KING SAUD UNIVERSITY

FELLOWSHIP PROGRAMME

IN PATHOLOGY
(MICROBIOLOGY)

Scheme of rotation for Residents training
in
Microbiology speciality (R1 to R5)
Revised 1425 H [2004 G]

King Saud University Fellowship
In Pathology
Rotation scheme (Diagram)
For
Residents Training
In Microbiology

R1		R2		R3		R4		R5	
Histo-4 wks		A – 26 Wks	A4-5wks ---------- A4-5wks ---------- A3 4wks ---------- A2-52ks	C – 5 wks		F – 6 wks		E – 5 wks	
Haema-4 wks				A – 24 wks					
Ch. Path-3 wks					A4 – 4wks	C – 4 wks			
A – 22wks	A5-2 -------- A3-4 -------- A4-4 -------- A2-4	A1 10wks		A1 10 wks	A5-2wks ---------- A3-4wks ---------- A2-4wks	D – 4 wks ---------- E – 4 wks		D – 5 wks ---------- - B – 4 wks ---------- C – 5 wks	
A1 8 wks									
C – 3wks		B – 5 wks		E – 5 wks		B – 3 wks		A – 24 wks	
		C – 5 wks		D – 5 wks		A – 23 wks		A4 –4wks	
D – 3wks		D – 5 wks		B – 3 wks		A1 10 Wks	A4-4wks ---------- A5-2wks ---------- A3-3wks ---------- A2-4wks	A1 10 wks	A5-wks ---------- A3-4wks ---------- A4-4wks
B – 3wks		E – 5 wks		F – 2 wks					
E – 3wks									

Note:

A = Bacteriology
B = Immunology
C = Parasitology
D = Virology
E = Mycology
F = Public Health Microbiology

A1 = General Bench
A2 = Sputum Bench
A3 = Stool Bench
A4 = Blood Culture Bench
A5 = Urine Bench

KING SAUD UNIVERSITY

Scheme of rotation for resident in medical Microbiology (R1-R5).
(Please see attached diagram).

R1:

The resident will spend most of the time in the microbiology department. Besides he will spend the following periods on other related pathology departments.

a. Histopathology - 4 weeks
b. Haematology - 4 weeks
c. Chemical pathology - 3 weeks

The rotation period in the microbiology department will be divided as follows:
(see attached diagram).

- Bacteriology - 22 weeks
- Virology - 3 weeks
- Immunology - 3 weeks
- Parasitology - 3 weeks
- Mycology - 3 weeks

R2:

The trainee will rotate in microbiology subspecialities as indicated in the attached diagram.

- Bacteriology
- Immunology
- Mycology
- Virology
- Parasitology

R3:

Same rotation as in R2 but the candidate will spend two weeks in the public health laboratory (see attached diagram).

R4:

Same rotation as in R3 but will spend more time in the public health laboratory (6weeks) (see attached diagram).

R5:

As in R3. The candidate will be encouraged to develop interest in one of the subspeciality. He or she may do a small project.

KING SAUD UNIVERSITY
FELLOWSHIP PROGRAM IN PATHOLOGY
(MICROBIOLOGY)

Methods and Objectives of training

The residency training period for the fellowship is 5 years (R1-R5). During this period the trainee microbiologist rotates in at least two of three main teaching hospitals in Riyadh i.e. King Khalid University Hospital (KKUH), King Faisal Specialist Hospital (KFSH) and Riyadh Armed Forces Hospital. This training is based on a teacher-student relationship. The trainee is expected to learn from the technical, scientific and medical staff.

The training program involves:

a. Attending formal lectures in the R1 period.

b. Learning laboratory procedures by daily bench work.

c. Dealing with clinical infectious cases and related problems during ward rounds.

d. Attending department weekly seminars and tutorials.

e. Case presentation and plate rounds.

f. Participation in hospital infection control committee activities and daily infection control team ward round.

g. Infectious disease unit ward rounds.

h. Journal club meetings.

i. Attending Riyadh microbiology and infectious disease club monthly meetings.

j. Learning public health microbiology in the public health laboratory.

In general, the training is geared to meet as much as possible the objective layed by the association of clinical pathologists of UK(ACP) in model training programme for medical microbiology.

These objectives are as follows:-

1. GENERAL

A. Laboratory safety

At the end of training, a microbiologist should:

1. Be familiar with National and International postal and packaging regulations for microbiology specimens and the safe transport of specimens within the District or Region which the laboratory serves.

2. Be able to identify safe containers and prepare a detailed local safety code including collection, transport, reception, handling, spillage and disposal of specimens and cultures.

3. Be familiar with the current requirements of control of laboratory acquired infections.

4. Understand the principles and uses of sterilizing procedures, disinfectants and their monitoring, and be able to formulate a policy on their use in the laboratory, hospital and community.

5. Be familiar with methods of ventilation, their application and monitoring in the laboratory and clinical areas, e.g. air sampling.

B. Specimens and reporting

At the end of training, a microbiologist should be able to:

1. Identify any mislabelled specimens and their derivatives (e.g cultures) and minimise those risks.

2. Interpret the results of microbiological tests, where necessary in the light of additional acquired information, e.g. the clinical condition of the patients, and produce reports which are helpful both to the clinician and to personnel involved in hospital and community epidemiology.

3. Identify those circumstances where urgent reports are required and be aware of the procedures which will expedite such results.

C. Range of materials

At the end of training, a microbiologist should:

1. Be able to process all specimens normally received from a General Hospital or the community (excepting those for which specialized laboratory tests are required) from receipt of the specimen to issuing the final report.

2. Develop an interest in a subspeciality. e.g. bacteriology, virology, mycology, parasitology.

3. Be able to initiate investigations in the light of clinical and microbiological indications.

4. Identify microbiology investigations which are not appropriate in a given situation and deal with the problem accordingly.

5. Be able to direct specimens requiring reference or special investigations to regional or reference laboratories and be aware of the circumstances in which it would be appropriate to make use of these facilities.

2. BACTERIOLOGY

A. Microscopy

At the end of training, a microbiologist should:

1. Understand the principle of light, dark ground, phase contrast, fluorescent and electron microscopy and be able to set up a light microscope with dark ground and phase contrast facilities.

2. Be able to perform all the staining techniques, including the use of fluorescent dyes, normally in the use in a routine bacteriology laboratory.

3. Be familiar with appearance of stained preparations and be able to recognise artefacts and their possible origin.

B. Culture

At the end of training, a microbiologist should:

1. Be familiar with media preparation, sterilization and internal quality control.

2. Be able to select appropriate media (selective, inhibitory, etc.) for the isolation of bacteria of medical importance from clinical and environment samples and undertake comparative studies in their use.
3. Be familiar with methods of varying environmental conditions in the culture of bacteria.

4. Have participated in the processing of specimens received from the National External Quality Assessment Scheme (NEQAS).

5. Be able to process specimens prior to culture were appropriate and to inoculate media competently.

C. Isolation and identification of bacteria

At the end of training, a microbiologist should:

1. Be able to identify to an appropriate level bacteria likely to be implicated in human disease.

2. Have a knowledge of the basis of identification systems, including biochemical tests, phage, typing, serotyping and able to perform those tests not requiring specialized facilities.

D. Antimicrobial investigations

At the end of training, a microbiologist should be able to:

1. Carry out sensitivity tests, including the determination of the MIC and MBC, by methods in common use, including the use of break points.

2. Carry out antimicrobial assays on body fluids.

3. Carry out back titrations and methods for the management of antimicrobial treatment, including the determination of synergistic combinations of antimicrobial drugs.

4. Evaluates new antibiotics in vitro and be able to draw up a protocol for their in vivo use prior to their introduction within the District.

E. Serological methods

At the end of training, a microbiologist, preferably by attachment at some time to a department virology, or to a laboratory carry out investigations in treponemal diseases, should be able to:

1. Carry out tests for antigen or antibody by slide agglutination, predipitation, including gel diffusion and CIE, haemolysis, complement fixation, haemaglutination and fluorescence.

2. Understand the principles of RIA and ELISA for the detection of antibody or antigen.

F. NEW TECHNOLOGY

At the end of training, a microbiologist should:

1. Have seen demonstrated, or have hand on experience on automated methods for blood culture, urinalysis, staining, identification of organisms and sensitivity testing.

2. Understand the principles and the potential of rapid methods, including the application of DNA probes, other nucleic acid amplification tests and monoclonal antibodies.

3. Be able to assess the need or otherwise for the introduction of new technology.

G. On –Call service

At the end of training, a microbiologist should:

1. Have experience of participating in an on-call rota, both for clinical advice and for the processing of specimens received out of hours.

2. Be able to define which tests should be carried out on an "on call" basis.

3. Be able to discuss with clinical colleagues any out-of –hours requests, and to comment on their usefulness.

4. Be able to offer advice on patient management in an emergency.

3. VIROLOGY

At the end of the training, a microbiologist should:

1. Be able to advise on the specimens required, their collection, transport and timing in cases suspected viral infections.

2. Understand the interpretation of the serology of viral infections.

3. Be able to offer advice on the management of viral infections with regard to isolation, examination of contacts, prophylaxis and treatment including immunisation and chemotherapy.

4. Know where laboratory facilities are obtainable, both during and out of hours.

5. Be familiar with standard diagnostic methods, including tissue culture and rapid diagnostic techniques.

4. MYCOLOGY

At the end of the training, a microbiologist should:

1. Advise on specimen collection and transport.

2. Prepare specimens for direct microscopy and culture, and recognize common fungi of medical importance by direct examination..

3. Identify common fungi by cultural methods and appropriate tests e.g. germ tubes, biochemical tests and stained preparations.

4. Advise on the role of serology in the diagnosis of fungal disease and be able to direct specimens appropriately.

5. Advise on chemotherapy and management of common fungal diseases.

5. PARASITOLOGY

At the end of the training, a microbiologist should be able to:

1. Advise on the collection of appropriate specimens and be capable of reliability carrying out procedures to recognise oparasites and endoparasites commonly found in the blood, gastrointestinal and genitourinary tracts of patients.

2. Judge when it is appropriate to make use of reference facilities for identification of other parasites and for the serological diagnosis of parasitic disease, and should be aware of the available reference facilities.

3. Offer competent advice on the treatment of prophylaxis of the common conditions.

EVALUATION

1. Examination and evaluation of residents at the end of each year will abide with the KSU examination regulations and any modification thereafter (copies are attached)

2. The resident after each rotation has to be evaluated and provided with a ticked check list to show the extent of the practical experience already covered, before going to the next rotation.

3. The supervisors are supposed to send to the coordinator, a full detailed reports as well as the filled official evaluation report on the residents performance at the end of each rotation.

The examination for different levels are conducted as follows:-

R1:

The examination consists of :

80% MCQ exam 40% at the end of first semester
40% at the end of the year

20% Supervisor's evaluation during rotation

R2:

An MCQ examination at the end of this year is made to cover 60% of the total mark. The remaining 40% for supervisor's evaluation.

R3:

The examination at the end of the year consists of :

a. A written examination

   1. MCQ (100) questions
   2. Essay questions paper.

b. A practical examination 30-40 spots

c. An oral examination if indicated.

R4:

The examination consists of two essay papers, 100 makes each:

Essay Paper I - mainly bacteriology
Essay Paper II – other sub- specialities

R5:

The final examination is composed of :

1. two 3 hours essay paper

   Essay paper I - Bacteriology
   Essay paper II – Other sub- specilaities

2. Practical examination:

   a. 30 -40 spots
   b. 2 ½ days practical examination
   c. oral examination

N.B.

- R3 & R5 candidates may not be allowed to proceed to the practical examination if they did not satisfy the examinations in the written papers.

PROF. A. M. KAMBAL
Course coordinator
Fellowship Course in Microbiology
Consultant and Head of Bacteriology Unit.

DETAILED TRAINING PROGRAMME

Bacteriology Section

1. Safety in the laboratory

   Handling and disposal of infectious material.
   Disinfection of infected apparatus. Use and storage of dangerous chemicals.

2. Instruction in proper collection of specimens including bed side inoculation.

3. Apparatus:

   The principles, general use and maintenance of apparatus including:

   - Centrifuges
   - Microscopes
     (phase contrast, dark field and fluorescent microscopy).
   - Balance
   - Water baths
   - Incubators
   - Gas cylinders
   - Safety hoods
   - Anaerobic jars, anaerobic cabinet
   - pH meters
   - Homogenisers
   - Bactec 60 T.B system
   - Anaerobic cabinet
   - Multipoint inoculator
   - Slit sampler
   - Automatic blood culture machines

4. Sterilization

The will include operation of equipment:

4.1 steaming and boiling
4.2 tyndallisation
4.3 autoclaving
4.4 hot-air oven
4.5 visits to C.S.S.D.
   a. membrane filter

* The selection of filters must depend on availability in the laboratory.

In addition to carrying out these sterilizing procedures, trainees must also apply the normal sterilizing controls, e.g. Bowie and Dick Test, use of thermocouples, Browne's tubes, bacterial spores, etc.

Trainees must also have the opportunity of visiting the hospital autoclaves and be acquianted with the other activities of C.S.S.D.

5. Microscopy

   5.1. Practical use and care of the light microscope.
   5.2. Preparation of materials for microscopy.

      a. Unstained preparations e.g. phase contrast, dark-ground microscopy.
      b. Stained preparations –making of films, simple stains, e.g. methylene blue, negative staining.
      c. Gram's stain, Ziehl Neelsen stain, Auramine fluorescent stain Albert's stain, spore stain.

6. Culture Media

   Trainee must have the opportunity of preparing or examining variety of culture media the and received instruction on the constituents. It is suggested that the media should include as many as possible of the following:

   Nutrient broth, nutrient agar, digest broth, peptone water, blood agar, serum agar, chocolate agar. MacConkey agar, D.C.A., X.L.D., T.C.B.S.; Alkaline Peptone Water, D.S.T.; Selenite F broth, Loeffler's serum Dorset egg, Lowenstein and Jensnen's media, peptone water sugars, Hiss's serum sugars, hoyle's Robertson's cooked meat medium, Brewerr's Sabouraud's, blood culture media, transport media. The majority of these may be made using dehydrated materials, but it is recommended that the underline media should be prepared form basic materials.

   Preparation of biological fluids for media making. Aseptic techniques. Quality control and batch recording of culture.

7. Bench Work:

Standard procedure which is being currently used in the diagnostic bacteriology laboratory for various specimens will be followed.

7.1 Inoculation of specimens and cultivation under various atmospheric conditions. The used in indicator media, selective media and enrichment media. Trainees should be made familiar with types of specimens examined, their appearance and the significance of abnormal forms. At this stage a reiteration of the occupational hazards of a microbiological laboratory should be made.

7.2 Microscopical examination of specimens of faeces, genital specimens, pus, sputum and CSF.

7.3 Examination of cultures; bacterial colony forms, significance of appearances, e.g. haemolysis rough colonies, etc. Use of the plate microscope. Use of straight wire loop and spreader.

7.4 Identification of isolated organisms, i.e. filming of colonies and provisional identification based on morphological appearances of the more routinely encountered bacteria, e.g.

7.5 Examples of simple methods of identification of bacteria. Staphylococcus-coagulate tests and other tests of pathogenicity. Streptococcus-precipitin tests, Salmonella agglution "O" and "H".

Enterobacteriacea-biochemical tests using API system. Isolation and identification of Campylobacter pylori, Helicobacter pylori and Gardnerella vaginalis. Isolation & identification of other non- fermentative bacteria.

7.6 Antibiotic sensitivity testing – Disc diffusion method using control organisms (Stoke's methods) and tube dilution techniques (MIC & MBC). Tests for antibiotic synergy.

7.7 Antibiotic assay- Biological, visit to the Pharmocokinetic laboratory, for EMIT.

7.8 Detection of Mycobacterium tuberculosis by microscopical and cultural methods. Trainees should receive instruction in the special precautions. Also trainees will gain experience in using Bactec 460 for the isolation, identification and sensitivity testing of M. tuberculosis and also conventional methods of sensitivity testing.

7.9 Anaerobic bacteriology
Trainees should receive instruction in specimen collection for anaerobic culture. Also they will be acquainted with the isolation and identification procedures using the anaerobic cabinet (Model 1024, Forma Scientific, Ohio). Anaerobic identification by various methods including API.

8. Public Health Microbiology

Trainees will spend sometime in Ministry of Health for public health microbiology which include examination of water, food, milk, sewage, etc.

9. Bacteriology serology – done on site (in immunology laboratory). Trainees will perform various routine tests. Details are given in a separate immunology guideline.

10. Animal House.

It is recommended that the trainee should visit the animal house where experience should begained in.

10.1 Safety in the animal house see appropriate section of "Safety in Pathology Laboratories" handbook.

10.2 Maintenance of records.

10.3 Care of animals.

10.4 Preparation of apparatus and materials for inoculation.

10.5 Humane methods of killing animals and preparation for postmortem examination.

10.6 Disposal of cadavers.

10.7 Collection of blood samples from laboratory animals.

N.B.

-R3 & R5: The candidates may not be allowed to proceed to the practical examination if they did not satisfy the examiners in the written papers.

PROF. A.M. KAMBAL
Course Coordinator
Fellowship Course in Microbiology
Consultant and Head of Bacteriology Unit

MYCOLOGY SECTION

I. General

A *Objective*

This is part of the programme for resident training in clinical microbiology. At the end of the programme the resident is expected to handle only the essential routine procedures involved in laboratory diagnosis of fungal infections.

B *Safety Measures in Mycology Laboratory*

The safety measures commonly observed in Microbiology will also apply here. These measures include biological hazards on handling clinical specimens and cultures of primary and opportunistic fungal pathogens, sterilization and disinfection of materials, tools and equipment accessories.

C. *Equipment*

The resident will be instructed and supervised on proper use of all equipments needed for routine procedures.

1. Incubators adjusted at different temperatures.
2. Microscopes
3. Biological safety cabinet
4. Sterilizer (Autoclaves & ovens)
5. Refrigerators.
6. Sonicators
7. Tissue grinders and homogenizers
8. Millipore filters
9. Fungal serology equipments e.g. counterimmunoelectrophoresis apparatus .
10. Balances and waterbaths
11. pH meters.

II. Laboratory procedures

A. Specimen Collection and transport. The resident will be familiarized with the various types of specimens commonly submitted for diagnosis and follow-up of fungal infections. He will practice on the proper methods of specimen collection, transport, and use of appropriate specimen containers. Common specimens received in Mycology laboratory include: sputum, endotracheal aspirates, bronchial brushing, bronchial lavage, swabs (from ears, eyes, geni) skin, Scraping nail, hair, fungal grains, tissue biopsies, pus, blood, CSF, urine, stools and aspirates (e.g from pleural, pericardial cavities, cysts and abscesses).

B. Processing of specimens. The importance of direct microscopic examination of specimens in the early diagnosis and management of fungal infections will be emphasized.

1. Direct Microscopic Examination:

The trainee will perform the following procedures for direct microscopy.

a. Unstained preparation

- Wet preparations.
- KOH mounts
- India Ink mounts.

b. Stained smears

1. - Geimsa stain
2. - Special fungal stains (Periodic Acid Schiff Stain, Gomori methenamine silver stain).
3. - Wright stain
4. - GM stain
5. - Z.N stain

In direct microscopy the trainee will be instructed and will gain experience in recognizing fungal elements in a particular specimen. (e.g.: septate hyphae, nonseptate hyphae, budding yeast cells, psendohyphae and others).

Prof. Al Hedaithy
Head and consultant
The Medical Mycology Unit

2. Culturing of Specimens

Preparation of specimens for culture: e.g. Concentration of fluids (centrifugation, millipore filtration), tissue grinding. Selection of media for culturing various specimens, incubation conditions.

C. Fungal Identification:

1. Recognition of common fungal contaminants and opportunistic fungal pathogens.

2. Recognition of primary pathogens, viz: Dermatophytes, agents of subcutaneous fungal infections (Moniliaceous and dematiceous), agents of systemic infections e.g. Histoplasma capsulatum, Blastomyces, Coccidioides, and actinomycetous agents.

3. For yeast indentification :

Morphology on CMA, germ tube test, pellicle formation, nitrate utilization, urease test, actidione inhibition, sugar fermentation and sugar assimilation (API 20C).

D. Fungal Serology

Residents will be instructed and will be involved in the performance and interpretation of common serological tests used for the diagnosis and follow- up of fungal infections. The serological tests employed routinely, immunodiffusion test, counterimmunoelectrophoresis (C.I.E.) and latex agglutination.

E. Antifungals

Tutorials will be given on antifungal drugs used for treatment of fungal infections.

Prof. Saleh Al-Hedaithy
Head and consultant
The Medical Mycology Unit

PARASITOLOGY

Objectives:

- To provide daily diagnostic service for patients complaining of parasitic disease.
- To incorporate the hospital activities with the teaching of the medical students by:
  a. Utilizing the available hospital facilities in the training programmes.
  b. Preparation of teaching materials from the incoming patient's specimens.
- Training may also entails personnel from their health institutions that require experience with special techniques and procedures.

Diagnostic of Parasitic Infections

1. Morphological demonstration of diagnostic stages is the principal means of diagnosis.
2. In tissues infection, immunodiagnostic techniques are generally more important.
3. Laboratory personnel must be competent to identify and exclude non-parasitic infection.
4. Identification may be by gross examination for adult helminth(e.g. full nematode worm like Ascaris Enterobius or segment of cestodes).

Safety

Safety measures need to be observed when handling specimens. Staff members and trainees should be aware about the possibility to infection hazard that might happen due to any negligence. Blood, faeces and other specimens as well as parasites cultures can be infective. Eggs of Ascaris spp. Can survive and embryonate even in formalin. In fresh fecal specimens, the cyst of E. histolytica and Giardia spp., the eggs of E vermicularis, Taenia solium. and Hymenolepis nana, and the larvae of S. stercoralis may be infective. In addition, faeces may contain other infectious agent, such as Hepatitis A; Rota virus; Salmonella spp., Shigella spp.and Campylobacter spp.

Blood and tissue specimens can be infectious contaminated by Trypanosomes, Leishmania spp.; malaria as well as non –A, non B hepatitis B and possible AIDS.

Reagents such as mercury, containing fixatives (schaudinn's and PVA fixatives) may be toxic and solvents such as ether may be flammable. These materials must be handled and discarded properly.

Specimens Receiving and Handling:-

Specimen have to be received through receiving section.

Sorting of Specimens:-

Each specimens should be accompanied by a request form signed by a clinician.

Specimens without requesting forms or with wrong form will not be accepted (returned to receiving section).

Specimen should be labeled with all required information specified on the vial.

Registration of Specimens

Each specimen will be given a laboratory number to be recorded on the request form as well as the vial.

Information about each specimen will be recorded in the appropriate lab. Book for reference.

Dispatching of Results

After examination result will be recorded in the request form, signed by the consultant. A copy will be sent to the receiving section for forwarding to treating doctors.

Filing

Extra copies of each request form will be kept in the appropriate cabinets in the lab in monthly serial numbers.

Collection of Stools Specimen:-

1. Fresh specimens are preferred for examination for trophozoites and for concentration of strongyloides larvae.

2. Watery or loose stool should be examined within one hour after passage for detection of any protozoan trophozoites.

3. Examination of formed stool may be delayed for a short time but must be completed on the day in which the specimen is received in the Lab.

4. If prompt examination or proper fixation cannot be carried out, formed specimen may be refrigerated for 1-2 days.

5. If specimens are delayed in reaching the lab. or if they cannot be examined promptly, such as those received on week-ends portions should be preserved in fixative such as 5% or10% formaline.

Handling/preservation of specimen for reference:-

SPECIMEN	HANDLING
Faeces, for Helminths & protozoa.	1. Fix in 10% buffered formaline. 2. Fix a portion in 10% buffered formaline and either fix a portion in PVA fixative or prepare Schaudinn's fixed fecal films.
Materials from suspected amoebic abscess	1. Place the last material aspirated in a sterile tube and send it quickly. 2. Prepare Schaudinn's fixed fecal films or fix a portion in PVA fixative. 3. Obtain serum for Serology.

Duodenal aspirates.	1. Centrifuge, and remove the supernatant. Prepare 2 films from sediment. Fix in Schaudinn's or PVA fixative. 2. Preserve the remainder of the sediment in 10% formal
Urine for Schistosomiasis.	1. Centrifuge entire mid-day urine. Add an equal volume of 10% buffered formalin to the sediment.
Sputum for nematode larvae or Paragonimus egg	1. Break up mechanically or digest of 3% NaOH for 1 hour centrifuge and preserve the sediment in an equal volume of 10% buffered formaline.
Amoeba	1. Prepare films fix in schaudinn's fixative or fix a portion in PVA fixative.
Sigmoidoscopic material	1. Fix films fixed in Schaudinn's fixative or mix material with PVA fixative.
Tissue	1. For impression smears when E. histolytica is suspected Fix in Schaudinn's or PVA fixatives.
Toxoplasma	When leishmania, pneumocytis spp. is suspected prepare multiple impression smear and fix in methyl alcohol.
Whole worms or proglottids	1. Wash debris from the specimen and send it in saline. If there are multiple worms or proglottids, some may be fixed in formalin.

Precautions during examination of stool specimens

1. It is advisable to examine multiple specimens before excluding parasites.
2. Generally it is recommended to collect 2nd or 3rd day.
3. A total of 3 specimens will be enough for final diagnosis.
4. Substances like oily materials, antacids and mineral oil may interfere with morphologic diagnosis. So specimens should not be submitted until the substances that has been cleared (5-10 days)
5. Fecal specimens should not be mixed with urine, or water during its collection.
6. Soft and liquid specimens should be examined promptly. While formed specimens can remain satisfactory for number of hours at room temperature.

Gross Examination of Stool:

1. Consistency of stool (watery or formed etc.)
2. Gross abnormalities (worms, mucus or blood).
3. Adult worms or segments should be carefully washed and diagnosed.

Summery of Guidelines for Examination of Faeces:

A routine faecal examination consist of 2 parts:

1. Preliminary macroscopic examination which includes:

    a. Noting stool consistency (water, loose, soft or formed).
    b. Looking for evidence of intestinal diseases (blood & mucus)
    c. Looking for contaminating substances (barium, mineral oil).

2. Microscopic examination which involves processing of faecal specimens by a combination of procedures designed to detect the greatest number of positive specimens. These procedures;

    a. Direct wet mount (both saline and iodine) of fresh material.
    b. Concentration (formalin-ether sedimentation, zinc sulphate flotation and Kato's thick smear).

3. I.H.A. for Bilharzia.

    Cases with 3 negative stool examination

4. False Parasitism

    Repeat stool examination 3 times on alternate days(2 days between each examinations).

5. Report non- pathogenic protozoa specially the heavy infection.

    a. Intestinal flagllates.
    b. Entamoeba coil
    c. Chilomastix mesnili.
    d. Iodamaeba butshilii.
    e. Endolimax nana.

6. Entamoeba histolytica –use trichrome stain.

7. Cases of pruritis ani – advise to do scotch tape.

Routine Stool Examination:

* Sedimentation by centrifugation is the method routinely used with each stool specimens.

* Each specimen is usually examined once.

* Based on the clinical information provided by the clinician, some specimens might be examined more than once either by the same or different techniques (e.g. Formol Ether concentration, zinc sulphate flotation etc.)

Specimen Obtained following Chemotherapy:

* The most common post-treatment examination are made in helminthic infection of the intestinal tract, particularly those due to hookworms, Ascaris, the tapeworms and intestinal trematodes.

* Depending on whether the antihelmintic is only anaesthetizing or is lethal in its effect, the worms will be evacuated in an undamaged living condition or may be damaged.

Residents shall be involved in the following types of activities :

1. Preparation of medium required for special types of diagnosis such as NNN medium for Leishmania culture.

2. Collection of specimens from patients that require special techniques such as Leishmania, Onchocerca.

3. Tutorial session on various topics on parasitic diseases will be presented regularly.

4. Follow- up of interesting cases of parasitic infection.

Occasional Visits to:

- Haematology laboratory for parasites seen in blood e.g. malaria.

- Dermatology clinic for cases of cutaneous parasitic infection such as Leishmania and Onchocerca.

Schedules of Training in Parasitology for Residents in Pathology for a Period of 4-5 weeks

CONTENTS	DURATION
* Prep. of stool specimens/Safety measure * Direct stool exam	2 days
* Direct stool exam. * Exam. of positive stool specimens	2 days
* Concentration techniques: * Sedimentation * Flotation	3 days
* Comparison of direct stool and cone technique on positive stool specimens.	3 days
Microscopic examination of routine stool specimens.	5 days
Kato smear technique for counting of Schisosoma ova. Hatching technique for Schistosoma ova. Cellotape swab technique for Enterobius	3 days
Preparation of bronchial smear for pneumocystis Preparation of smear and culture inoculation for Leishmania.	3 days
Blood films staining. Parasitic serology	2 days
Exam of Hydatid cyst content. Baerman's technique for detedtion of larvae	2 days
Permanent mount (Trematodes).	1 days
Permanent mount (Intestinal & Tissue Protozoa)	1 days
Permanent mount (Cestodes)	1 days
Permanent mount (Blood protozoa)	1 days
Permanent mount (Nematodes)	1 days
Permanent mount (Arthropods)	1 days

VIROLOGY SECTION

Rotation Schedule: Virology

Student:----------

Supervisors

Date: ----------*Remarks*:----------

1st Week:

- Discussion of principles in virology to include specimens-disease relationship, specimen collection and suitable host systems for isolation. Student is informed of a availability of laboratory manuals and reference books.

- Demonstration of media and solution preparation utilizing both the autoclave and filtering as means for sterilization.

- Student will watch preparing media and solutions used in his/her training period.

- Demonstration and / or participation in the preparation of primary cell cultures.

- Discussion and demonstration of passing techniques employed in maintaining continuous cell cultures. This will include preparation of equipment and supplies methods of cell cultivation, detective of contaminants and preservation of cells by freezing.

- Demonstration and / or participation in preparation of different cell culture line to maintain cells.

- Freeze ∝ thaw of cells.

2nd Week:

- Demonstration and discussion of cell culture inoculation and viral cytophatic effects and the used of cytophatic effect for preliminary viral identification.

- Demonstration and / or participation of inoculation of known of viruses and studying their progressive cytophatic effect by microscopy
examination of cell cultures.

- Discussion and demonstration of inoculation of cell culture for chlamydia isolation.

- Follow-up of inoculated culture and staining for chlamydia inclusion bodies with different stain.
- Discussion of viral titration and identification procedures. Utilization of hemaabsorption,

hemaabsorption –inhibition, neutralization, complement fixation and hemagglutination-inhibition is incorporated into the discussion.

- Student may be given unknown specimen to work up and identify.

- Discussion of techniques employing embryonated hen's eggs, specific reference will be made to routes of information versus specific viruses.

- Discussion and demonstration of hamagglutination and hemagglutination-inhibition assays. Specific reference will be made to test procedures for rubella.

3rd Week:

- Discussion and demonstration of direct and indirect immunofluorescence. Specific reference will be made to viral diagnosis using monoclonal Ab.

- Student will examine slides from direct IF for Ag detection e.g. measles, RSV parainfluenza and an indirect immunofluorescence assay for Ab detection.

- Diagnosis of congenital infection (TORCH).

4th Week:

- Discussion and demonstration of the enzyme linked immunosirbent assay (ELISA).

- Application of the assay in the detection of

  Anti-HCV

  - HBs AG
  - Other hepatitis markers: HBeAg/anti HBe,anti-HBcAg and anti-HBsAg.
  - Anti-Delta agent.
  - Anti-HIV – 1, anti HIV – 2
  - Anti-CMV
  - Anti-Rubella IgG and Ig M
  - Rotavirus
  - Chlamydia
  - Interpretation of the hepatitis profile.
  - Interpretation of HIV profile.
  - Interpretation of Western blot.
  - Interpretation of line immunoassays.

5th Week:

- Quality control of media and equipment.

- Participation in administration and supervisory duties with the Supervisor and Chief Technologist.

IMMUNOLOGY SECTION

Introduction:

The objections of the Residency Training Programme in Immunology is to offer in depth knowledge on the performance of various immunological procedures and their application in the diagnosis, prognosis and management of patients with immunological disorders.

The programme includes:

1. Introductory sessions on basic immunology and laboratory procedures.
2. Routine laboratory work (both bench work and data interpretation).

The training programme in Immunology comprise 4 sections. Candidates will rotate from one section to the other according to the supervisor's satisfaction.

Trainees will be under continuous assessment as they rotate and a check list will be held and signed at the end of rotations.

Discussion with candidates on technical problems will be conducted as they perform their bench work.

The practical training programme include:

I 1. Introductory sessions and basic laboratory procedures:

A. Instruction on safety measures in the laboratory.

1. Potential hazards.
2. Handling human sera and infectious materials
3. Instruction on type of specimens and containers required from different tests.
4. Handling radioisotopes.
5. Familiarization with potentially explosive material and posions.

B. Introduction to principles, design and operation of equipment in Immunology Laboratory. This also include briefing on maintenance, ordering and costing reagents.

II. Routine diagnostic procedures:

3. Detection of auto-antibodies

- Anti-DNA using a haemagglutination test and ELISA
- Anti-thyroid antibodies using a haemagglutination test.
- Anti-nuclear Anti bodies (ANA) using Hep-2 cell cultures in an indirect fluorescent antibody test.
- Anti-mitochondiral antibody test (AMA) by using indirect

Immunofluorescence on kidney tissues substrates.

- Anti-smooth muscle antibody test (ASMA) by using indirect Immunoflorescence on rat stomach tissue substrate.

- Detection of extractable nuclear antigen (ENA)

3. Techniques for diagnosis of allergic diseases:-

Technique of skin testing for immediate hyper sensitivity and for measurement of delayed type hypersensitivity. These includes:-

1. Skin prick test
2. Patch test
3. Estimation of total and specific IgE (RAST) using the CAP system.
4. Measurement of mediators by the CAP system.

1. Bacterial and parasitic serology

A. Widal test for Salmonella and Brucella applying both micro and macrotitration techniques.
B. Syphilis Serology:

- V.D.R.L. Rapid agglutination card test.
- T.P.H.I. Treponema Pallidum Haemagglutination test.
- FTA/ABS. This applies a fluorescent treponemal antibody test.

C. Toxoplasmosis:

- Screening test using latex agglutination or haemaglutination techniques.
- Application of ELISA for measurement of specific antibodies Toxo IgG and Toxo IgM.

D. Hydatid disease, using an indirect haemagglutination test.

E. Bilharzia, using indirect haemagglutination test.

F. Amaebiasis, using indirect haemagglutination test.

G. Leismanisis, using indirect haemagglutination test.

4. Cellular Immunology and special procedures.

Flow cytometry for:-

A. T-lymphocyte counts

B. Enumeration of T-cell subsets.

C. Enumeration of B-cells

D. Flow cytometry for leukemia immunophenotyping.

E. Flow cytometry for lymphoma immunophenotyping.

F. Assessment of phagocyte function by flow cytometry.

5. HLA Typing:

- HLA-ABC typing and HLA-DR tissue typing.
  This implies isolation of T and B lymphocytes and then performing a lymphocytotoxicity test procedures using Terasaki plates.

- HLA-DR typing by PCR.

C. Basic laboratory organization regarding-

- Work flow
- Keeping accurate records
- Retrieve data

D. Quality control procedures including necessary information on internal and external programmes.

II. Basic procedures

- Preparation, preservation, lyophilisation and storage of sera.
- Inactivation or maintenance of complement.
- Separation of cell populations using Hypaque ficoll.
- Preparation of cell suspensions and tests of viability.
- Preparation and staining of blood films, counting white blood cells And performing differential white cell counts.

2. General Immunology:

This section include the routine general immunology investigations . if offers good training on the practical application of the commonly used basic immunological reactions. This comprise the following procedures:

A. Test for the Rheumatoid factor, C-reactive protein using latex particle agglutination techniques.

B. Quantitation of Immunoglobulins IgG, IgA, IgM and complement C3, C4 by single radial immunodiffusion. Candidates will also be instructed on preparation of gels for double immunodiffusion and the various applications of this method.

C. Measurement of antistreptolysin O titre by a microtitration method based on the orginal procedure of Todd.

D. Pregnancy testing both qualitative and quantitative, the method used is a highly sensitive technique using monolonal antibodies.