid fever. A recent publication from another high impact factor journal also cited Tigecycline as a possible treatment option for multi and extensively drug resistant strains of *Salmonella* Typhi **[1]**. This is quite unanticipated as both these classes of drugs are ineffective against *Salmonella* spp causing Typhoid Fever considering its intracellular nature.

Clinical and Laboratory Standards Institute (CLSI) had warned against the use or reporting of Aminoglycosides in *Salmonella* spp as early as 2007 in the M-100 approved guidelines which specifically cited the warning - "For *Salmonella* spp and *Shigella* spp, Aminoglycosides may appear active *in vitro*, but are not effective clinically and should not be reported as susceptible". Despite this, multiple in vitro susceptibility studies have been published giving a wrong interpretation about the clinical use of Aminoglycosides for *Salmonella* spp. The reason for this warning is justified because *Salmonella* is a facultative intracellular bacterium, present in the selectively fused vacuole with a low pH and oxygen tension. Aminoglycosides have to pass through two obstacles, the macrophage cell membrane and the bacterial cell membrane to reach the 30S ribosome and inhibit protein synthesis. Since the oxygen tensions are low and entry in the bacterium itself is oxygen dependant, intra-protoplasmic accumulation of aminoglycosides in such intracellular organisms is very low and does not reach cidal concentrations despite in vitro susceptibility. **[2]** Therefore, in vitro testing, reporting or clinical use of Aminoglycosides against *Salmonella* spp is not good clinical practice.

Similarly, Tigecycline was approved by the FDA specifically for skin and soft tissue infections, complicated intra-abdominal infections and community acquired pneumonias. Tigecycline has a very large volume of distribution (Vd). After its parenteral administration, it gets widely distributed in tissues and serum concentrations fall immediately. Hence, it is of no clinical use in primary bacteraemia and urinary tract infections and therefore, despite its good in vitro response, it is not to be prescribed for Typhoid fever or Sepsis. **[3]**

Clinical Microbiologists need to strictly follow the latest clinical interpretive guidelines from CLSI or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). CLSI M-100 approved guidelines also warn that, for *Salmonella* spp and *Shigella* spp 1st and 2nd generation cephalosporins and cephamycins may appear active in vitro but are not effective clinically and should not be reported as susceptible. Therefore, the reporting clinical microbiology teams must always bear in mind, the triad of - the antimicrobial agent, the bacterial isolate under test and the site of infection during clinical treatment. In vitro susceptibility cannot always be extrapolated as in vivo clinical response and this needs to be highlighted diligently to prevent wrong antibiotic prescription habits which are central to development of antimicrobial resistance.

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COMMON DIAGNOSTIC PROCEDURE USED IN MICROBIOLOGY

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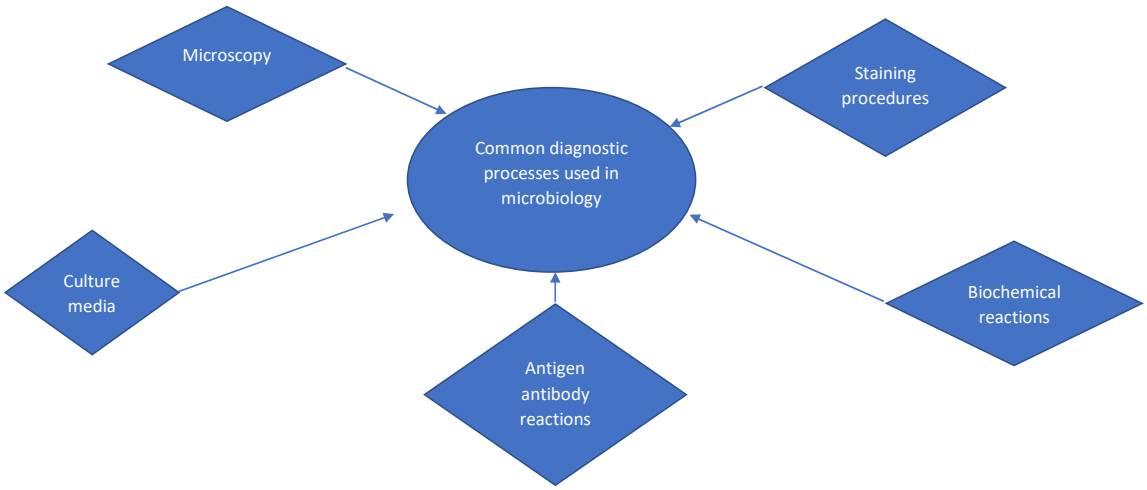
Microbiologist utilize various diagnostic tools to identify causative organisms of disease process. A variety of routine and advanced diagnostic tools are available. here we discuss the various types of common diagnostic tools used in medical microbiology.



1. **Microscopy:**
   - Bright-field or light microscopy:
     i. This forms a dark image against a brighter background.
     ii. Principle:
        1. the rays emitted from the light source pass through the iris diaphragm and fall on the specimen.
        2. The light rays passing through the specimen are gathered by the objective and a magnified image is formed
        3. The image is further magnified by the ocular lens to produce the final magnified virtual image.

   - Dark ground microscopy:
     i. Principle:
        1. Here the object appears bright against a dark background. This is made possible by use of a special dark field condenser
        2. The dark field condenser has a central opaque area that blocks light from entering the objective lens directly and ha a peripheral annular hollow area which allows the light to pass through and focus on the specimen obliquely

3. Only the light which is reflected by the specimen enters the objective lens. as a result, the specimen is brightly illuminated, but the background appears dark

ii. Application:

1. It is used to identify he living unstained cells and thin bacteria like spirochetes which cannot be visualized by light microscopy

- Phase contrast microscopy:
  - i. It is used to study the properties of living cells without staining as though staining enhances contrast it kills the microbes
  - ii. The microscope visualizes the unstained living cells by creating difference in contrast between the cells and water.
  - **iii. Principle:**
    1. The condenser is similar to that of dark field microscope consists of an opaque central area with a thin transparent ring which produces a hollow cone of light.
    2. As this cone of light passes through a cell some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about one fourth of the wave length.
    3. The un deviated light rays strike a phase ring in the phase plate while the deviated rays miss the ring and pass through the rest of the plate
    4. The phase ring is constructed in such a way that the undeviated light passing through it is advanced by one fourth of a wave length, the deviated and undeviated waves will be about half wave length out of the phase and will cancel each other when they come together to form an image.
    5. The background formed by undeviated light is bright while the unstained object appears dark and well defined
  - iv. **Applications:** useful for studying:
    1. Microbial motility
    2. Determining the shape of living cells
    3. Detecting microbial internal cellular components

- Fluorescence microscopy:
  - **i. Principle:**
    1. When fluorescent dyes are exposed to UV rays, they become excited and are said to fluoresce i.e they convert this invisible short length into light of longer wave length

2. The excitation filter is so designed that It allows only short wavelength UV light to pass through blocking all other long wave length
3. The exciting rays then get reflected by a dichromatic mirror in such a way that they fall on the specimen which is previously stained by fluorescent dye and focused under the microscope.

4. The fluorescent dye absorbs the exciting ray's short wavelength gets activated and in turn emits rays of higher wavelength
5. A barrier filter positioned after the objective lens removes and remaining UV light which would otherwise damage the viewers eyes or blue and violet light which would reduce the images contrast

ii. Applications:

1. Epifluorescence microscope:
    a. **Auto fluorescence**: certain microbes directly fluoresce when placed under UV Lamp e.g. *Cyclospora* (a protozoan species)
    b. **Microbes coated with fluorescent dyes**: certain microbes fluoresce when they are stained non-specifically by fluorochrome dyes
        i. Acridine orange dye is used for the detection of parasites such as Plasmodium and filarial nematodes by a method called as quantitative buffy coat examination
        ii. Auramine phenol is used for the detection of tubercle bacilli
    c. Immunofluorescence: it uses fluorescent dye tagged immunoglobulins to detect cell surface antigens or antibodies bound to cell surface antigens
2. Confocal microscope: It uses point illumination and a pin hole in an optically conjugate plane to eliminate out of focus signal and to get a better resolution of the fluorescent image.
3.

2. <u>Staining methods:</u>

i. Staining techniques:
    1. Vital staining
    2. Supravital staining
        a. Simple staining
        b. Negative staining
        c. Differential staining
            i. Gram staining: modifications:
                1. Bruke's modification

                2. Kopeloff and bermann's modification

3. Jensen's modification
4. Weigert's modification
5. Preston and Morrells modification
6. Hucker's modification
7. Brown and Brenn modificaton

ii. Ziehl-Neelsen staining
1. Hot staining techniques:
a. Z n staining
b. Hot modified acid-fast stain for cryptosporidium in stool
2. Cold staining technique's:
a. Kinyoun's cold stain method
b. Coppers modification
c. Gabbett modification
d. Mueller-Chermock Carbol fuschin-tergitol cold stain method
e. Brucella differential stain

iii. Romanowsky staining
1. Leishman's stain
2. Giemsa's stain
3. Jenner's stain
4. JSB Stain
5. Field's stain

d. Impregnation staining:
i. Silver solutions
ii. Fontana's method
iii. Levaditi's method

e. Special staining
i. Staining of spores
1. Modified ZN staining method
2. Schaeffer and Fulton's staining method
3. Dorners method
4. Modified Dorners method
5. Writz and Conklin method
6. Abbot stain

ii. Staining of capsules
1. Negative staining methods:
a. The wet film India ink method
b. Dry film negatives staining method
c. Congo red capsule stain
2. Positive staining methods
a. Hiss method
b. MacNeal method
c. Lawson method

d. Anthony method
e. Muirs method
f. Howie and Kirkpatric method

iii. Staining of granules
1. Staining of volutin granules
a. Albert's stain
b. Neisser's stain
c. Ljubinsky's stain
d. Gohar's stain
e. Methylene blue stain
f. Ponder's stain
2. Staining of lipid granules:
a. Burdon's method
b. Lipid spore stain (Holbrook and Anderson)
3. Staining of polysaccharide granules/polysaccharides:
a. Periodic Acid Schiff method

iv. Staining of flagella:
1. Leifson's method
2. Modified Leifson's stain
3. Leifson-Hugh modification
4. Silver plating stain
5. Grey stain
6. Wet-mount flagellar stain

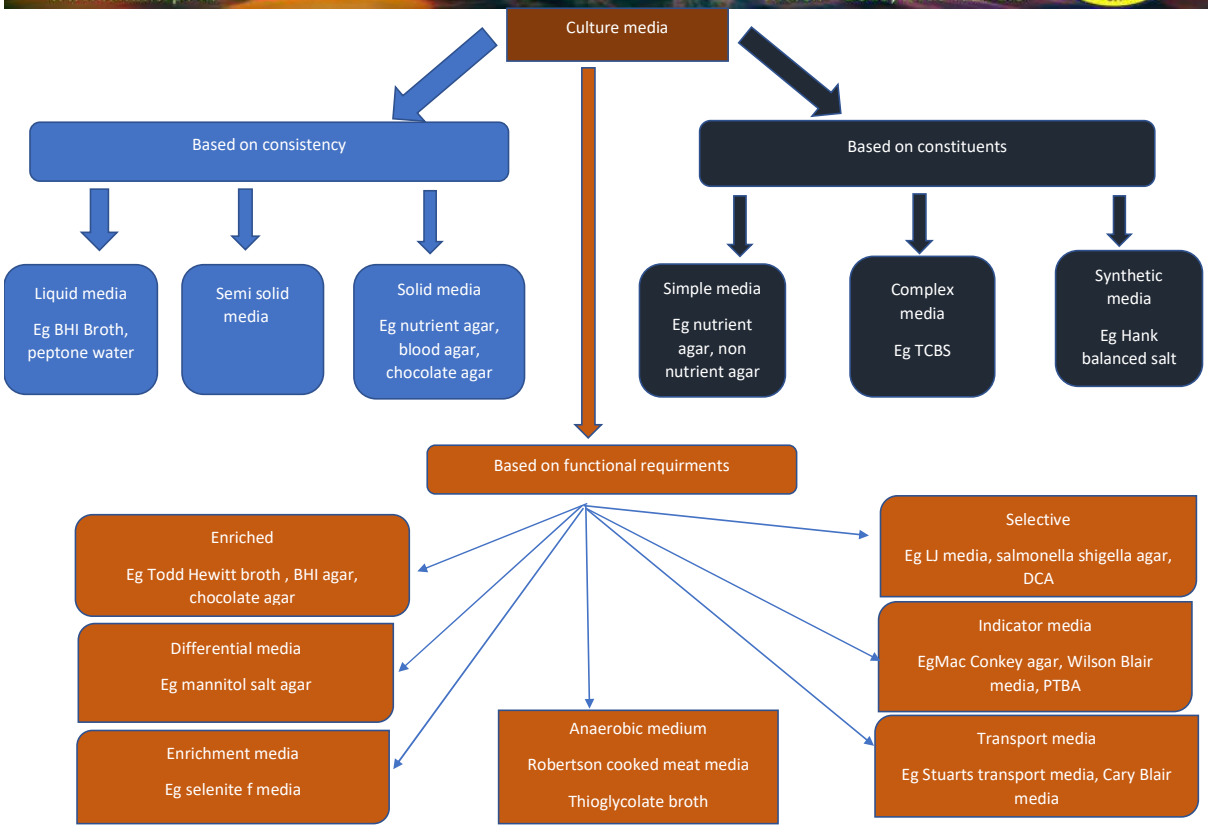
ii. Staining procedures in mycology:
1. Potassium hydroxide mounts
2. Saline wet mount
3. India ink stain
4. Modified India ink
5. Nigrosine stain
6. Calcofluor white stain
7. Lactophenol cotton blue stain
8. PHOL ono and lee stain
9. Neutral red stain
10. Diazonium blue B reagents
11. Differential stain:
a. Gram's stain: brown and Brenn modification
b. Acid fast stain: Kinyoun's stain
12. Hematoxylin and eosin stain
13. Giemsa stain
14. Periodic Acid-Schiff stain
15. Gridelys fungal stain
16. Grocott Gomori's methenamine-silver stain
17. Mayers mucicarmine stain

18. Masson-Fontana silver stain
19. Toluidine blue o stain for *P.jirovecii*
20. Acridine-Orange stain
21. Fluorescent -Antibody stain

iii. Staining procedures in parasitology:
   1. Temporary methods:
      a. Wet mounts:
         i. Saline preparation
         ii. Iodine preparation
         iii. Lactophenol cotton blue mount
         iv. Eosin mount
         v. Buffered methylene blue mount
   2. Permanent methods:
      a. Modified ZN techniques:
         i. Hot modified acid-fast staining method
         ii. Kinyoun's cold stain method
      b. Safranin methylene blue technique
      c. Trichrome stain
      d. D'Antoni's iodine
      e. Modified trichrome stain
      f. Iron hematoxylin stain
   3. Methods for staining blood films:
      a. Temporary preparations:
         i. Wet mount
      b. Permanent preparations:
         i. Romanowsky's stains
         ii. Hematoxylin stain

iv. Staining procedure in virology:
   1. Demonstration of cytopathic changes in infected host cells: a. Giemsa's stain; b. Papanicolaou stain
   2. Demonstration of virus inclusion bodies:
   3. Demonstration of virus by direct immunofluorescence
   4. Demonstration of virus by electron microscopy
      a. Negative staining technique
      b. Positive staining technique
   5. Demonstration immunoelectron microscopy

3. Culture media:			
Functional requirements	Differential		• Mannitol salt agar (MSA) differentiates *Staph aureus* from *Staph epidermidis*
	Enriched	• Nutritional requirements such as blood serum or eggs are added to basal media to cultivate exacting or fastidious bacteria	• Todd Hewitt broth • Blood agar • Chocolate agar • Brain hearth infusion broth
	Enrichment	• These media are used to supress commensal bacteria while allowing pathogen to remain viable to grow	• Selenite F medium (for faecal samples to supress gut commensals) in dysentery • Tetrathionate broth
	Selective	• An inhibiting substance added to solid medium enable required bacterium to form colonies while inhibiting other bacteria's	• Salmonella-shigella agar • TCBS for *Vibrio cholerae*
	Indicator	• These media contain an indicator that changes colour when a bacterium grows on them	• Mac Conkey agar • In Wilson Blair media sulphite • PTBA (Potassium tellurite blood agar)
	Transport	STM is soft agar gel containing a reducing agent (sodium thioglycolate) to prevent oxidation and charcoal to neutralise certain bacterial inhibitors	• Stuarts transport medium (for transporting throat and genital tract swabs collected to isolate pathogens from)
	Anaerobic media		• Robertson cooked meat media



4. Biochemical reactions:

Test	Purpose
Beta-glucuronidase	To identify *E. coli*
Bile solubility	To differentiate *S. pneumoniae* from other alpha hemolytic streptococci
Catalase	To differentiate staphylococci from streptococci
Citrate utilization	To differentiate enterobacteria
Coagulase	To identify *S. aureus*
DNA ase	To identify *S. aureus*
Indole	To differentiate gram negative rods particularly *E. coli*
Litmus milk decolorization	To help identify enterococcus and some clostridia
Lysine decarboxylase	To assist in the identifications of salmonellae and shigella
Oxidase	To help identify *Neisseria, Pasteurella, Vibrio, Pseudomonas*
Urease	To help identify *Proteus, Morganella, Y. enterocolica H. pylori*
KIA and Rosco sugar fermentation	To screen for shigella and salmonella in fecal specimens
Carbohydrate fermentation test	Peptone water sugars and Rosco sugar fermentation tablets for identification of bacteria
PYR test	To identify *S. pyogenes*

5. **Antigen antibody reactions:**
    - Conventional techniques:
        i. Precipitation reactions:
            1. Slide flocculation test: for syphilis
            2. Elek's gel precipitation test (detecting diphtheria toxin)
        ii. Complement fixation test
        iii. Agglutination reaction:
            1. Direct agglutination test:
                a. Slide agglutination
                b. Tube agglutination
                c. Microscopic agglutination
            2. Indirect or passive agglutination test (for antibody detection):
                a. Indirect hemagglutination test (IHA)
                b. Latex agglutination test/(LAT) (for antibody detection): used for detection of ASO
                c. Reverse passive agglutination test (for antigen detection):
                    i. Reverse passive hemagglutination assay (RPHA): used in past for detection of hepatitis B surface antigen, now obsolete
                    ii. Latex agglutination test for antigen detection: used widely for detection of CRP, RA, Capsular antigen detection in CSF
                    iii. Co agglutination test: staphylococcus aureus acts as carrier molecule; was used to detect antigen from clinical specimen; now obsolete
                d. hemagglutination test:
                    i. Direct hemagglutination test:
                        1. Paul Bunnell test: to detect Epstein Barr virus antibodies in serum
                        2. Cold agglutination test: uses human RBC as antigens to detect Mycoplasma antibodies in serum
                        3. Blood grouping: ABO and Rh grouping
                        4. Coombs test or antiglobulin test : it is performed to diagnose Rh incompatibility by detecting Rh antibody from mothers and baby's serum
                    ii. Indirect (IHA): obsolete now
        iv. Neutralization test:
            1. Viral neutralization test:
            2. Plaque inhibition test: done for bacteriophages
            3. Toxin- antitoxin neutralization test:

                a. Schick test: diphtheria toxin antitoxin neutralization test

b. Nagler's reaction: detection of alfa toxin of *Clostridium peferingens*
c. ASO test: now replaced by latex agglutination.

4. Hemagglutination inhibition test:

- Newer techniques:

i. ELISA: enzyme linked immunosorbent assay
   1. Direct ELISA
   2. Indirect ELISA
   3. Sandwich ELISA
   4. IgM antibody capture (MAC) ELISA
   5. Competitive ELISA
   6. ELISPOT test
   - ELISA used for antigen detection: HBsAg, HBeAg, NS1 antigen for dengue
   - ELISA used for antibody detection: against Hepatitis B, Hepatitis C, HIV, dengue, EBV, HSV, toxoplasmosis leishmaniasis etc.

ii. ELFA: enzyme linked fluorescent assay

Can be used to detect various parameters

   1. Infectious diseases: markers if hepatitis viruses and HIV Ab to TORCH infection, Measles, Mumps. Varicella, SARS-CoV 2 etc

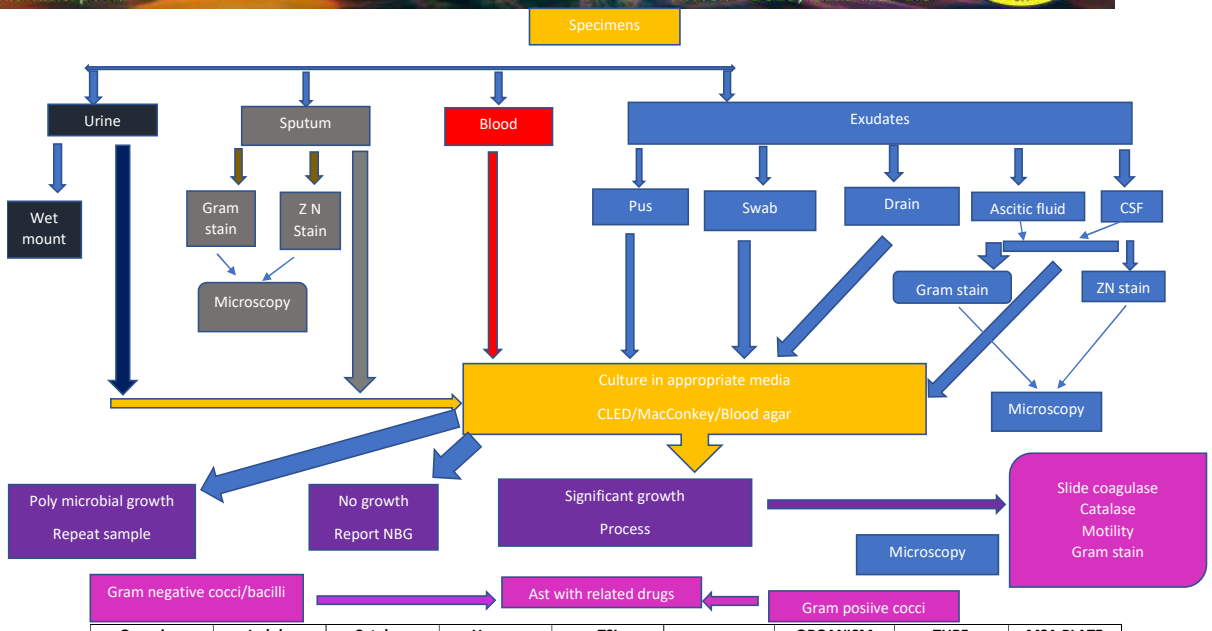
iii. IFA: Immunofluorescence assay
   1. Direct immunofluorescence assay
   2. Indirect immunofluorescence assay
   3. Flowcytometry: CD4 T cell count in HIV
   4. Detection of leucocytes with specific marker

iv. CLIA: chemiluminescence linked immune assay; used for Ag and antibody detection in HIV, TORCH, hepatitis virus etc

v. Immunohistochemistry

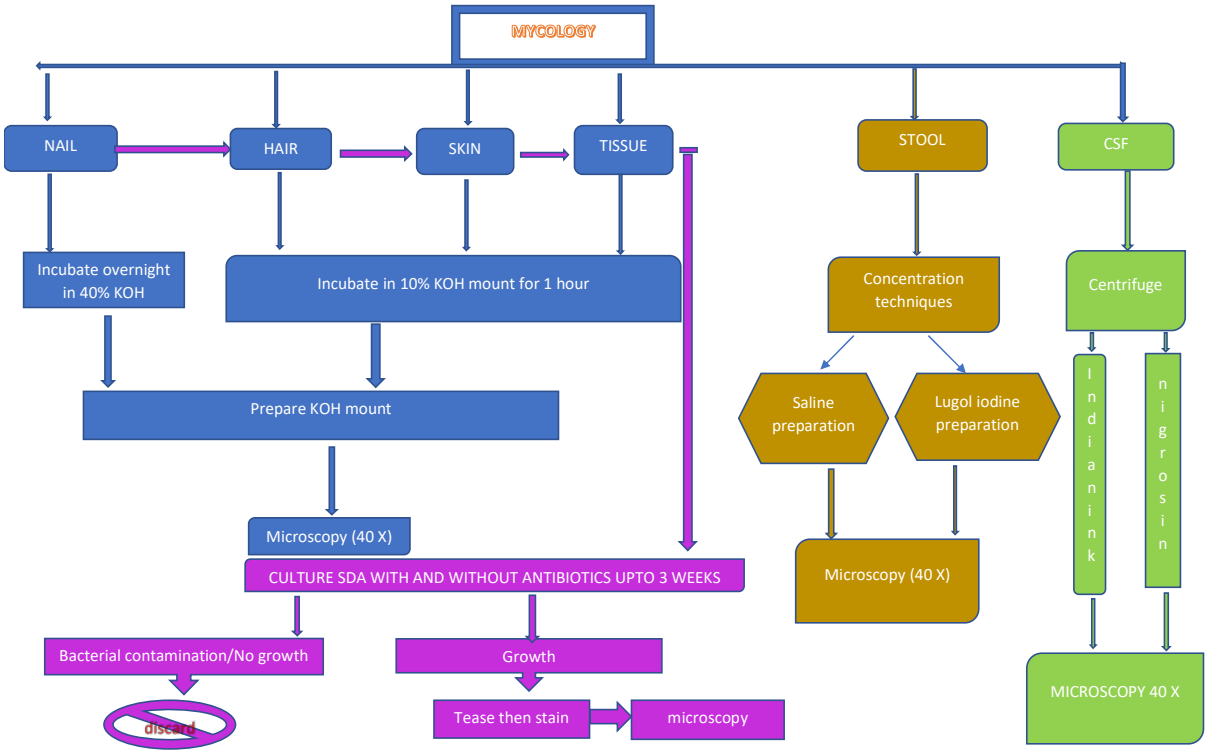
vi. Rapid tests:
   1. Lateral flow assay
   2. Flow through assay

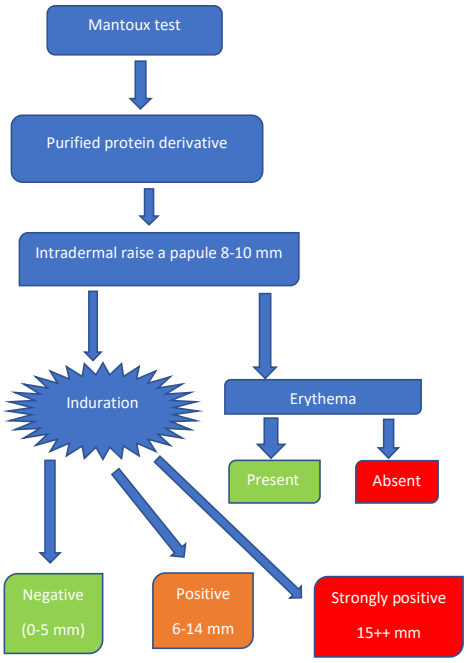
vii. Western blot: used to confirm results of ELISA

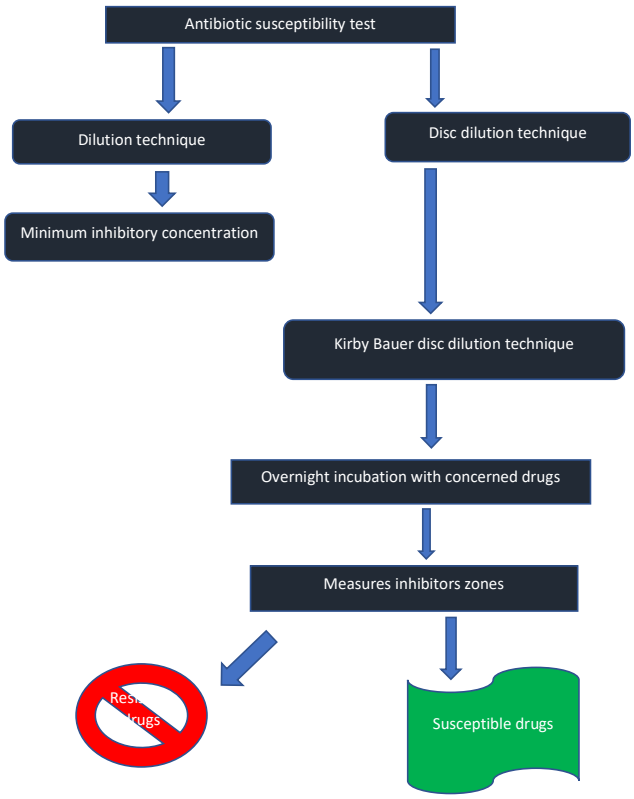


Organism	Indole	Catalase	Urease	TSI
Pseudomonas	Negative	Positive	Variable	K/NC
E.coli	Positive	Negative	Negative	A/A
Klebsiella	Negative	Positive	Positive	A/A with gas

ORGANISM	TUBE COAGULASE	MSA PLATE
Staphylococcus	Positive	Positive
Enterococcus	Negative	Negative







Newer methods:

1. PCR: (polymerase chain reaction):

This rapid automated method for amplification of specific DNA sequence or genes is widely used now and is a versatile tool useful in diverse areas such as diagnosis of infectious, genetic or neoplastic diseases, in forensic investigation etc.

PCR consists of several cycles of sequential DNA replication where products of the first cycle become template for the next cycle.it makes available abundant quantities of specific DNA sequence starting from sources containing minimal quantities of the same.

2. MALDITOF:(Matrix-assisted laser desorption/ionization-time of flight) here identification is based on unique protein composition of bacterial cell/ yeast cell. This type of technology is useful in identification of microorganisms by a mass spectrometric profile of the proteins largely rRNA of the molecules.
3. VITEK: this system is used to perform automated antimicrobial susceptibility test.it has been further modified to improve accuracy.
   VITEK 2 system is an integrated molecular system with several technical improvements which automates many manual procedures involved previously
4. GeneXpert: CBNATT**:** it is based on real time PCR technique simultaneously detects

I. MTB complex DNA
II. Rifampacin resistance

5. Line probe essay:it involves probe-based detection of amplified DNA in the specimen. It has been useful in TB diagnostics
    I. Identification of MTB complex
    II. Detection of resistance in first line antitubercular drugs
    III. Speciation of MTB complex and NTM
    IV. Detection of resistance to second line ATDs
    V.

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3. Essentials of medical microbiology by Apurba S Sastry 3rd edition
4. Ananthanarayan and Panicker textbook of microbiology 13th edition /9th edition
5. Stains and staining procedures in microbiology by BS Nagoba, Megha Rastogi
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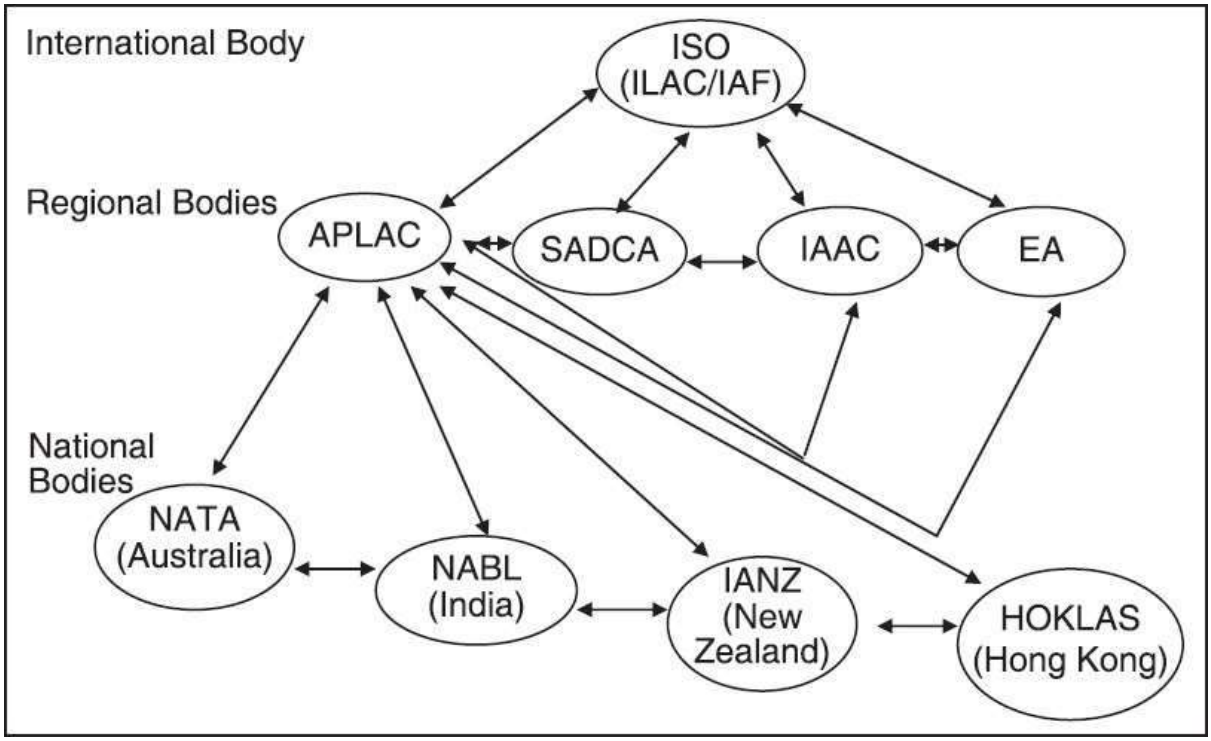
ACCREDITATION OF CLINICAL MICROBIOLOGY LABORATORY

Dr. Y. Joshua Swaroop, 2nd Year PG **Mentors:** Dr. Y. Saritha, Asst. Professor, Dr. B. Venkat Rao, Professor, Dept. of Microbiology, Siddhartha Medical College, Vijayawada

Introduction

In the present times the responsibilities of the Microbiology laboratories have increased and medical microbiologists are expected to provide quality services for management and surveillance of infectious diseases and monitoring of antimicrobial resistance. A step in the direction is the accreditation of the laboratories, which will ensure the reliability and acceptability of the results.

The MRA (Mutual Recognition Arrangement) partner and a full member of APLAC (Asia Pacific Laboratory Accreditation Cooperation) in India is the National Accreditation Board for testing and calibration laboratories (NABL). NABL is an autonomous body under the aegis of Department of Science and Technology, Govt. of India, registered under the societies act is the sole authorized laboratory accreditation body in India. Its objective is to provide third party assessment of quality and technical competence.



Accreditation is a procedure by which an accreditation body (like NABL) gives formal recognition that a body (laboratory) is competent to carry out specific tasks. The procedure imparts official credit, authorization and registration of a laboratory and that it has demonstrated its capability, competence and credibility to carry out its specified scope.

Advantages of accreditation include the following:

- Reports are accepted internationally implying the concept "once tested accepted everywhere"
- Overseas business is improved
- Quality of reports is not affected by individuals once the systems are in place
- User confidence increases
- Productivity increases as error and wastage decreases.

The road map to article is as follows: accreditation bodies, need for accreditation, existing practices in different laboratories, Pre-requisites, Getting started, Management requirements, Quality management applying for accreditation, Laboratory area, Organization chart, List of equipment, Details of last Internal audit, quality plan and preparation of documents, internal QC in examination procedures, facing accreditation, EQAS & ILC, Reporting of results, post – assessment ,New amendments 2022.

1) Accreditation bodies

An accreditation body is statuary organization that is usually established by an act of parliament and is internationally recognised through 'Mutual Recognition Arrangement' (MRA). It promotes development and maintenance of good practices in testing and calibration

2) Need for accreditation

Successful QA (Quality Assessment) program can deliver reliable reports in an agreed upon time frame. Systemized process can be assessed, evaluated and improved upon which is comprehensively called the lab QMS (Quality Management System). Accreditation re-in forces and reassures quality by creating an opportunity for the laboratory to look back upon the pitfalls.

3) Existing practices in different laboratories

Laboratories may be divided into two broad categories:

- those which are run by government organizations among which a few are attached to medical colleges
- private sector laboratories which are further divided as
    a) independent laboratories run by big corporate giants or the in house laboratories of
    Private /corporate hospitals

    b) small scale laboratories

4) Pre-Requisites for laboratory

- Legally registered
- Adequate facilities
- Technically competent qualified staff
- QM has attended 4-day internal audit training
- Should comply with standard ISO 15189, other NABL documents
- Equipment should be calibrated
- Completed one Internal audit covering all clauses of ISO15189
- Management review
- Satisfactorily participated in at least one proficiency testing program conducted by NABL in accordance with the International standard ISO17043
- Internal quality plan

5) Getting started

First requirement is appointing quality manager (QM). The QM should be sent for training on LAB QMS and internal audit program as per ISO 15189:2012 Standard. These programs are earlier conducted by NABL, but now a few NABL accredited government and private training institutes independently conduct it as a 4 day course. The QM should write down all documents as per ISO 15189:2012 standard and train other lab personnel.

6) Management requirements

- Legal entity: Entity that can be held legally responsible for its activities
- Laboratory director: A person with the competence and delegated responsibility for the services provided which include scientific, consultative, organizational, administrative and educational matters. The lab director has ultimate responsibility for the overall operation.
- Quality policy lab management shall define the intent of its quality management system in quality policy it provides a framework for establishing and reviewing quality objectives.
- Quality manager ensures that processes needed for the quality management system are established, implemented and maintained

7) Quality management system

The laboratory shall establish, document, implement and maintain a QMS and continually improve its effectiveness in accordance with the requirements of international standard. The QMS shall provide for the integration of all processes required to fulfil its quality policy and objectives.

8) Applying for Accreditation

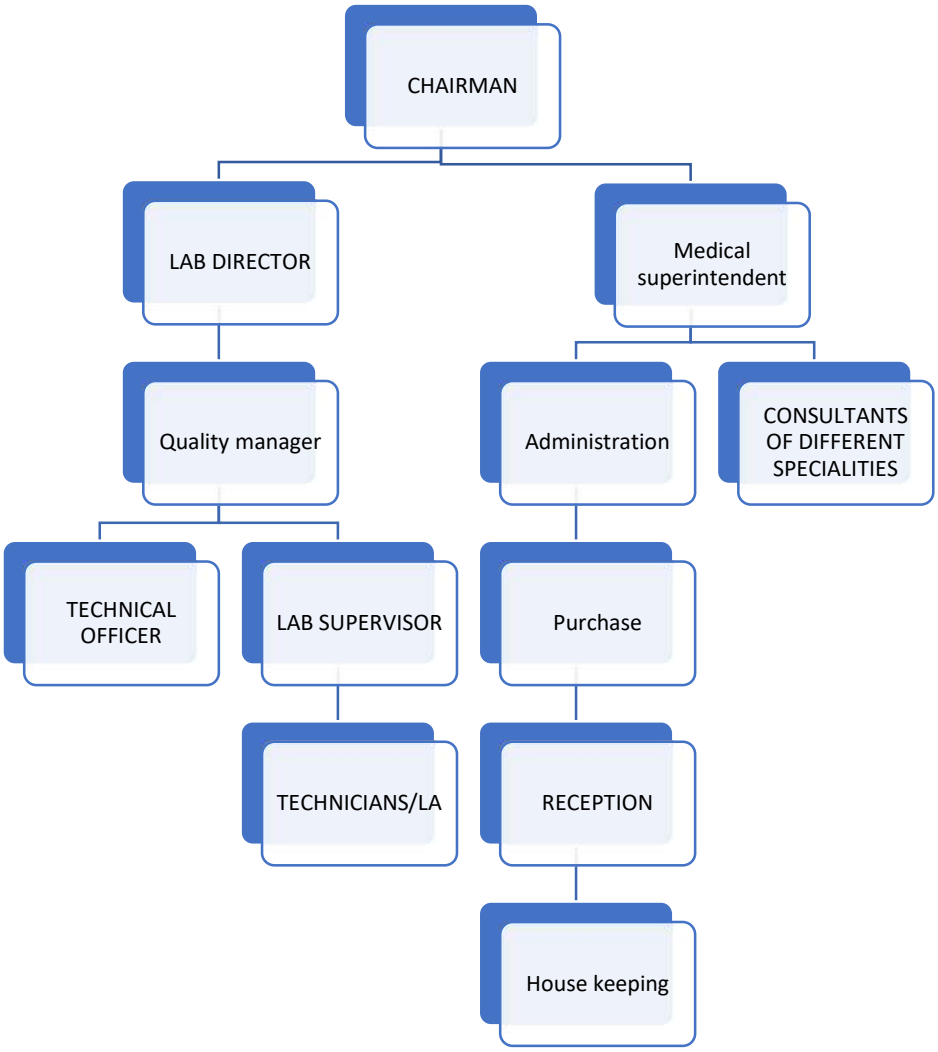
One must first set up a laboratory, establish a lab QMS, start processing, perform an internal audit, improve the system as per ISO 15189 2012, apply for External Quality Assurance Scheme (EQAS) and once having achieved satisfactory results, apply for accreditation.

9) Laboratory area

- Front office: OPD patients make the first contact here, this should display all the tests carried out in-house and /or those out-sourced. Turnaround time, names of the various consultants and working hours of the laboratory. There should be a system of lodging complains and receiving feedback from the customers.
- Sample collection room and toilet
- Processing area

Laboratory	Area specification	Equipment
Bacteriology	Separate laboratory, staining station, handwash station, self-closing doors, BSL-2 Compliance	Biosafety cabinet (BSC) CLASS II A, incubator set at 35°C Incubator at 56°C ,refrigerator, automated system for culture and identification of isolate
Serology	Separate laboratory, ELISA testing area, rapid serological testing area, Hand wash station	ELISA reader & washer, incubator, centirifuge, Rapid kids
Immunology	Separate laboratory & CD4 testing area, Hand wash area	Sysmex CD4 Counter, Computer with printer
Mycology	Same as above	Same as above + BOD at 25°C Incubator set at 37°C
Parasitology	Separate laboratory, microscopic area	Binocular Microscope, Centrifuge, concerned stains
Virology	Separate laboratory for RTPCR testing, Doffing &Donning area and hand wash	Automated Extraction machine & PCR, Biosafety cabinet class IIB
Mycobacteriology	Same as above but it should be BSL – 3	Biosafety cabinet class IIB, incubator set at 37°C and 25°C
Media preparation	Separate Media Pouring Area	Laminar flow (for pouring), weighing scale, pH meter, distillation plant, water bath, inspissator, autoclave and refrigerator
Washing room	Separate area, deep sink	Autoclave, hot air oven
Store	separate area with shelves	Computer with printer

10) Organization chart: It clearly depicts the hierarchy of the laboratory personnel. It needs to be present in the Quality Manual and may also be displayed in the laboratory front office.



11) Equipment

A list of equipment has to be provided to the NABL office in a format. The format should include manufacturer's name, model no., year of installation, availability of operation manual and procedure, details of periodic maintenance/calibration/validation of all equipment being used in the laboratory.

12) Quality plan and preparation of documents

Preparation of documents is easier if one begins with the lowest tier among the hierarchy of documents i.e. 'End point documents'. This would include the pre-analytical, analytical and post analytical documents along with others like feedback/complaint forms and formats for QC tests. Start with the end point documents and once they are streamlined start writing SOPs.

Obviously, one would keep on improving the end point documents as and when one gains more experience

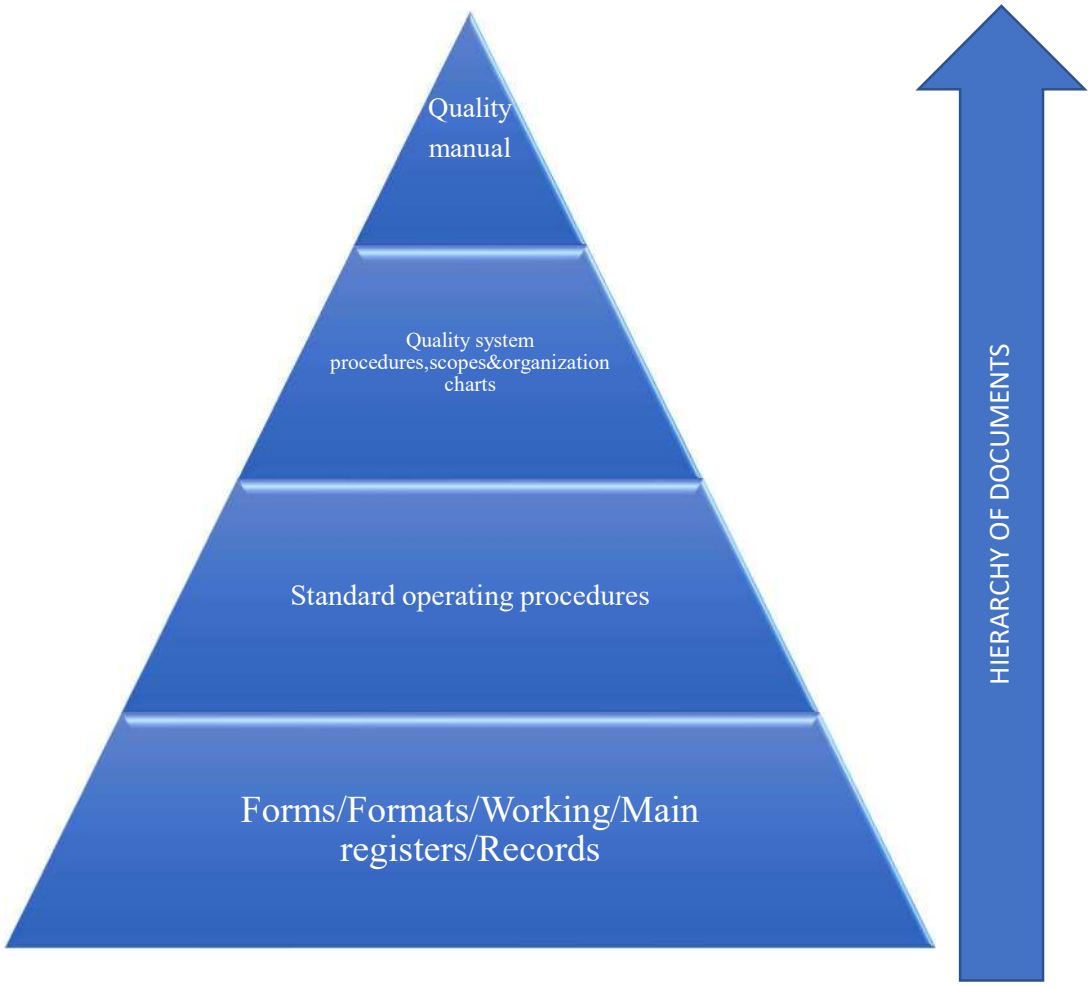


Figure - hierarchy of documents in lab qms required for accreditation

13) Standard operating procedures

An SOP should incorporate in every testing procedure. They should be available at work bench and should be strictly followed

14) Control of non-conformities, improvement and preventive action:

To handle this important aspect, one has to nominate a person (may be deputy QM) who would note any non-conformity brought to the notice by any personnel of the laboratory. Once the problem has been identified the next step is to take corrective action which at first go is stopping the release of results. All these activities should be documented.

15) External services and supplies:

- Review of contracts**:** the laboratory should have written policy on what all services they are providing so that the requests can be addressed according to the laboratory ability.
- Selection of referral laboratory: While selecting a referral laboratory, one must make sure that the test outsourced falls within the scope. A Memorandum of Understanding (MOU) has to be signed between the two laboratories.
- Purchasing services and supplies: The laboratory has to design for selection of its vendors. This may include price, market reputation, maintenance of cold chain and ability to deliver products in a proper timely manner. List of suppliers is to be made along with the evaluation scores using above criteria

16) Pre Analytical procedures

The laboratory has to prepare QSP's (Quality System Procedures) for sample reception area which should include sample rejection criteria, sample numbering system, billing system, labelling of urgent requests, written policy on verbal requests, delivery of critical information, storage of samples and handling of complaints to monitor quality, one should prepare records of incompletely filled forms, rejected samples, sample label errors and lost samples

Internal quality control in examination procedures.

17) Analytical

- QC tests should be conducted: for all testing procedures. The QC must be done for all the sections like Bacteriology, Serology, Mycology, Parasitology, Immunology, Mycobacteriology, and Virology.
- EQAS and Inter Laboratory Comparison (ILC): NABL recognises EQAS program run by various institutes like CMC Vellore, one can perform ILC by sending a portion of sample to NABL accredited laboratory

18) Post Analytical

- Reporting of results: Reports shall include the information necessary for the interpretation of the examination results. The lab shall have a process for notifying the requester when an examination is delayed. Report content shall include a clear, unambiguous identification of the examination including, where appropriate, the examination procedure, identification of all examinations that have been performed by a referral laboratory
- Release of Results: The lab shall establish documented procedures for the release of results, including details of who may release results and to whom/When examination results fall within established 'alert' or 'critical' intervals a physician is notified immediately, record is maintained of action taken that day including date and time. Results provided orally shall be followed by a written report, there shall be a record of all oral results provided.

- The QM holds the key to uphold quality by remaining vigilant and creating a system for periodic self-audit and a continuous laboratory technology education. Human resource department should also chip in with policies on retaining good staff by linking their appraisals to their privileging.

19) Accident record & Preventive record: The Corresponding records must be maintained

20) Details of Last internal audit and Management review

Management review meetings (MRMs) can be conducted by the QM in the presence of technical manager, chairman and consultants of various specialities. The minutes of the meeting have to be documented. Internal audits have to be conducted for each speciality by internal auditors from a different department, at least yearly and non-compliances as well as the corrective action taken have to be documented. The laboratory is required to keep a photocopy of the auditor's certificate issued by one of the recognized internal audit programs conducting agency.

21) New amendments 2022

- Alignment with ISO/IEC 17025:2017 resulted in the management requirements
- Requirements for point of care testing (POCT) Previously in ISO 22870 have been incorporated
- Increased emphasis on risk management

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4. Eurachem Accreditation for Microbiological Laboratories: second edition 2013

5. National Accreditation Board for Testing and Calibration Laboratories NABL 112: Special criteria for accreditation of medical laboratories

6. International standard ISO 15189 2022, ISO Reference number ISO 15189:2022(E)

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WHONET & ANTI-MICROBIAL STEWARDSHIP

Dr. E. Priya, Postgraduate, Dept. of Microbiology, Guntur Medical College, Guntur

WHONET is a free desktop Windows application for the management and analysis of microbiology laboratory data with a particular focus on antimicrobial resistance surveillance developed and supported by the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance at the Brigham and Women's Hospital in Boston, Massachusetts,

CO-FOUNDERS:
Dr. Tom O'Brien
In 1995, Dr. O'Brien became a Co-Founder of the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance.
He is Infectious disease specialist in Boston, Massachusetts.
He received his medical degree from Harvard Medical school and has been in practice for 61 years.

DR. JOHN STELLING
Co-Director of the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance based at the Brigham & Women's Hospital in Boston.
He received his undergraduate degree from Johns Hopkins University and Master's in Public Health from the Johns Hopkins School of Hygiene and Public Health.

ADAM CLARK
He is the principal WHONET software engineer. He has been working with the project since 2011.
Since then, he has greatly refactored and extended the desktop applications WHONET and BacLink, improved the web version of WHONET, created the WHONET Automation Tool, and the AMR Interpretation Engine among other accomplishments.

ROB PETERS
Principal WHONET Software QA Analyst
he has contributed to stream-lining the user experience, modeled WHONET's ability to analyze multiple laboratories at the local and state levels, modernized and rebranded the WHONET logo, website, documentation, and training materials.

WHONET, available in 44 languages, supports local, national, regional, and global surveillance efforts in over 2,300 hospital, public health, animal health, and food laboratories in over 130 countries worldwide.

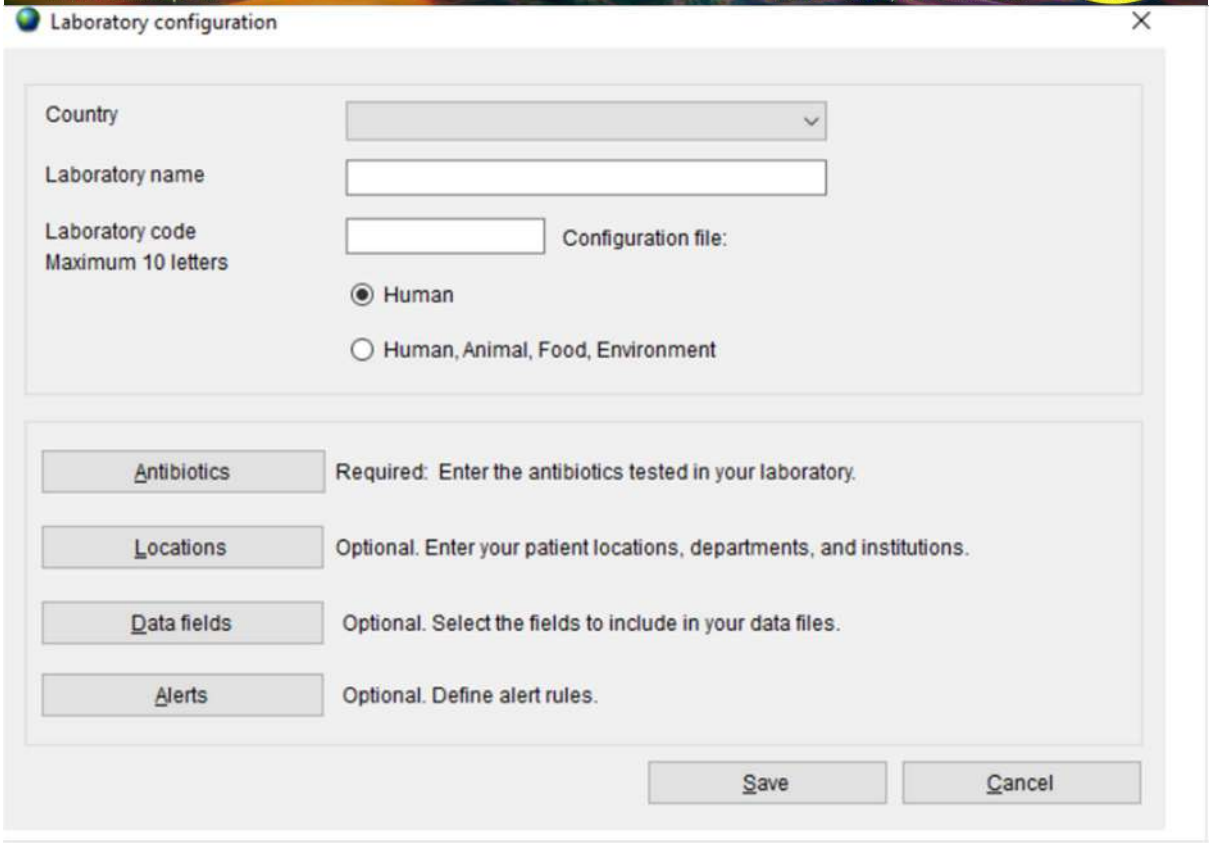
WHONET 2022

MODULES

- Laboratory configuration
- Data entry
- Data analysis
- Public health reporting
- Data encryption among others

LABORATORY CONFIGURATION:

This includes sections like
Describing your laboratory
Selecting your antibiotics
Configuring your antibiotics
Patient locations
Data fields
Isolate alerts
Finishing laboratory configuration



DATA ENTRY:

This includes

Creating a new data file

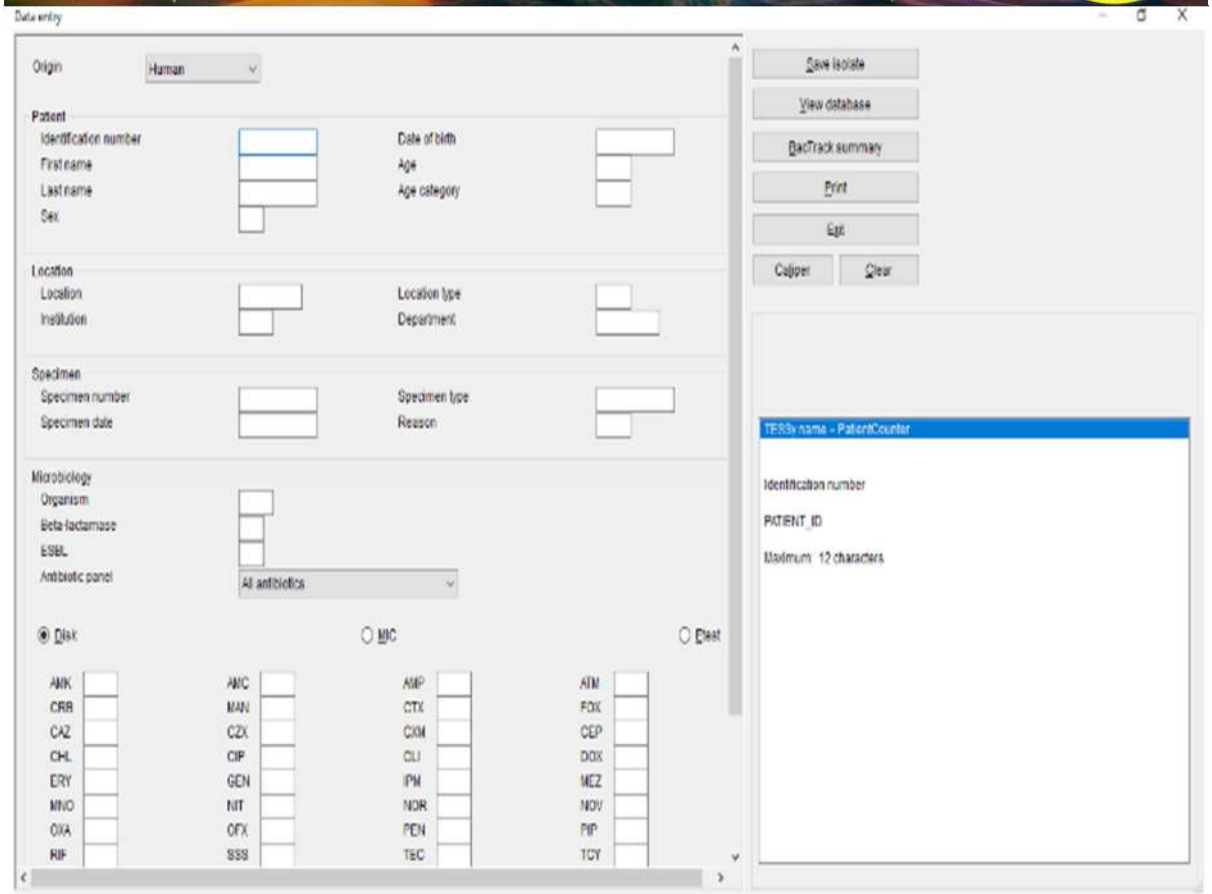
Data entry

Viewing the database

Clinical reports

Finishing up

Other options for managing data files



DATA ANALYSIS

This includes

Getting started

Setting up an analysis: %RIS and test measurements

Running the analysis and interpreting the results

Transferring WHONET results to Excel and other software

%Susceptible summary

Isolate listing and summary

Important features of these tutorial

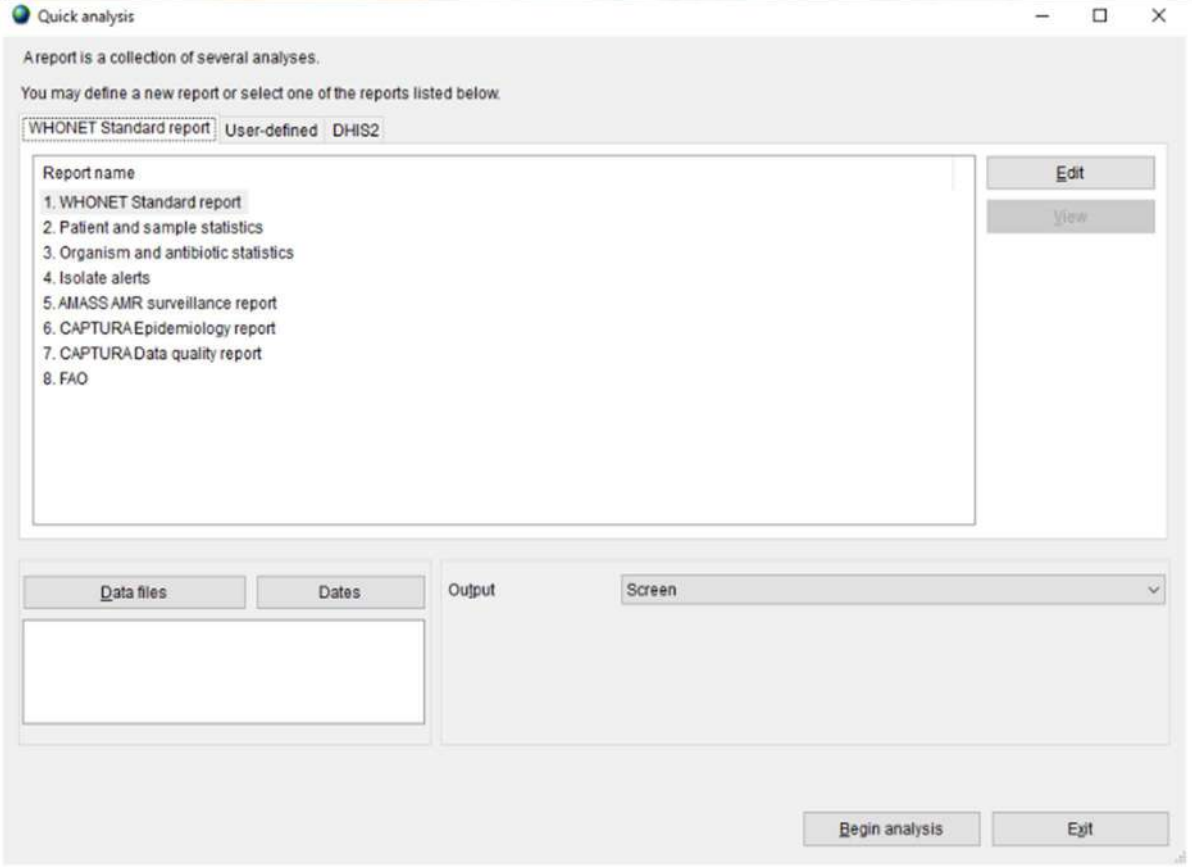
Continuous quality improvement – assessing laboratory test practices and utilization of laboratory services by clinical departments.

Describing trends in epidemiology of microbial populations and antimicrobial resistance.

Characterizing the molecular epidemiology of antimicrobial resistance strains

Guiding antimicrobial therapy recommendations and policy.

Supporting infection control interventions, in particular the early identification of hospital and community outbreaks.



DATA EXPORTS TO SURVEILLANCE NETWORK

This feature includes the following sections.

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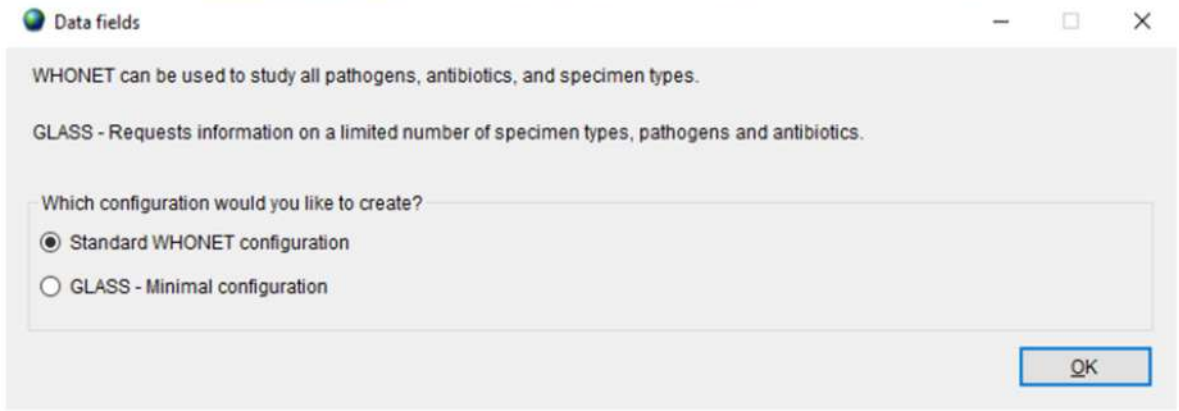
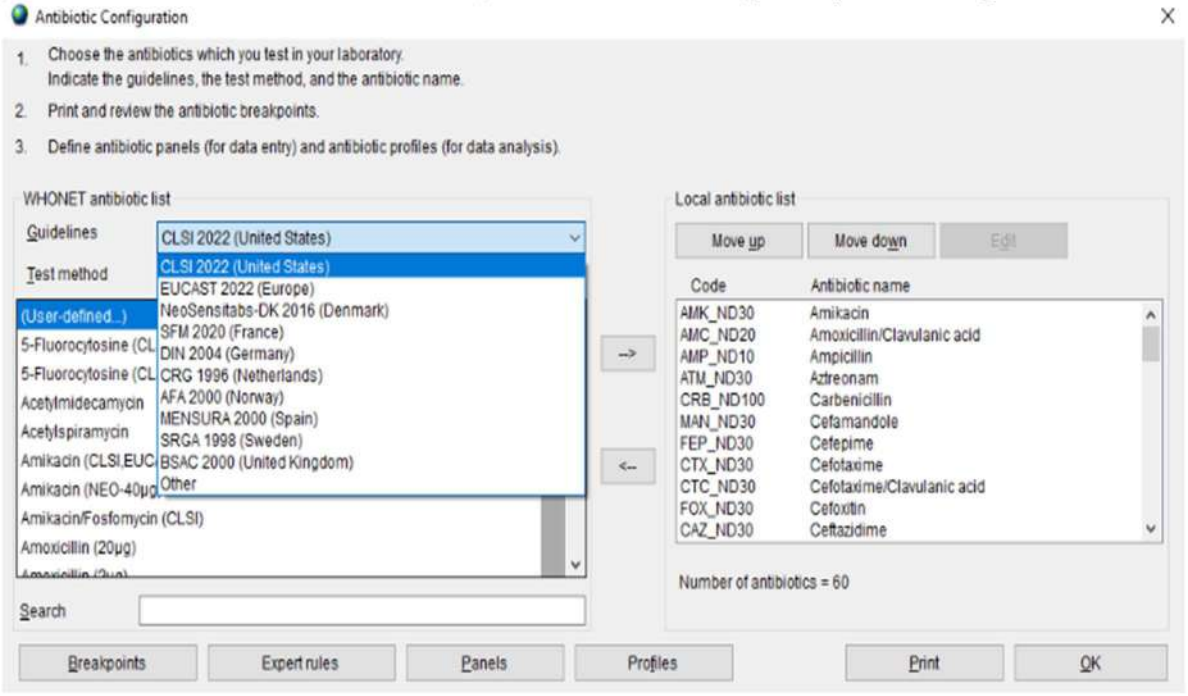
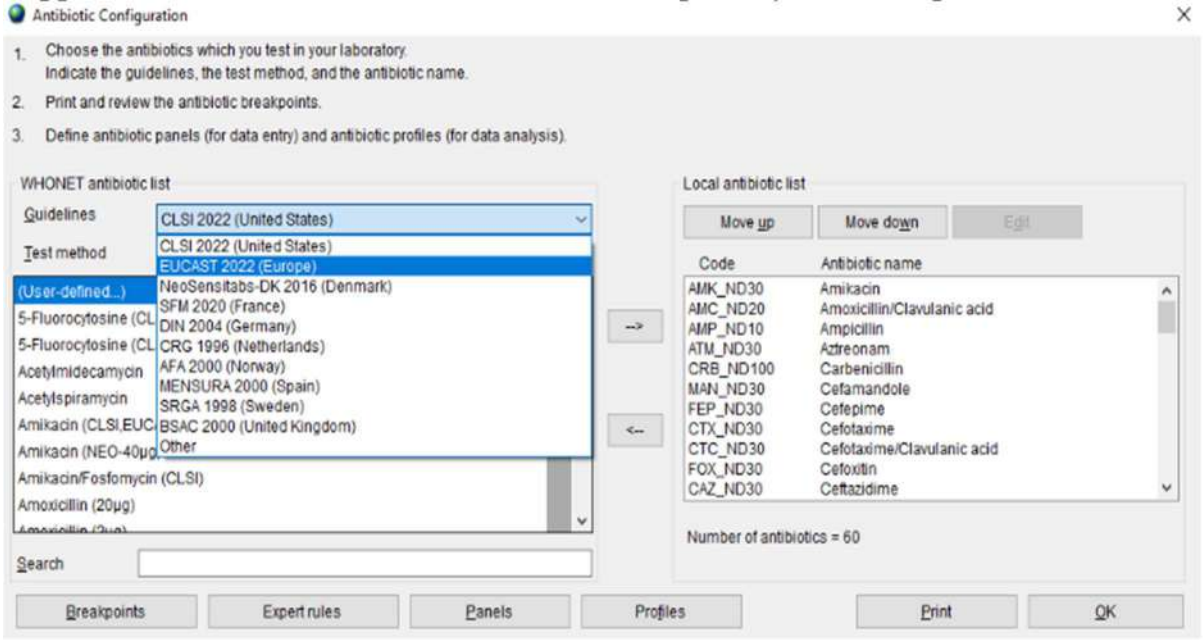


SUPPORT FOR CLSI HUMAN, VETERINARY AND ANTIMICROBIAL SUSCEPTIBILITY TEST BREAKPOINTS:

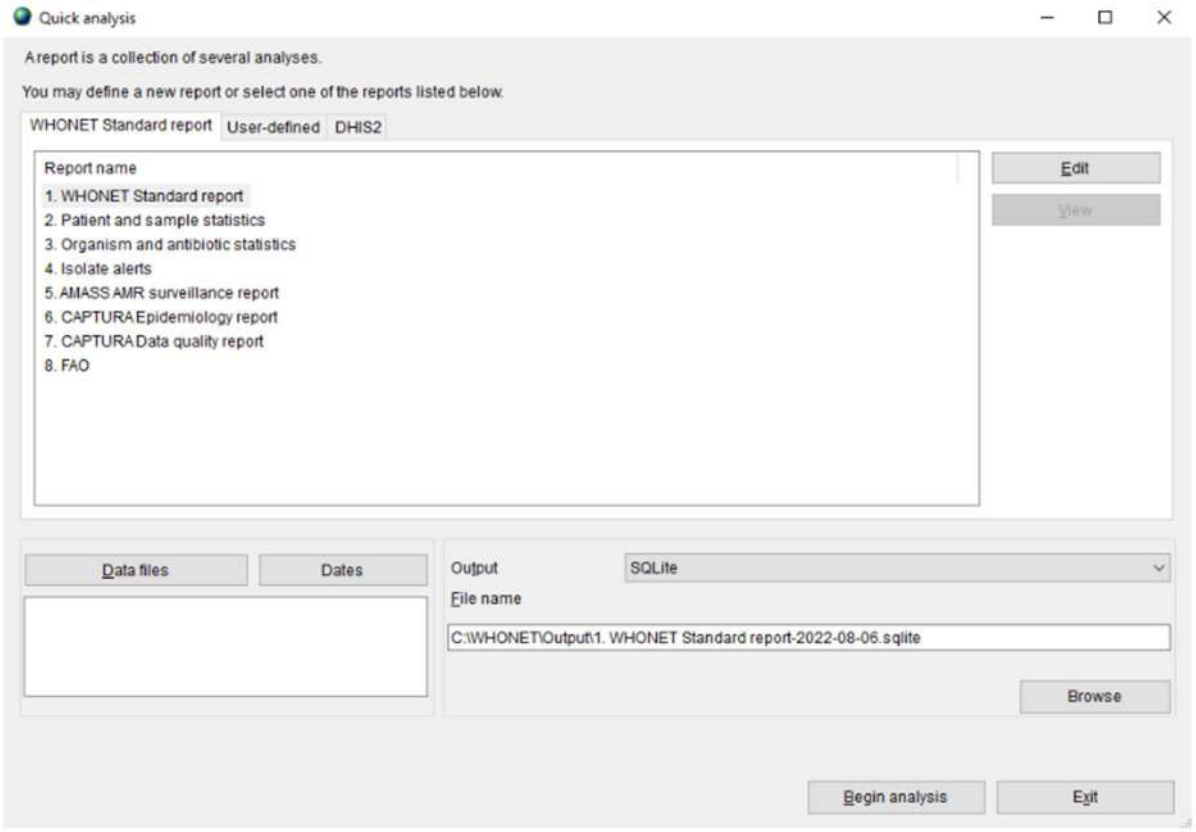
Support for CLSI human (M100, M45, M60, M61, access free resources) and veterinary (VET01, VET03, VET04, and VET06) antimicrobial susceptibility test breakpoints.



Support for EUCAST human antimicrobial susceptibility test breakpoints



New option for saving WHONET data as SQLite files



BacLink – Data import configuration

This tutorial includes

What is BackLink?

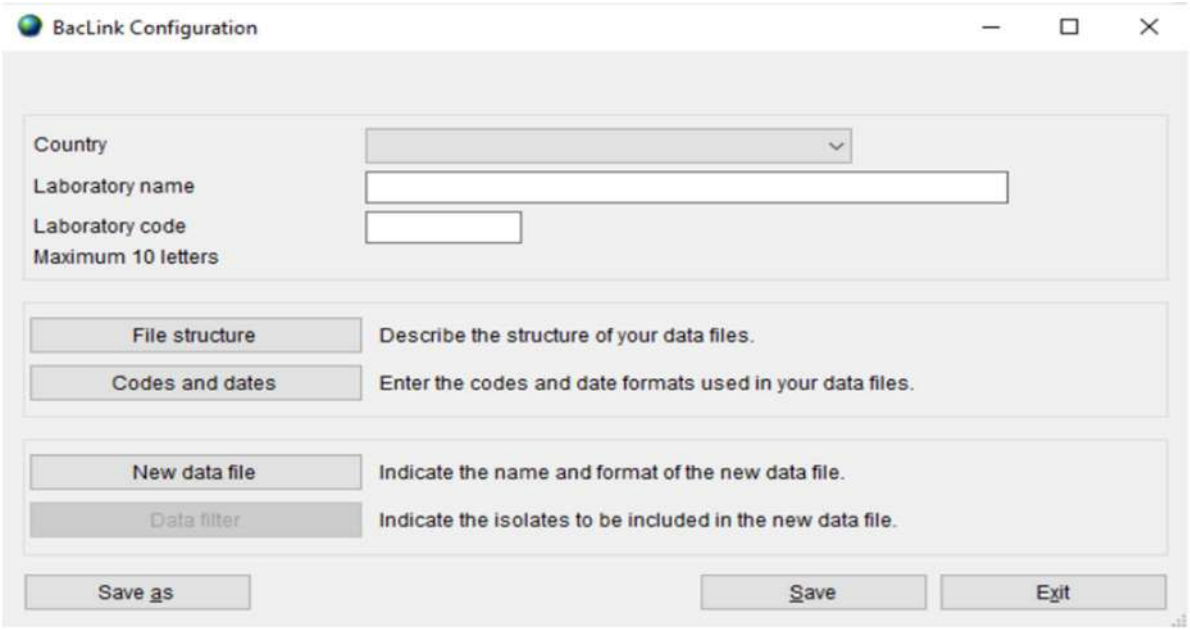
What systems are compatible with BackLink?
How does BackLink work?
What's the next step?

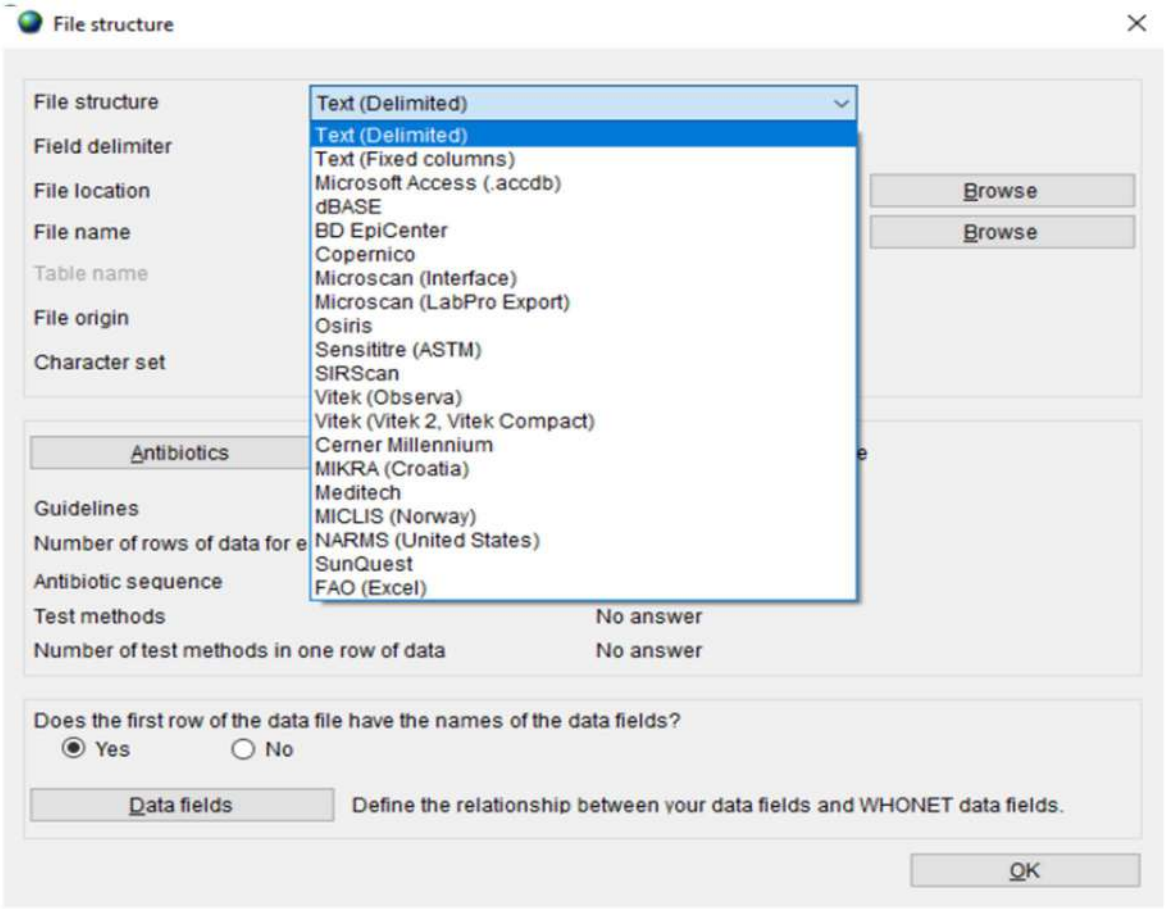
Part 1. What is BacLink?

Many laboratories around the world already have well-established computer databases that meet the day-to-day needs of clinical reporting, specimen processing, and long-term data storage. Unfortunately, most of these systems have limited capacity for sophisticated data analysis. It is in these areas that WHONET is a valuable supplement to existing systems.

This purpose of the BacLink software is the conversion and standardization of microbiology data from existing systems into WHONET. You can convert data on a weekly, monthly, or *ad hoc* basis, or in a number of institutions, it has also been possible to automate and schedule the entire process. Both WHONET and BacLink are available free-of-charge from the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance website: https://whonet.org.

By converting data to WHONET, laboratories have the benefits of: 1. flexible data analysis capabilities; and 2. the ability to share data with other laboratories, for example in a national surveillance network.


BacLink Configuration
Country
Laboratory name
Laboratory code
Maximum 10 letters
File structure
Describe the structure of your data files.
Codes and dates
Enter the codes and date formats used in your data files.
New data file
Indicate the name and format of the new data file.
Data filter
Indicate the isolates to be included in the new data file.
Save as
Save
Exit


File structure
File structure
Text (Delimited)
Field delimiter
File location
File name
Table name
File origin
Character set
Browse
Browse
Text (Delimited)
Text (Fixed columns)
Microsoft Access (.accdb)
dBASE
BD EpiCenter
Copernico
Microscan (Interface)
Microscan (LabPro Export)
Osiris
Sensititre (ASTM)
SIRScan
Vitek (Observa)
Vitek (Vitek 2, Vitek Compact)
Cerner Millennium
MIKRA (Croatia)
Meditech
MICLIS (Norway)
NARMS (United States)
SunQuest
FAO (Excel)
Antibiotics
Guidelines
Number of rows of data for e
Antibiotic sequence
Test methods
No answer
Number of test methods in one row of data
No answer
Does the first row of the data file have the names of the data fields?
Yes
No
Data fields
Define the relationship between your data fields and WHONET data fields.
OK

ANTIBIOTIC STEWARDSHIP PROGRAMME (AMSP)

Bacteria have become increasingly resistant to various antimicrobials. AMR is a global threat to public health.

STEWARDSHIP:

It is defined as "the careful and responsible management of something entrusted to one's care", (originally applied in health care settings).

"ANTIMICROBIAL STEWARDSHIP" (AMS):

AMS is one of the 3 "pillars" of integrated approach to health systems strengthening. Other pillars are infection prevention & control and medicine & patient safety.

AMS principles also apply to use of antimicrobials in animal and agriculture sectors (focus on public health challenges of bacterial resistance to antibiotics although viral, fungal and parasitic resistance are in increasing levels).

GLOBAL ACTION PLAN ON AMR:

Sets out five strategic objectives.

Improve awareness and understanding of AMR through effective communication, education and training.

Strengthen the knowledge and evidence base through surveillance and research.

Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures.

Optimize the use of antimicrobial medicines in human and animal health.

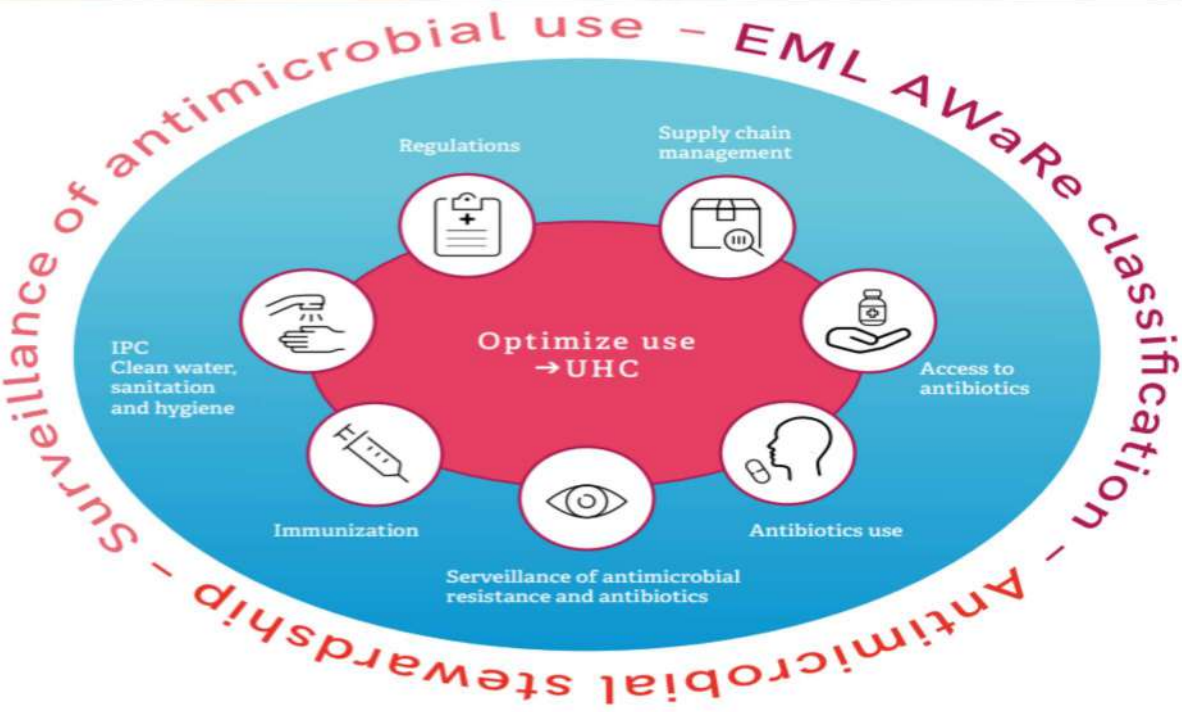
Develop the economic case for sustainable investment that takes account of the needs of the all countries and increase in new medicines, diagnostic tools, vaccines and other interventions.

ANTIMICROBIAL STEWARDSHIP PROGRAMME:

It optimizes the use of antimicrobials, improve patient outcomes, reduce AMR and health-care-associated infections and save the healthcare costs among others.

AIMS:

- to optimize the use of antibiotics;
- to promote behavior, change in antibiotic prescribing and dispensing practices;
- to improve quality of care and patient outcomes;
- to save on unnecessary health-care costs;
- to reduce further emergence, selection and spread of AMR;
- to prolong the lifespan of existing antibiotics;
- to limit the adverse economic impact of AMR; and
- to build the best-practices capacity of health-care professionals regarding the rational use of antibiotics.

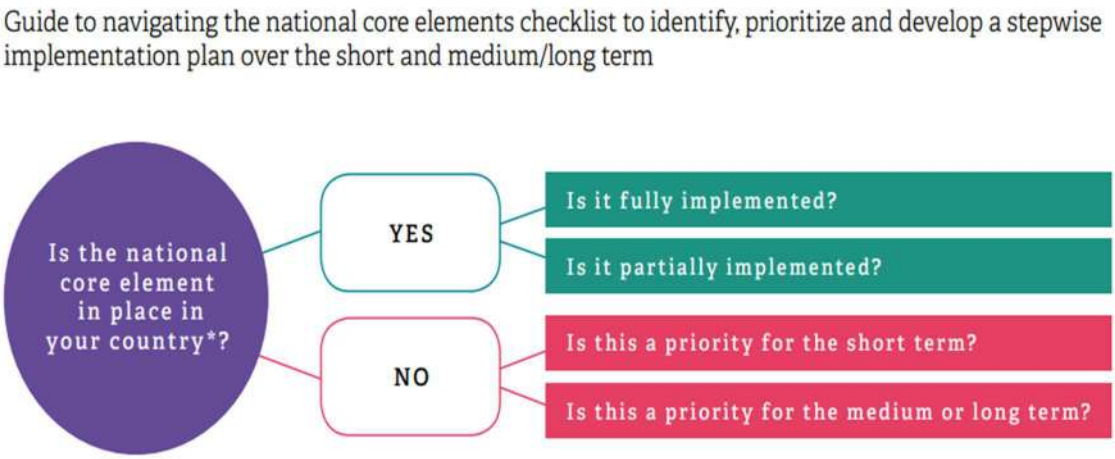


AMS PROGRAMME – NATIONAL (STATE / REGIONAL) CORE ELEMENTS:

It is a guide for AMS program implementation at national level.

The core elements have been stratified as basic (requires fewer resources) and advanced (require more resources).

HOW TO USE THE NATIONAL CORE ELEMENTS LIST:



HEALTH-CARE FACILITY LEADERSHIP, AMS COMMITTEE AND/OR AMS TEAM:

Formulating AMS Team:

Antimicrobial stewardship team (AMS team) is a multidisciplinary committee which is responsible for framing, implementing and monitoring the compliance to antimicrobial policy of the hospital.

AMS team is led by the antimicrobial steward who may be an infectious disease physician or infection control officer or clinical microbiologist.

Antimicrobial steward is the central driving force behind this program. A larger hospital may require more than one antimicrobial steward.

Other members of AMS team include stewardship nurses, clinical pharmacists and officer in-charge of pharmacy.

Infrastructure Support Infrastructure support is essential to initiate appropriate pathogen-directed antimicrobial agent at the earliest. Support from the microbiology laboratory

Automations: Facility for automated culture (e.g., BACTEC, BacT/ALERT or Virtuo), identification (MALDI-TOF) and sensitivity (e.g., VITEK) should be available. This reduces the turn-around time to 24– 48 hours; compared to conventional cultures which takes 2–5 days

Biomarkers: Facility for testing biomarkers such as procalcitonin and C-reactive protein (CRP) must be available (discussed subsequently).

Molecular tests: Facility to perform rapid molecular tests must be available; e.g. Biofire FilmArray multiplex PCR.

Emergency laboratory: Emergency lab functioning round the clock is a marker of a quality microbiology laboratory.

FRAMING ANTIMICROBIAL POLICY

Every hospital should frame their own hospital antimicrobial policy which is usually a pocket handbook, comprising of system/syndrome wise indications for antimicrobial choice and their dosage.

It should be prepared by AMS team after discussing with all the clinicians, microbiologists and administrators.

The policy must be compliant to the standard national and international antimicrobial guidelines and local antibiogram pattern.

Common consensus between all clinicians must be arrived, before framing the policy; which facilitates better adherence to policy.

IMPLEMENTING AMS STRATEGIES

Two types of strategies are available for implementing AMSP.

Front end strategies (formulary restriction)

Back-end strategies (prospective audit and feedback).

FRONT END STRATEGY (FORMULARY RESTRICTION)

This involves classifying antimicrobial agents into restricted, semi-restricted and non-restricted antimicrobials with indications for their use combined with an approval system regulated by the AMS team (Table 26.1).

This strategy sounds more attractive, impact is immediate and appears to be the most ideal way to achieve antimicrobial stewardship, but practically implementing formulary restrictions is challenging and a difficult task.

It creates a lot of confusion as it directly compromises the clinician's freedom to choose antimicrobials.

More so availability of the AMSP consultants to give approval all the time further complicates the problem, especially in emergency situations.

BACK-END STRATEGY

This is carried out by prospective audit and feedback. Though difficult to perform, but it is the most effective strategy to implement AMSP.

The AMS team goes for stewardship round during which they discuss with the clinical team in detail about the compliance to the antimicrobial policy in terms of appropriateness of the antimicrobials used, dosage with renal adjustment, compliance to susceptibility report, etc. The clinical team gives justification about the noncompliance occurred, if any.

The prospective audit and feedback is a mutually agreed upon constructive discussion between AMS team and the clinical team on the cases with daily follow up.

Although the back-end strategy is more labor-intensive, it has several advantages:

It is more widely practiced.

It is more easily accepted by clinicians.

It provides a higher opportunity for educating and training health care professionals.

Impact is delayed but sustainable improving the overall quality of antimicrobial prescribing practice.

MONITORING THE COMPLIANCE TO ANTIMICROBIAL STEWARDSHIP PROGRAM

It is said that "If you cannot measure it, you cannot improve it". Measurement of the compliance to AMSP is achieved by looking at both process and outcome indicators.

Policy adherence indicator (process indicator): This is achieved by conducting antimicrobial stewardship audit as described under backward strategy. Both prescription and administrative compliance can be calculated.

2. Antimicrobial usage outcome indicators such as defined daily dosage (DDD) and days of therapy (DOT). These indicators are used to estimate the antibiotic consumption. They are discussed below

3. AMR outcome indicator: The change in AMR pattern is analyzed by conducting periodic AMR surveillance.

4. Clinical outcome indicators such as morbidity (e.g. length of stay) and mortality (e.g. infection-related deaths) indicators.

5. Financial outcome indicators such as antimicrobial cost per patient day or per year or per admission.

RATIONAL USE OF ANTIMICROBIAL AGENTS

When prescribing antimicrobial agents,

the clinicians should consider the following advice. Prescribe Only when Indicated Prescribe antibiotics only when it is indicated. There are various conditions where antibiotics are not required.

Diarrhea: Oral rehydration solution is the mainstay of treatment, not antibiotics. More so, the most common cause of diarrhea is of viral etiology.

Upper respiratory tract infections such as common cold and sore throat, where the primary cause is viral infections (except when bacterial infections such as streptococcal sore throat or diphtheria are strongly suspected).

When an **alternative diagnosis** is suspected/confirmed such as dengue, chikungunya, malaria, etc.

Prophylaxis: Routine antibiotic prophylaxis should not be given to prevent infection, except for particular situations such as cotrimoxazole prophylaxis in HIV infected individuals.

ESCALATION VS DE-ESCALATION APPROACH:

There are two approaches by which antimicrobial agents are prescribed—escalation and de-escalation.

The approach needs to be chosen based on local antimicrobial resistance pattern and the spectrum of activity of the antibiotic.

Antibiotics prescribed for an organism can be ranked based on their spectrum of activity and local antimicrobial resistance pattern.

For example; In hospital X, the antibiotics given for Gram-negative organisms such as E. coli are ranked according to decreasing order of susceptibility: colistin (rank-1) → tigecycline → carbapenems → piperacillin-tazobactam → cefoperazone sulbactam → amikacin → cefepime → ceftazidime → cotrimoxazole → ceftazidime → ciprofloxacin → ceftriaxone (lowest rank).

MIC-GUIDED THERAPY:

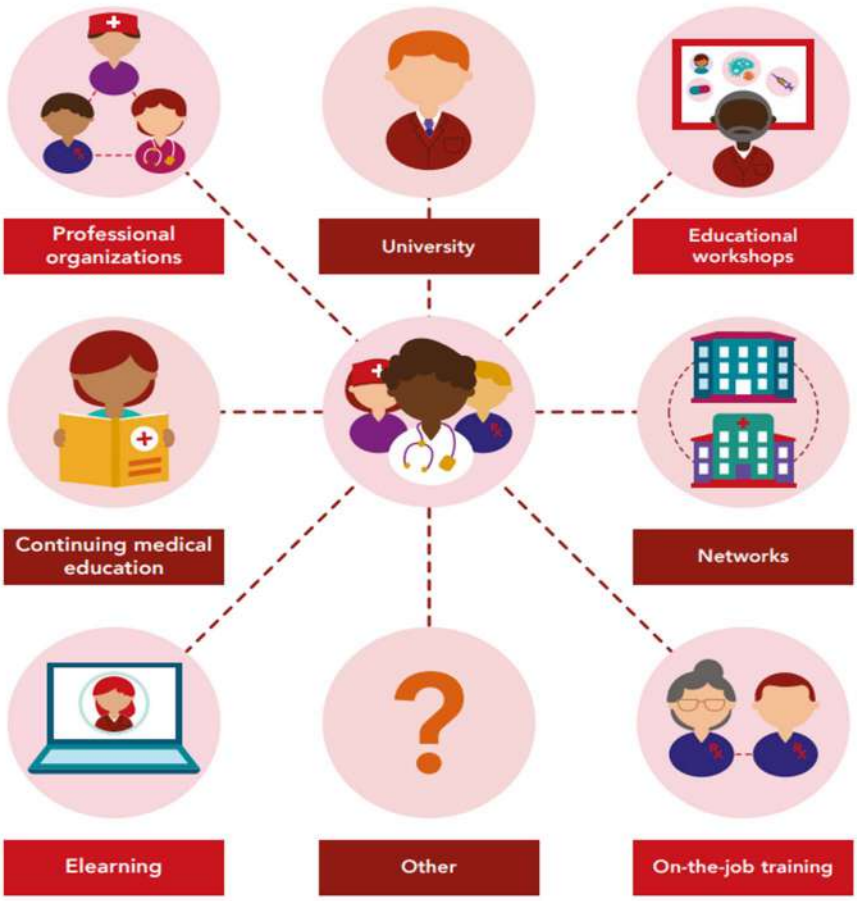
The AST can be performed by disk diffusion or by MIC (minimum inhibitory concentration)–based method; the latter being more accurate and reliable. There are certain situations, where the antibiotic treatment is MIC-guided.

Clinical conditions such as endocarditis, pneumococcal meningitis/pneumonia, etc.

Vancomycin for S. aureus: Vancomycin should be avoided if MIC is >1 µg/mL.

EDUCATION AND TRAINING:

Education and training delivery modes for AMS-related competencies



TEACHING METHODS FOR AMS INTERVENTIONS

PASSIVE

- Printed educational materials
- Clinical practice guidelines
- Formal lectures
- Seminars, conferences
- Educational courses
- Reminders
- Distance learning, e-learning

ACTIVE

- Discussion groups, journal clubs
- Educational outreach visits and academic discussions
- Audit and feedback
- Interactive role play, case scenarios, interactive educational workshops
- Sequenced educational sessions (learn-work-learn), learning by working (practice)
- Distance learning, e-learning.

CONCLUSION:

As the Microbiologist we should make our full effort to prevent antimicrobial resistance and to avoid unnecessary prolonged hospital stay and unjudicious use of antibiotics.

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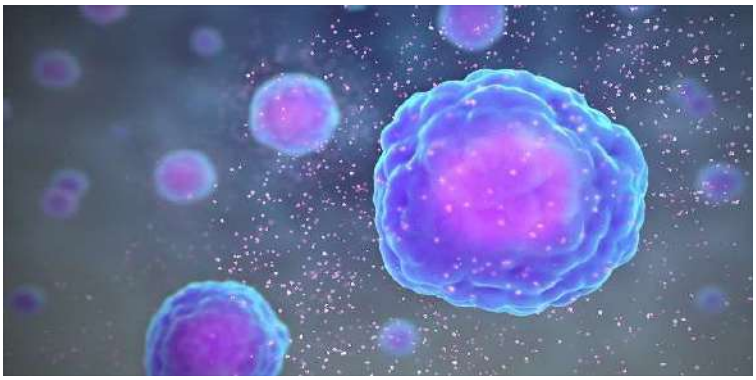
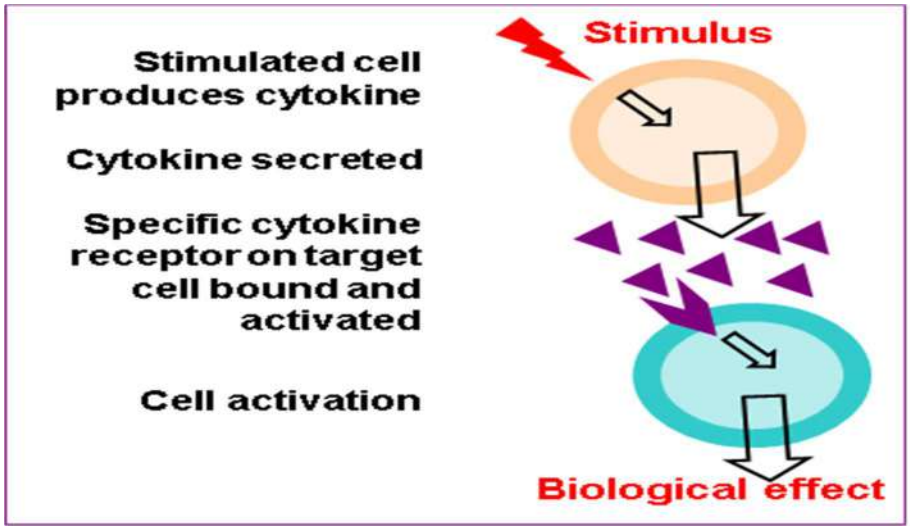
ROLE OF CYTOKINES IN IMMUNE RESPONSE



Dr. Hema Preethi, Postgraduate, Dept. of Microbiology, Guntur Medical College, Guntur

Cytokines are chemical substances which serves as a messenger, mediating interaction and communication between various cells of immune system.
The mechanism of innate immunity was discovered by Elie Metchinknoff- awarded noble prize in physiology or medicine in 1908.He coined phagocytic cells.
It has a high degree of alpha helix structure but, no beta structure.



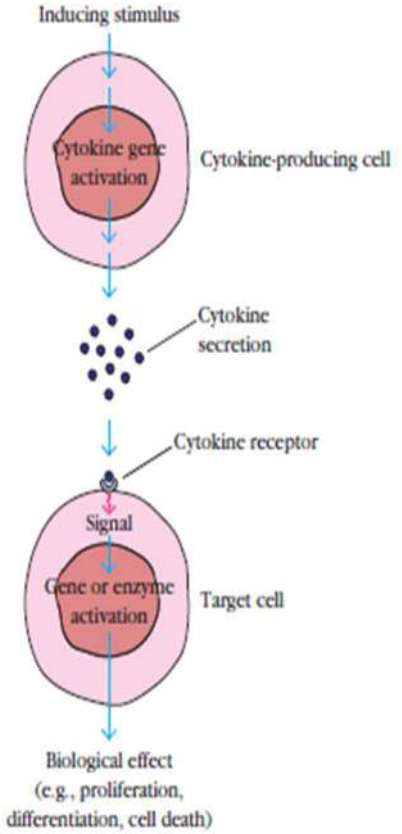
FAMILY OF CYTOKINES-Six families

Cytokines	Action
Interferon family	Antiviral proteins
Chemokine family	Direct cell migration, adhension and activation
Tumour necrosis factor family	Regulate inflammatory and immune responses
Interleukin family	Variety of actions dependent upon interleukin and cell type
Haematopoietins	Promote cell proliferation and differentiation
Transforming growth factor beta family	Regulation of immune cells

CLASSES OF CYTOKINES

CLASS	FUNCTION
LYMPHOKINES	Produced by lymphocytes
MONOKINES	Produced by monocytes and macrophages
INTERLEUKINS	Produced by white blood cells and acting on the same or different WBC s
CHEMOKINES	Involved in chemotaxis and other leukocyte behavior

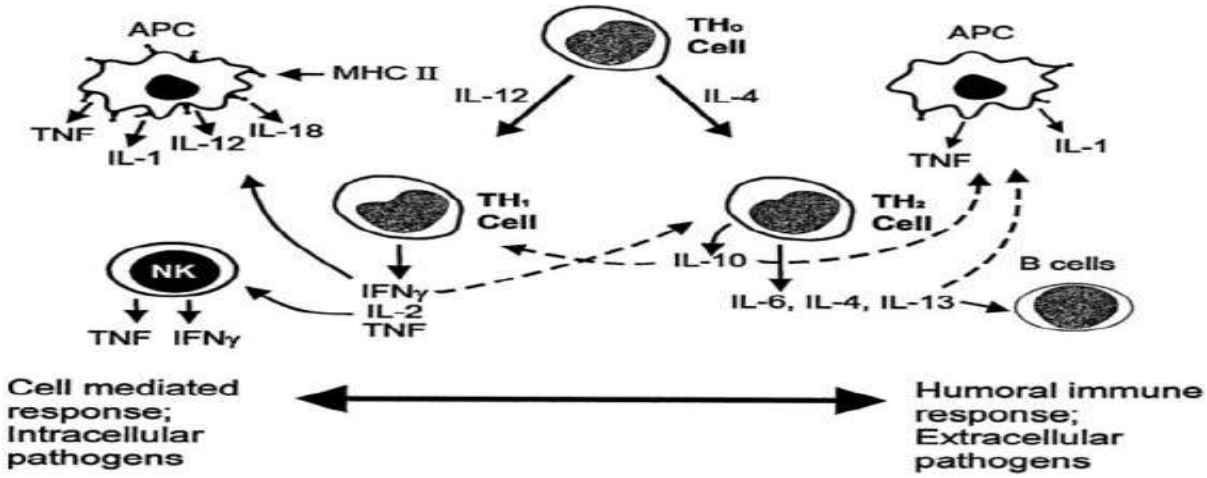
INDUCTION-CYTOKINE



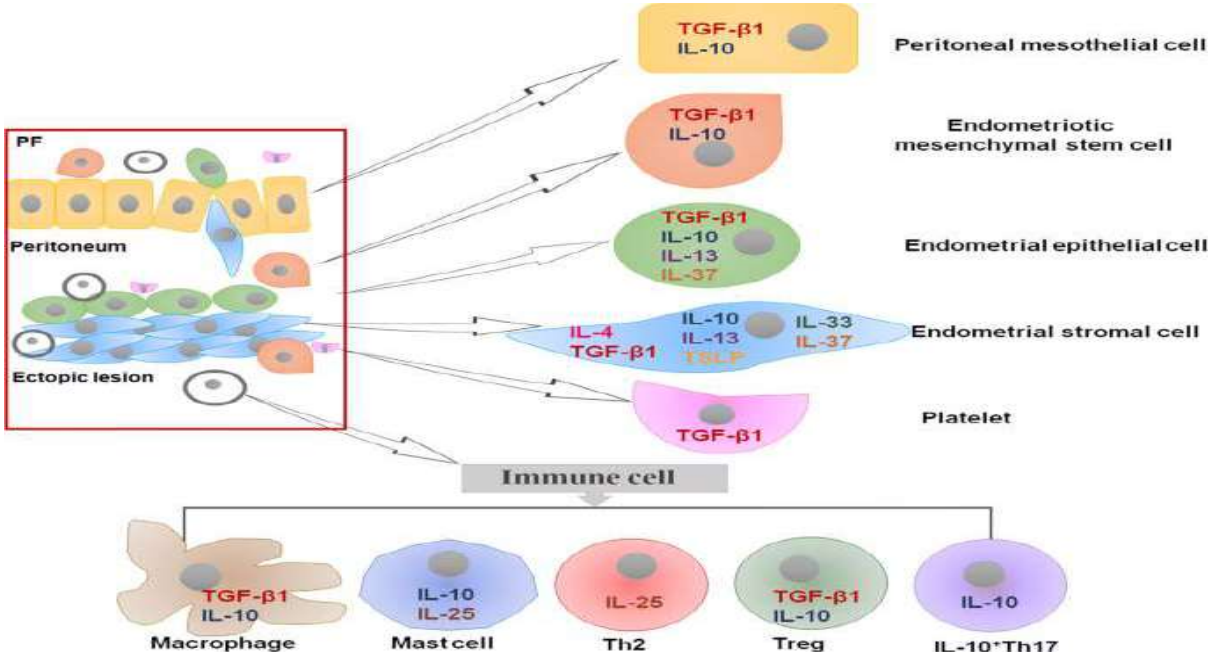
FUNCTION OF CYTOKINES

1.Promote development of cellular and humoral responses of adaptive immunity.
2.Promotes various responses of innate immunity.

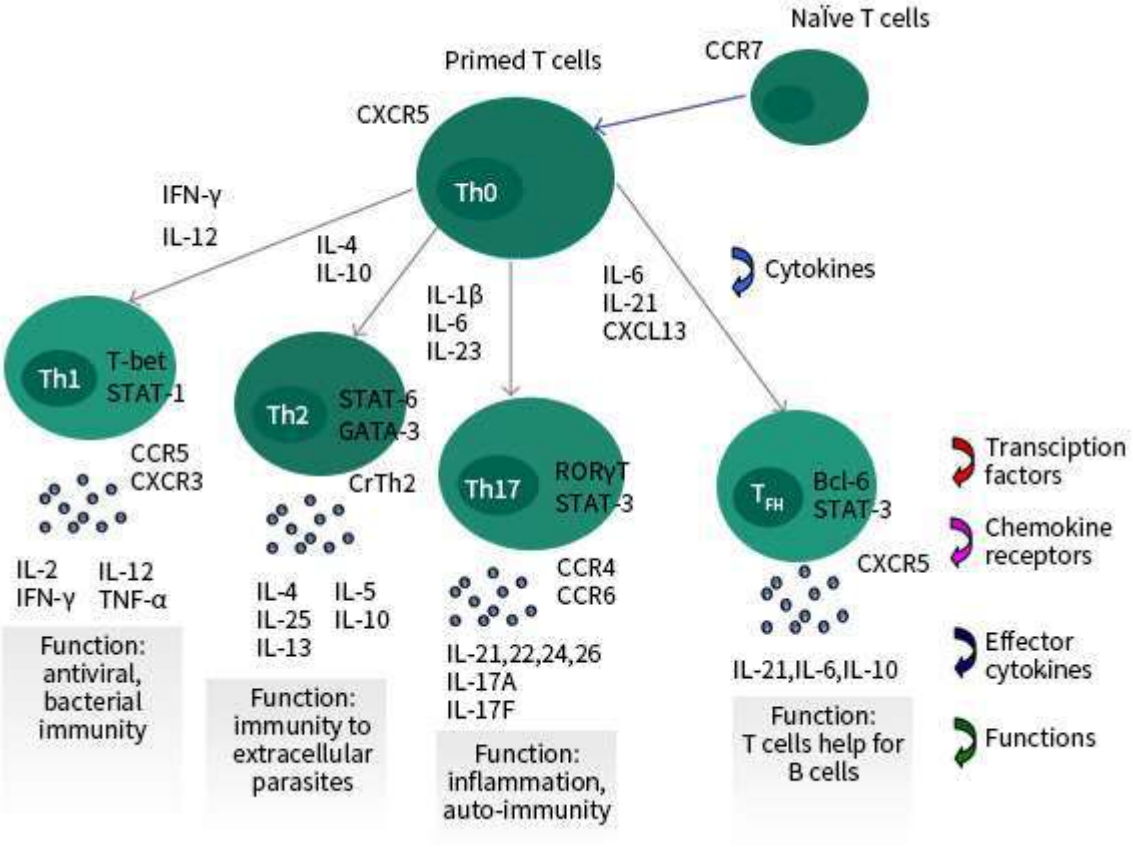
IMMUNE RESPONSES OF CYTOKINE



SOURCES OF CYTOKINES



MECHANISM OF CYTOKINE



PROPERTIES OF CYTOKINES

Compared to growth factors and hormones,

Growth factors are produced consistently while cytokines are inducible

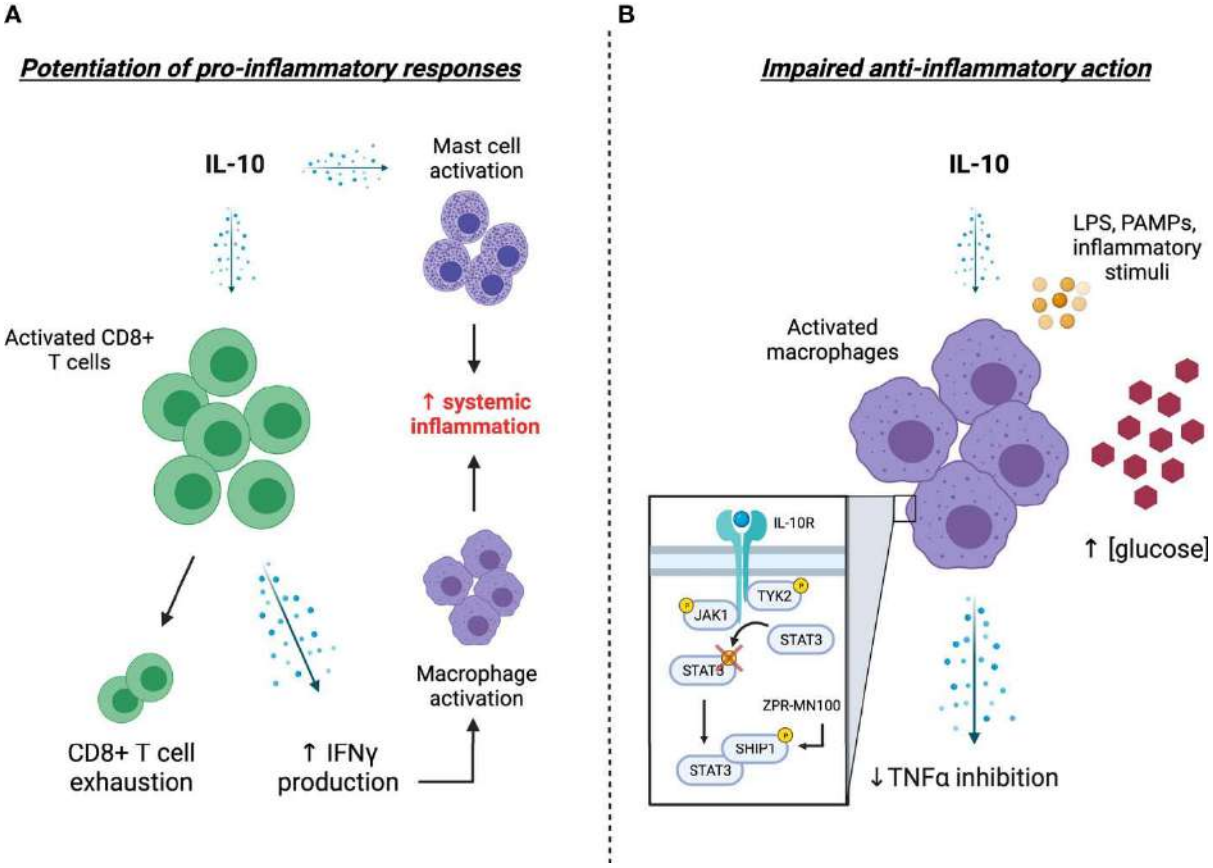
Hormones have mostly endocrine effect and also has paracrine and autocrine effect

Pleiotrophy and redundancy effect

Synergy and antagonism effect

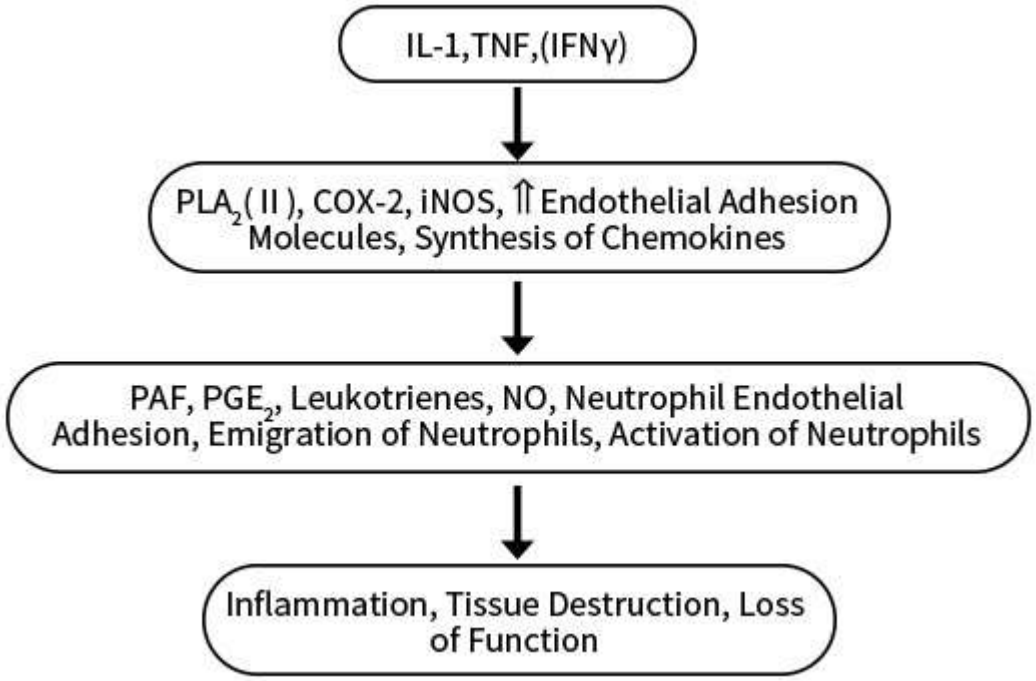
Cascade effect

PRO-INFLAMMATORY AND ANTI-INFLAMMATORY EFFECT



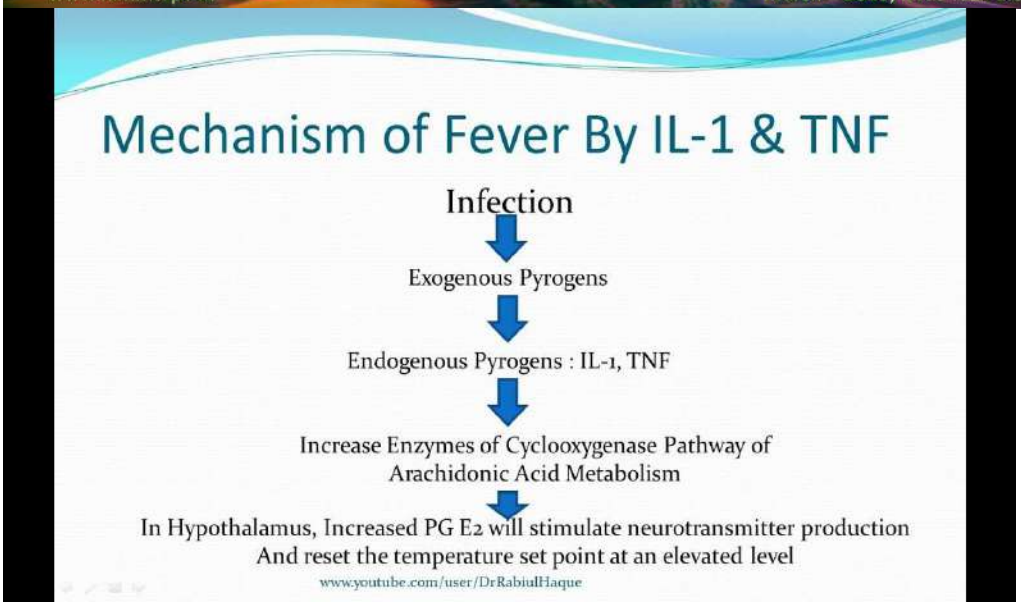
PROINFLAMMATORY CYTOKINES

Macrophages on exposure to inflammatory stimuli secret, cytokines such as TNF, IL1, IL6, IL8, IL12 and also releases Chemokine's, Leukotriene ,Prostaglandins and Complements. Cytokines produced in appropriate amount leads to inflammatory response, when produced in dysregulated manner leads to toxicity.



1.TNF-aplha
Stimulates an acute immune response.
Responsible for septic shock
Induces vasodilation and loss of vascular permeability
In connection with IL 17-with increased CxCR2 dependent neutrophil migration, induces the inflammatory response leading to excess release of TNF-plays an important role in Inflammatory bowel disease, Rheumatoid-arthritis, asthma, carcinoma, psoriasis and autoimmune diseases.

2.IL1
IL1 alpha and beta binds to IL1 receptor.
IL1 beta synthesized as precursor, cleaved by inflammasome activated caspase 1.
IL1 beta-endogenous pyrogen, released as immune response to infections, lesions and stress.
IL1 alpha synthesized de-novo, actively secretly or passively released from apoptotic cells.



3.IL 6
Pleiotropic cytokine- has both proinflammatory and anti-inflammatory functions.
B cells converted to plasma cells via IL6.
It activates cytotoxic T cells and regulates bone homeostasis.
It is implicated in Crohn's disease and rheumatoid arthritis.
It is an endogenous pyrogen which also promotes fever.
4.IL12
It is a Heterodimeric cytokine which hasP35 and P40 subunits.
Produced by monocytes, macrophages and other APCs.
It is essential for fighting infectious diseases and carcinoma.
Deletion within P40 gene observed in patients suffering from concurrent multiple bacterial infections.
5.IL18
It is an inducer of IFN gamma production.
It synergizes with IL12 to Activate T cells and NK cells.
It is not a pyrogen.
Lack of fever induction is explained by fact that IL18 signals through MAPK p38 pathway instead of NK-KB pathway.
6.IL 23
It is a T cell activator, involved in various disease ranges from psoriasis to schizophrenia.
It is an INF γ inducer and induces inflammation.
7.IL 27
It is produced in early monocytes and macrophages.
Due to impaired INF γ production it is responsible for bacterial and parasitic infections.
It inhibits differentiation of Th 17 cells.
It has an inflammatory property.
It Has P28 subunit and Epstein Barr virus induced gene 3.

ANTI-INFLAMMATORY CYTOKINES

1.IL10

Produced by activation of B macrophages, B and T cells.

It suppresses MHC 2 expression and inhibits APCs.

Inhibits the production of INF γ by Th1 and NK cells and induces the growth, differentiation and secretion of immunoglobulins by B cell.

Decreased IL10 leads to development of GI pathologies.

2.TGF-Beta

It is a potent suppressor of Th1 and Th2 cells.

It is implicated in hematopoiesis and has a crucial role in embryogenesis, tissue regeneration, cell proliferation and differentiation.

3.Chemokines

It is a heparin binding cytokine

It has role in promoting angiogenesis.

4.CXCL1 and CXCL2

They are macrophage inflammatory protein 2α.

Secreted by monocytes and macrophages for recruiting neutrophils and hematopoietic stem cells.

Promotes development of tumor such as melanomas.

5.CXCL8(IL8)

Is a chemoattractant for neutrophils.

Keratinocytes, endothelial cells, eosinophils and basophils responds to this chemokine.

Responsible for inflammatory diseases such as psoriasis, cancer and Crohn's disease.

6.CXCL9(MIG)

Known as monokine induced by γ interferon.

It inhibits neovascularization.

It has antitumor and antimetastatic effect.

7.CXCL10(IP 10)

It is interferon induced protein 10.

Secreted by monocytes, macrophages and also by fibroblast and endothelial cells.

8.CXCL11(IP9)

It is an inducible cytokine.

It mediates T cell.

Inhibits angiogenesis and tumor formation.

OTHER USES OF CYTOKINE

Genetic defects in cytokines, their receptors, or the molecules involved in cytokine directed signal transduction led to immunodeficiency disorder.

Pathogenesis of many diseases is characterized by increased expression of cytokines

E.g., Septic shock

Toxic shock syndrome

Cancers

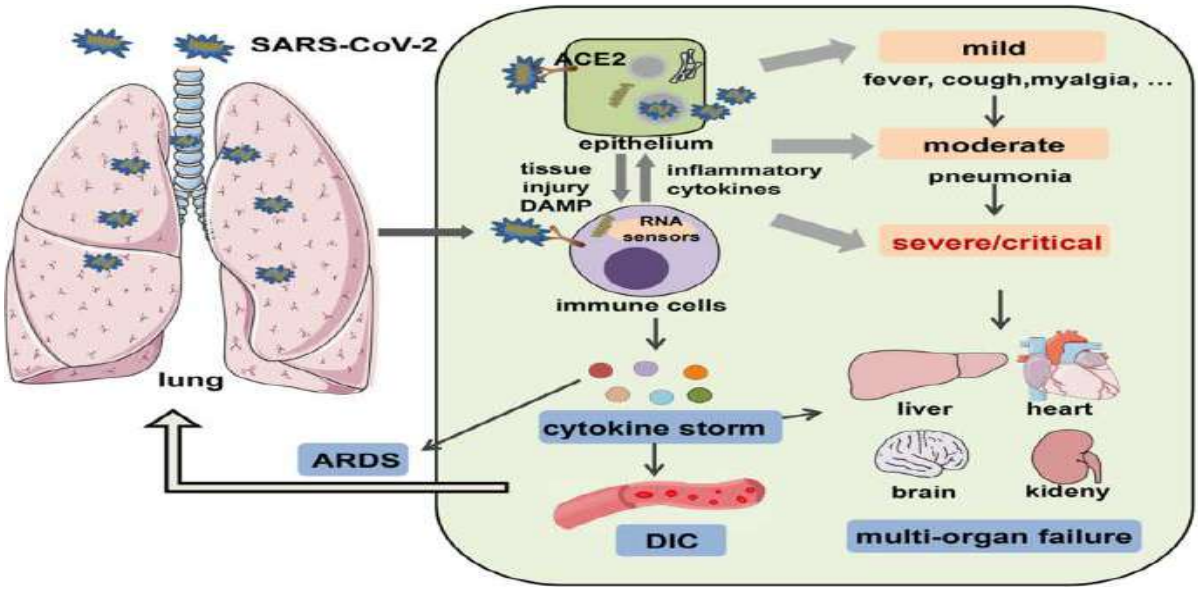
TOXIC SHOCK SYNDROME:

TSST-1 gets absorbed into circulation from the tampons, then being a superantigen it stimulates the T-cells nonspecifically (by binding to Vβ region of T-cell receptor) causing excessive production of cytokine (cytokine storm), leads to potentially fatal multisystem disease.

Toxin causes capillary leak, aggressive parenteral fluid replacement and vasopressor to reverse hypotension should be initiated at the earliest.

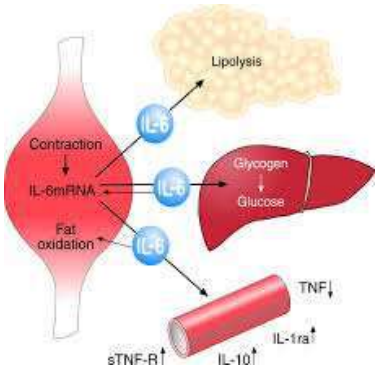
CYTOKINE STORM IN COVID 19

When cytokine produced in excess it leads to hypercytokinemia, causes significant damage to body tissues and organs.

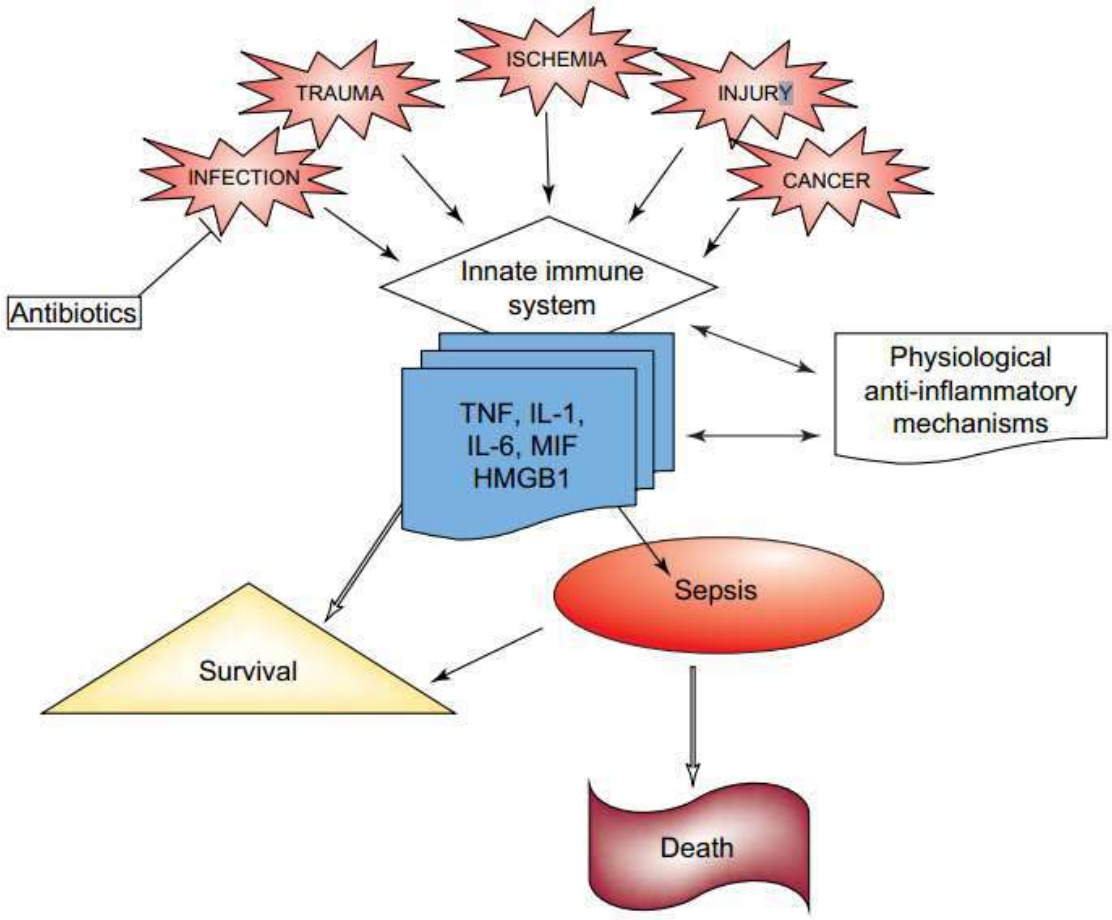


CYTOKINE STORM IN COVID 19

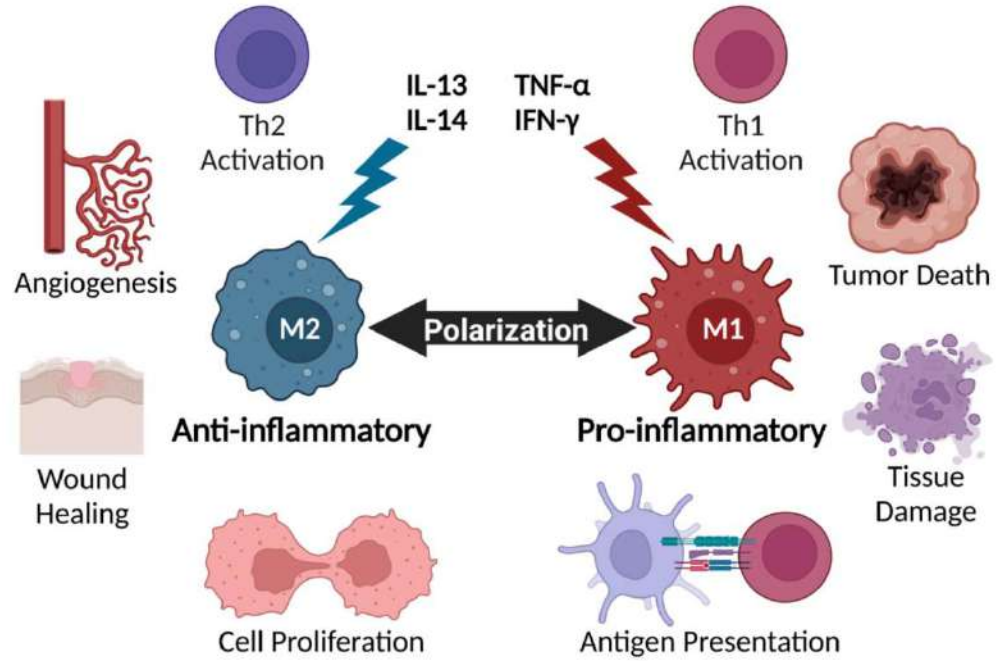
ROLE OF CYTOKINE IN EXERCISE



ROLE OF CYTOKINE IN SEPSIS

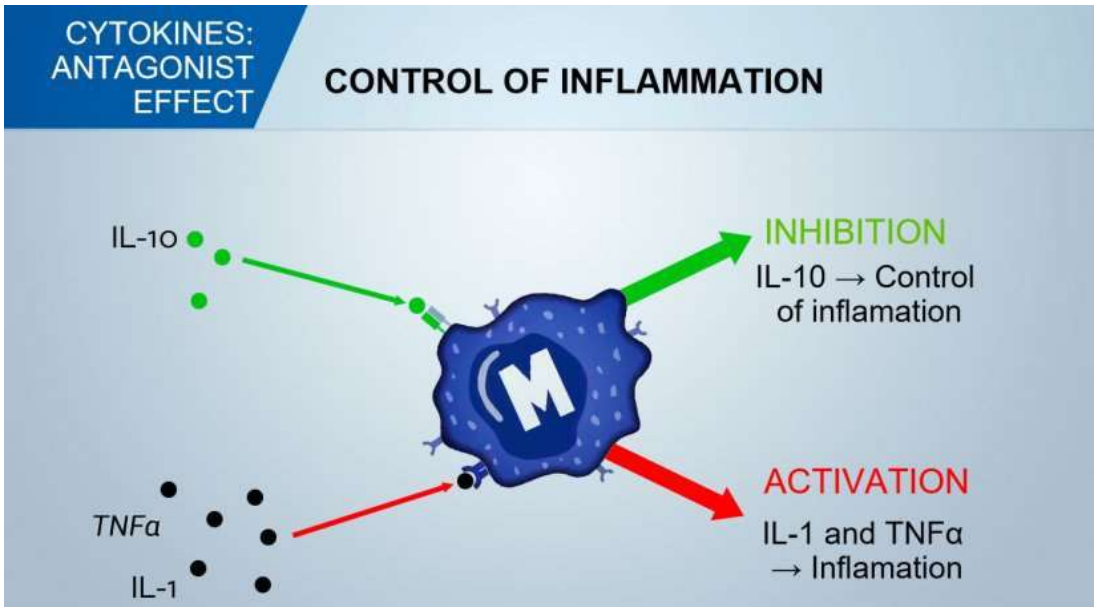


ROLE OF CYTOKINE IN CANCERS



ANTAGONITIC EFFECT OF CYTOKINE

A number of proteins that inhibit the biological activity of cytokines. These proteins act in one of two ways: either they bind directly to a cytokine receptor but fail to activate the cell, thus blocking the active cytokine from binding, or they bind directly to the cytokine itself, inhibiting its ability to bind to the cognate receptor.



ROLE OF CYTOKINE IN TREATMENT MODALITY

INTERFERON-α

IFN α(also known by its trade names Roferon and Intron A) has been used for the treatment of hepatitis C and hepatitis B.

A type of B-cell leukemia known as hairy-cell leukemia responds well to IFN.

CML, a disease characterized by increased numbers of granulocytes, begins with a slowly developing chronic phase that changes to an accelerated phase and terminates in a blast phase, which is usually resistant to treatment. IFN is an effective treatment for this leukemia in the chronic phase.

INTERFERON-β
IFN β has emerged as the first drug capable of producing clinical improvement in multiple sclerosis (MS).

Treatment with IFN β provides longer periods of remission and reduces the severity of relapses.

MRI studies of CNS damage in treated and untreated patients revealed that MS induced damage was less severe in a group of IFN β treated patients than in untreated ones.

INTERFERON-γ
IFN γ has been used, with varying degrees of success, to treat a variety of malignancies that includes non-Hodgkin's lymphoma, cutaneous T-cell lymphoma and multiple myeloma.

THERAPUETIC USES OF CYTOKINES

AGENT	NATURE OF AGENT	CLINICAL USE
Enbrel	Chimeric TNF-receptor/IgG constant region	Rheumatoid arthritis
Remicade or Humira	Monoclonal antibody against TNF α receptor	Rheumatoid arthritis, Crohn's disease
Roferon	Interferon α-2a	Hepatitis B, Hairy-cell leukemia, Kaposi's sarcoma, Hepatitis C
Intron A	Interferon α-2b	Melanoma
Betaseron	Interferon β–1b	Multiple sclerosis
Avonex	Interferon β–1a	Multiple sclerosis
Ankinra (kineret)	Recombinant IL-1Ra	Rheumatoid arthritis
Daclizumab	Humanized monoclonal antibody against IL-2R	Prevents rejection after transplantation

VARIOUS ANTI-CYTOKINE STRATEGIES USED BY VIRUSES:

• The generation of viral products that interfere with cytokine secretion.
• The generation of cytokine homologs that compete with natural cytokines or inhibit anti-viral responses.
• The production of soluble cytokine-binding proteins.
• The expression of homologs of cytokine receptors.
• The generation of viral products that interfere with intracellular signaling.
• The induction of cytokine inhibitors in the host cell.

E.g.: EBV IL-10–like molecule (viral IL-10 or vIL-10) that binds to the IL-10 receptor. Just like host-derived IL-10, this viral homologue suppresses TH1-type cell-mediated responses that would be effective in fighting a viral infection.

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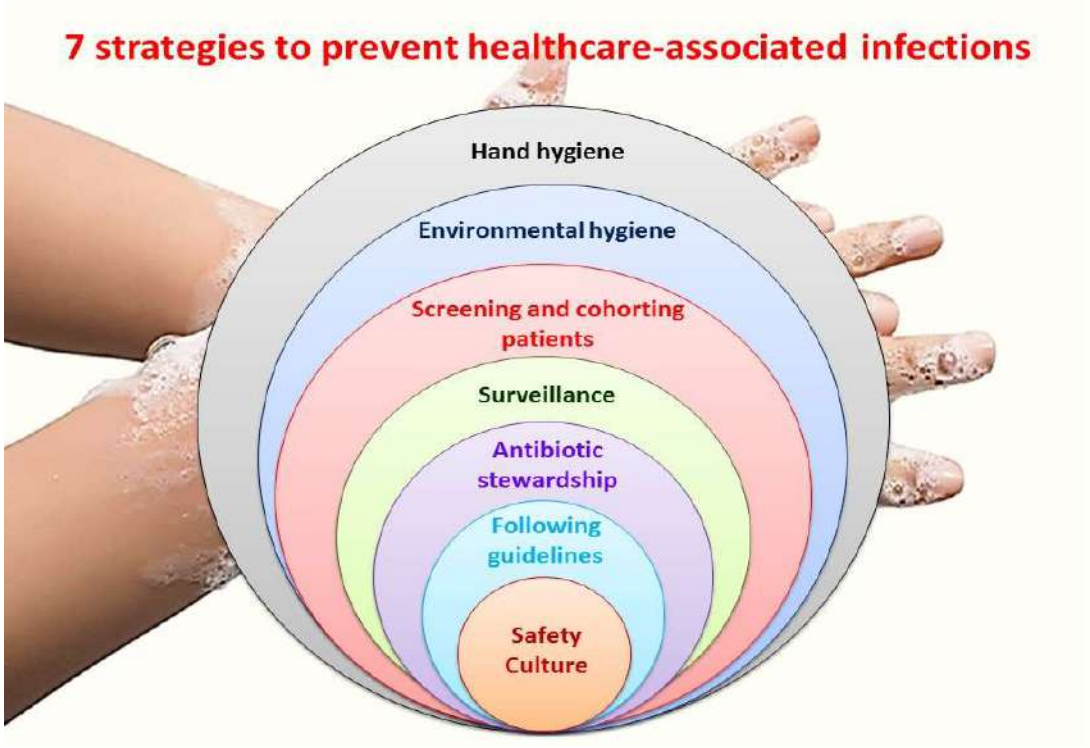
ROLE OF CLINICAL MICROBIOLOGIST IN HOSPITAL INFECTION CONTROL PROGRAM

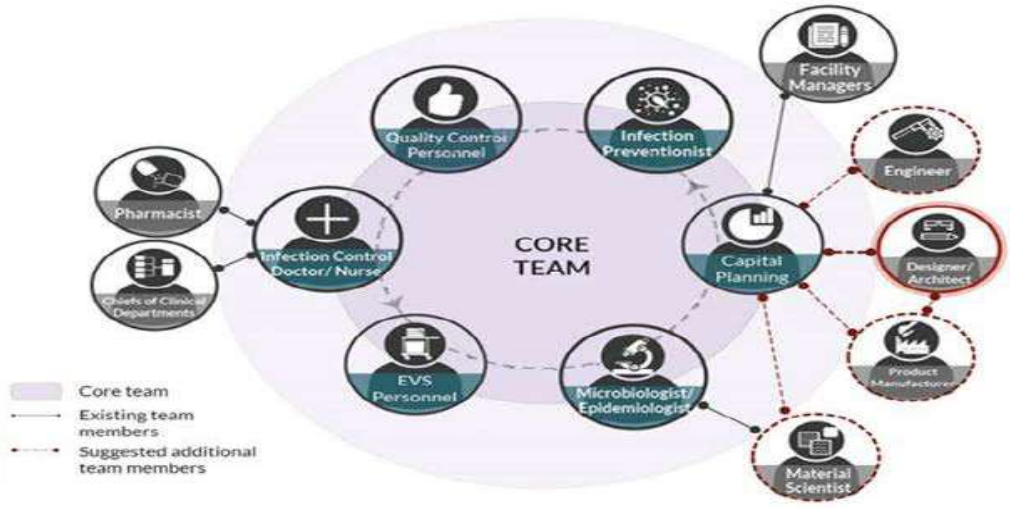
Dr. P.V. Sujitha Priya, Post Graduate, Dept. of Microbiology, Rangaraya Medical College, Kakinada, Andhra Pradesh.

Health care associated infections (HAIs) defined as infections occurring after 48hours of admission or procedures in the hospital and up to 30days after procedures/discharge from the hospital. HAIs continue to present a major problem in hospitals today which increases the mortality, morbidity and burden of cost to the patient. microbiologist has the responsibility of supporting activities related to HAI surveillance, control and prevention.



Clinical microbiologist role is to minimum the occurrence of HAIs in the following seven ways:

1) Participating in hospital wide infection control activities especially those of the hospital infection control committee (0r) service team
2) Recovering and accurately identifying responsible organisms
3) Determining antimicrobial susceptibility of selected HAI pathogens
4) Reporting in timely fashion laboratory data relevant to infection control and participation in HAI surveillance
5)Providing additional studies, when necessary to establish the similarity (or) difference of organisms
6) Providing on occasion, microbiological studies of the hospital environment
7) Training infection control personnel



1) PARTICIPATION IN HOSPITAL WIDE INFECTION CONTROL ACTIVITIES:

A) RELATIONSHIP OF THE MICROBIOLOGIST TO THE INFECTION CONTROL COMMITTEE:

Microbiologist role is very important in assessing the importance of culture data, determining the validity of laboratory techniques used to identify HAI's, designing, implementing investigations and survey projects

Microbiologist primarily engaged in the evaluation of cultures related to infection, because these are the crucial data for successful infection control, the laboratory activities should be closely coordinated with the infection control committee e.g.: The adequacy of the basic techniques for primary isolation, speciation and antimicrobial susceptibility testing should be discussed by the microbiologists.

B) BUDGETARY CONSIDERATIONS:

Microbiologist should have a contingency fund to enable personnel, materials and space to be temporarily assigned to epidemic aid support. an investigation of an outbreak should not be financed by charging individual patients for cultures.

2) ACCURATE IDENTIFICATION OF ORGANISMS INVOLVED IN HAI:

Enterobacteriaceae, Staphylococcus aureus, *Pseudomonas aeruginosa* and CONS remain frequently associated with HAIs. Microbiologist constantly searching for evidence that a common organism spread from patient to patient (or) from staff to patient, this information permitting the successful tracing of organism.

A) COLLECTION AND TRANSPORT OF SPECIMENS:

Specimen collection, transport and handling must be sufficiently high quality to provide valid data. Microbiologist must monitor specimen handling continually and work closely with both inpatient and Ambulatory care units to ensure minimization of the possibility of contaminated specimens.

B) INITIAL EVALUATION OF SPECIMENS:

Assessing specimens at the time they are received in the laboratory is one of the best ways to evaluate their suitability. Repeat specimen collection should be requested for inadequate specimens. Microscopy at the time of specimen submission can help other aspects of

microbiological diagnosis e.g.: Examination of Gram stain for morphology can identify organisms that might be epidemiologically important

C) ISOLATION OF ISOLATES:

Once the specimen has been received, microbiologist must be processed in a way that maximizes the likelihood of recovering older and newer agents causing HAI. Gram positive cocci and Gram-negative aerobic bacilli are the cause for the HAI. among the GNB *Klebsiella, Enterobacter, Pseudomonas* and more recently *Acinetobacter* and *Pseudomonas species* have become increasingly predominant.

D) NEED FOR COMPLETE IDENTIFICATION:

Organism identification routinely is carried to be important to HAI control efforts. Microbiologist constantly searching for evidence that a common organism has spread from patient to patient. Microbiologist should have the capability to identify the Gram-negative bacilli to genus level. Species level identification is made when special problems in a given institution make such information useful for dealing with HAI problems. Incomplete (or) incorrect identification of organisms may obscure real problems and make retrospective epidemiologic investigation impossible. E.g.: *Klebsiella,* Enterobacter group fails to distinguish between two species (*Klebsiella* or *Enterobacter species*) that have different epidemiological patterns of infection within the hospital.

E) NEED FOR ACCURACY AND CONSISTENCY:

Many spurious outbreaks have been traced to inaccurate microbiological procedures
E.g.: Outbreak of staphylococcus aureus infection may be caused by delayed reading of coagulase test resulting in misidentification of coagulase negative organisms as coagulase positive

F) INTRODUCTION OF NEW PROCEDURES:

Microbiologists must consider whether additional laboratory techniques can make testing results more relevant e.g.: Cultures of intravenous catheter tips may be positive because of contamination at the time of catheter removal. In order to avoid these several semiquantitative and quantitative methods for culture of IV catheter have been useful.

G) QUALITY CONTROL:

Quality control program begins with a comprehensive procedure manual that establishes standards for performance, including the definition of acceptable and unacceptable quality of specimens and delay between collection and receipt of the specimen. Minimum standards for identification of isolates, including a list of the equipment and reagents to be monitored and the measures to be made to ensure accurate performance should be provided. Periodic evaluation of skills of all employees, including evening, night, and weakened workers should be included in the program.

3) ACCURATE CHARACTERIZATION OF ANTIMICROBIAL SUSCEPTIBILITY OF HEALTH CARE ASSOCIATED INFECTION PATHOGENS:

A) SELECTION OF STRAINS FOR SUSCEPTIBILITY:

Application of susceptibility tests to bacteria that are doubtfully related to infection must be avoided e.g.: Request for testing of susceptibility should be carefully evaluated when the organisms isolated are endogenous flora present at sites in which they are not normally pathogens.

B) SELECTION OF DRUGS TO ROUTINE AND SPECIAL TESTING:

Microbiologist should undertake the selection of drugs for routine testing after consultation with infection control committee, pharmacy and therapeutics committee. The chosen agents should reflect both the common usage practices of physicians in the hospital.

C) QUALITY CONTROL:

Consistent and accurate identification of organisms over time is necessary for susceptibility data to be useful for clinical and epidemiologic purposes. To avoid errors, microbiologists must maintain quality control procedures for all elements of susceptibility testing process. Special attention must be given to the storage of reagents, control of batch-to-batch variation in media, use of control strains of testing and monitoring of incubation temperatures and atmospheres.

4) TIMELY REPORTING OF LABORATORY DATA AND PARTICIPATION IN SURVEILLANCE OF HAI:

To deal with individual problems of HAIs in the hospital, control measures must be taken as quickly as possible and must be based on accurate assessments of the problems and their causes.

A) SURVEILLANCE:

Review of laboratory records is done and is the most common method for surveillance of hospital infection. More than 80% of infections defined by other criteria as nosocomial may be identified by review of positive cultures by the microbiologist. Computer programs have been developed to identify clusters of infections with the same organism and susceptibilities that occur at the same time in the same ward. Microbiologist must provide clinical data to determine whether organism found in culture indicate infection (or) colonization.

B) REPORTING OF RESULTS:

To facilitate HAI surveillance of all infections requiring isolation (or) notification of public health authorities, a copy of positive culture results should be provided to infection control personnel, occasions for reporting include accidents such as presumptive identification of certain agents in meningitis, isolation of *Salmonella* (or) *Shigella species* from stool specimens.

C) LABORATORY RECORDS:

Microbiologist should maintain the laboratory records in such a way that they facilitate such retrospective epidemiologic investigations and quality control activities. source of each specimen, date of collection, patient identification hospital number, and organisms identified in the final report should be recorded . culture data of inpatients and outpatients should be maintained separately. Records can be maintained in simple, inexpensive and epidemiologically useful bound log books that are kept chronologically for each specimen.

D) RETENTION PERIOD OF RECORDS:

Microbiologist maintain the records in some accessible format for a reasonable period e.g.: computer file, disk storage. Length of time such records can be maintained depends on the hospital size and laboratory work volume, available storage facilities with computer storage it may be possible to maintain data for long periods.

5) ADDITIONAL STUDIES TO ESTABLISH SIMILARITY OR DIFFERENCE OF ORGANISMS:

A) METHODS FOR TYPING OF ISOLATES:

Two methods are there 1) Phenotypic techniques

2) Genotypic techniques

TABLE 1: TYPING SYSTEMS FOR ORGANISMS CAUSING NOSOCOMIAL INFECTION: SOME PHENOTYPIC METHODS AND EXAMPLES OF USE OF SPECIFIC ORGANISMS

Pattern of susceptibility to antimicrobials: *Klebsiella* *Legionella*
Pattern of susceptibility to heavy metals: MRSA *Candida albicans*
Bio typing: *Serratia marcescens* Enterococci
Phage typing: MRSA
Serotyping: *Klebsiella* *Serratia*
Bacteriocin production: *Enterobacter* *Serratia*
Immunoblotting: MRSA-south blot hybridization after protein electrophoresis

TABLE 2: GENOTYPIC METHODS AND EXAMPLES OF USE FOR SPECIFIC ORGANISMS

Plasmid profile analysis: *Serratia marcescens* Coagulase negative staphylococci
Restriction endonuclease analysis: Plasmids-MRSA Chromosomes - *Clostridium difficile*
Ribotyping: *Escherichia coli* MRSA
Pulsed field gel electrophoresis: Enterococci *Pseudomonas aeruginosa*

Analysis of nucleic acid amplification products: DNA typing by PCR assay - *Streptococcus pneumoniae* Whole cell repetitive extragenic PCR- *Acinetobacter*
Nucleotide sequence analysis Hepatitis B virus

B) STORAGE OF STRAINS:

Microbiologist should subculture and save epidemiologically important isolates (from outbreaks or single instances of unusual) stored by placing a small amount of growth on a blank paper disk that is placed in a 2ml glass screwcap vial containing a few granules of silica gel. If the vial is kept tightly closed, isolates may hold up to 6 months, they can be easily retrieved by placing a disk in the broth.

6) OCCASIONAL MICROBIOLOGICAL STUDIES OF HOSPITAL PERSONNEL OR ENVIRONMENT

A) ROUTINE ENVIRONMENTAL SAMPLING:

TABLE 3:

RECOMMENDED FOR ROUTINE PRACTICE:	PROCEDURES NOT RECOMMENDED
Monitoring sterilization	Routine culture surveys of patients
Monitoring dialysis fluids	Routine culture of commercial products that is labelled as sterile
Elective environmental monitoring	Routine culture of blood units
Surveys to investigate a specific problem of patient infection	Routine testing of antiseptics and disinfectants
Surveys for educational purposes	

B) MONITORING OF STERILIZATION:

Microbiologist should check all steam sterilizers at least once each week with suitable live spore preparation. Ethylene oxide gas sterilizer should be checked with each load of items that come into contact with blood (or) tissues. Dry heat sterilizers should be monitored at least once each month.

C) SAMPLING OF INFANT FORMULA AND OTHER FOOD PRODUCTS PREPARED IN THE HOSPITAL:

Infant formula prepared in the hospital kitchen should be monitored on weekly basis. Guideline for interpreting culture suggests that <25 organisms per milli meter indicates no virulent bacteria (e.g.: *Salmonella* and *Shigella*).

D) CULTURE OF DIALYSIS FLUID:

Dialysis fluid should be tested by the microbiologist at least once per month for colony count. If <200 viable organisms per milli litre present in water used to prepare dialysis and <2000 cfu per milli litre for reprocess dialyzers considered as negative

E) ELECTIVE ENVIRONMENTAL MONITORING:

It includes the following:

1) Support for investigation of specific problems of HAI
2) Culture of blood products after a transfusion reaction
3) Culture of parenteral fluids and intravascular therapy equipment
4 Methods for culturing hands and skin
5) Methods for culturing tubes and containers
6) Sampling of respiratory therapy equipment
7) Sampling of air
8) Culture of floors and other surfaces
9) Sampling of water and ice

F) DEVELOPING SELECTIVE MEDIA FOR SURVEYS:

Selective survey media should be used whenever possible for culturing specimens during outbreak investigations. Susceptibility data of unknown strains may be used to identify an appropriate selective medium

G) SURVEY OF EDUCATIONAL PURPOSES:

Sampling techniques that are not directly related to epidemiologic surveys may be useful in educational programs, evidence of contamination of hands, clothing, and equipment may serve to teach the need for effective technique and sanitation.

7) TEACHING MICROBIOLOGICAL ASPECTS OF HAI TO INFECTION CONTROL PERSONNEL:

The key to success in infection control efforts is communication, It is necessary that all involved speak the same language. For this purpose, training infection control personnel in the language of the clinical laboratory is important. Microbiologist must learn some of the concepts of infection control and exposure to newer techniques used for measuring frequency of infection and the concept of colonization versus infection.

REFERENCES:

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