Kingdom of Saudi Arabia
King Abdulaziz University
Faculty of Science
Biochemistry Department

SUMMER TRAINING

Reported by:
Student. Khairiah Khedher Al-Moshre

Al-Qunfudah General Hospital
Cooperation with staff in the hospital

CONTENTS

#	Topic
1.	Reception and specimen collection
2.	Hematology
3.	Biochemistry
4.	Microscopy
5.	Blood Bank
6.	Serology

First section

RECEPTION AND SPECIMEN COLLECTION

Reception and Specimen collection:

1. Collection of blood sample.
2. Collection of urine sample.
3. Collection of stool sample.
4. Bleeding time and clotting time.

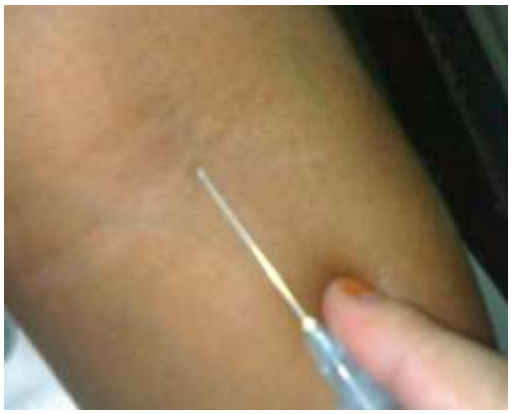
Specimen collection:

Usually collected specimens are blood, urine, and stool samples. Before specimens collection, selection of vactainer or container.

If sample is blood ⟶ vactainer

If sample are urine or stool ⟶ container

1. Collection of blood samples



1. If plasma is selected, vactainer with an appropriate anticoagulant has to be used. And if serum is selected, without anticoagulant has to be used.
   Note: (selection of vactainer depend on type of test).
2. Check on the patient is a fasting or not fasting (depend on test).
3. Position the patient properly.
4. Select a suitable vein for punctureand or syringe.
5. Apply a tourniquet several inches above the puncture or syringe and cleans the vein puncture site with 70% isopropyl alcohol.
6. Anchor the vein firmly both above and below the puncture.
7. Perform the venipuncture. Enter the skin with the needle at approximately 15° to the arm. Insert the needle smoothly and fairly. If using a syringe pull pack on the barrel with slow even tension as blood flows into the syringe.
8. Release turniquet.
9. After drawing blood, the patient may be release his/her fist.
10. Clean the place and take sterile cotton put on site drawn.

11. If test required serum no need mix but if test required plasma or whole blood is need mix with anticoagulant.
12. Initial the labels and record the time of collection.

2. Collection of urine sample

There are three types of collection of urine samples: random, timed, 24°total volume.

Random

Give the patient clean container and it is most desirable for bacteriologic examinations. Labeled with patient name, date and time of collection.

Timed

Starting time zero and are noted on each subsquent container time collection.

24°total volume

For 24° total volume collection one should remind the patient to discard the first morning specimen, record the time. And collect every subsequent voiding for the next 24 hours, with the last to be 24 hours after timing commenced.

3. Collection of stool sample

Give the patient clean container, labeled with patient name and date due to collection of the sample.

Bleeding time

1. Clean the finger by alcohol and allow drying.
2. Puncture deeply by using a sterile needle and start stopwatch.
3. Allow the blood to flow freely without squeezing.
4. After30 sec. Collect the drop of blood at one corner of the filter paper without touching the skin, then repeat the step to

formation of bleeding ceases then stop the stopwatch and record the time.

Note; if the bleeding time is prolonged for more than 10min. Discontinue the test and apply pressure to the bleeding spot.

4. Clotting time

1. Clean the finger by alcohol and allow drying.
2. Puncture deeply by using a sterile needle
3. Wipe off the first drop of blood and collect the second drop on the slide. Start the stopwatch.
4. Using the needle check for fibrin string every 30 sec. When the fibrin string appears, stop the watch and record the time.

Second section

HEMATOLOGY DEPARTMENT

Type of sample

Whole blood (EDTA tube)

Routine testes that uses the type of sample

CBC
BG
BFM
Retic.
Diff.
St
Hb alk. Electro.
Pre-marriage testing

Plasma (sodium citrate)

Routine testes that uses the type of sample

ESR
PT
APTT
FDP

Equipments used in the department b:

1. Blood cell analyzer
   SYSMYX SE-9500
   COULTER HMX
2. HELENA ELECTROPHORESIS EQUIPMEBT
3. Blood coagulation
   BCT
   ST4

SYSMYX SE-9500

Method for running the sample:

1. Turn ON the power starting.
2. Set the button CBC, and then enter.
3. For manual mode; select ID number, enter.
4. Feed the sample be pressing the green button and wait for the beep. Release sample after the beep.
5. Result will be printed automatically.

Control

1. Press F4, look for QC menu.
2. Press 2 from small window, select the file.
   File I ⟶ Normal
   File II ⟶ Low
   File III ⟶ High
3. Feed the control. Look for +/_ sign.

Calibration

1. Press shift and F7 at once, look for change 3.
2. Press 4 for calibration

Shut down

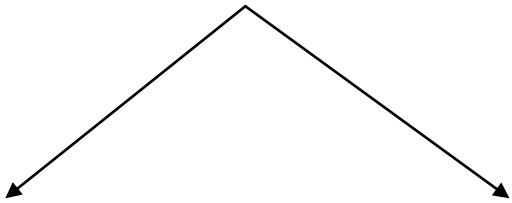
1. Select from main menu.
2. Press shutdown.
3. Place the clean cell in the aspirator.

COULTER HMX

Method for running the sample:

1. Switch ON the power button at the back of the mechaine.
2. Switch ON the reset button at the left corner of machine.
3. Wait till the access screen appears on the monitor.
4. Do start-up procedure daily before running the samples.
5. Press F9 and go to Diluter Function.
6. Select start up from Diluter Function and enter.
7. If the background results are OK, proceed to run controls. If any problem exists, see Trouble shooting guide.
8. Procedure for control run
   Select f2 and enter.
   Look for control File.
   Select the desired control runs (Normal. Abnormal I and II) and press Enter.
9. Run the desired controls and look for the values. If it is OK, proceed to run samples. If any Problem, see Trouble shooting guide.

Sample analysis



Sample on primary mode

1. Quantity of the sample must be more than 1ml.
2. Select function.
3. Sample ID.
   Look for work list on the screen by pressing f1 and enter cassette ID number and patient ID number.
4. Take out the casssettes and put the samples and keep it in the loding place. The machine will automatically run the samples and print the results.

Sample in secondary mode

1. Select F1 and F3 twice and select secondary Mode,
2. Enter the ID number on the screen.
3. Open the sample and immerse the aspirator tip on the sample.
4. Press and the sample bar then remove the sample after the beep.

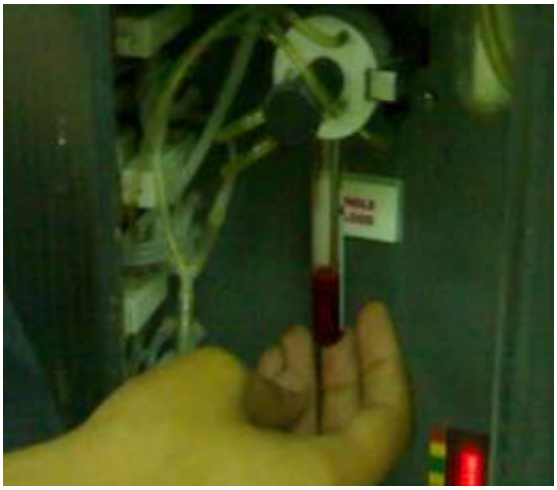
Closing the Machine

1. Look for Select Function.
2. Select diluter Function, SHUTDOWN.
3. Press enter to page.
4. Close the machine by switching OFF the main button.

Complete Blood Count (CBC)

Before make any test for hematology should be the following:

1. Cheking the sample by the patient name, test requested and other details.
2. Enter the patient name, File number and word in the logbook.
3. Looking for any discrepancy in sample ex; inadequate volume, blood clotting and information of patient on the tube and which it in the requested.



CBC including are hemoglobin, white blood cells, red blood cells, hematocrite, platelet, main cell value, main cell hemoglobin, and main cell hemoglobin value. Measure the CBC by Equipments for analyzer blood cells. Enter the sample in Equipment then running the sample and the results in the logbook.

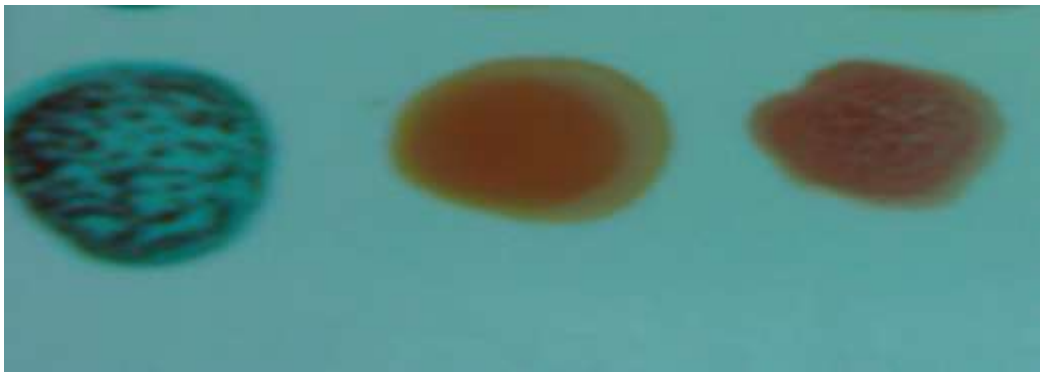
Blood group

Group	A	B	AB	O
Anti.A	+	_	+	_
Anti.B	_	+	+	_
RH	$A+/A^{-}$	$B+/B^{-}$	$AB+/AB^{-}$	$O+/O^{-}$

Method:

One drop from blood + one drop Antiserum ⟶ clotting formation (+)

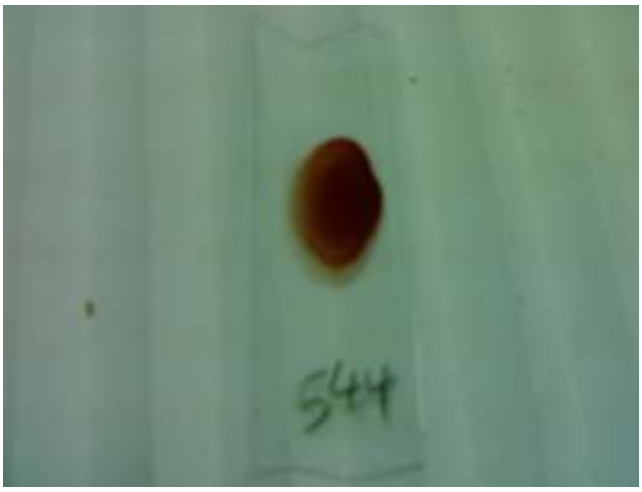
One drop from blood + one drop Antiserum ⟶ non clotting formation (-)



Blood Film for Malaria (BFM)

Detection of parasites in the blood.

1. A drop of blood from the finger is placed on a slide, spread and dried.
2. It is stained and examined under the microscope for the following:
   Malaria parasites
   Trypanosomes
   Microfilariae
   Borreliae



3. During staining of the drop of dried blood, the hemoglobin in the red cells dissolves and is washed out by the water in the staining solution (1in 10 dilution Giemsa stain).
   Note*:* All that remain are the parasites and the white blood cells which can be seen under the microscope.

Reticulocyte count

The number of reticulocyte in the blood indicates the degree of activity of the bone marrow and when the marrow is very active (as in anaemia) their number increases.

Method:

1. Take the 2 drops of blood + 2 drop of filtered brilliant cresyl blue solution.
2. Mix by using the pipette.
3. Take the 1 drop of the mixture, and then make a thin smear.
4. Examine the smear using the oil-immersion objective. Look at the end of the smear where the red cells should be well separated from each other.

Differential count

Thin blood films

Method:

1. Put one drop of blood on glass slide then make thin smear, Stand to dry it.
2. Fix the thin blood film with methanol for 2-3 minutes.
3. Put already filtered Leishman stain over the slide.
4. Put double the amount of distilled water over the slide and blow it if for mixing.
5. Leave it for10-15 minutes.
6. Wash the slide in distilled water and tip the water off and stand slide in a draining rack to dry.
7. Examine under the microscope.

Sickling test

Preparation of reagent:

1. Weigh 0.5 g of sodium metabisulfite.
2. Using a graduated cylinder, measure 25 ml water.
3. Mix.
4. Transfer to a clean amber bottle and cover with parafilm.

Method of the test:

1. Put small drop of blood on center of a slide.
2. Add an equal drop of the reagent.
3. Mix, Cover with the coverslip, making sure that no air bubbles form.
4. Put in a petri dish that has wet filter paper in the bottom. Support the slide in two sticks.
5. Wait for 1 hour.
6. Examine under microscope (X40 objective) After 1 hour.

Results:

Negative ⟶ the red cells remain round.

Positive ⟶ the red cell becomes sickle-shaped or banana-shaped.

Note; if result is positive should be make Hb electrophoresis due to design type of patient, is treat or disease.

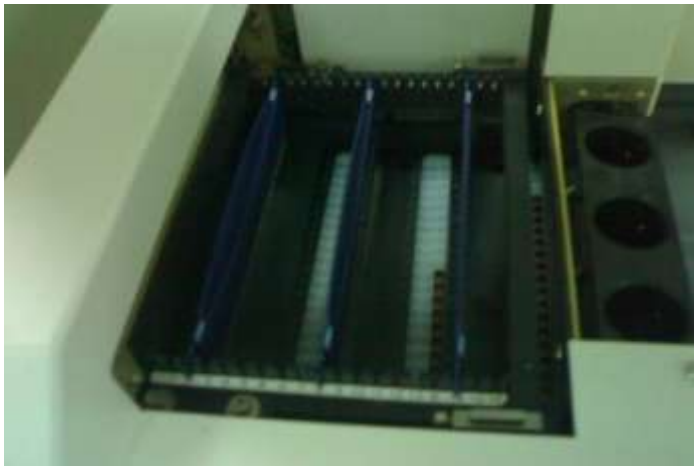
HELENA ELECTROPHORESIS EQUIPMEBT

Hb Alkaline Electrophoresis

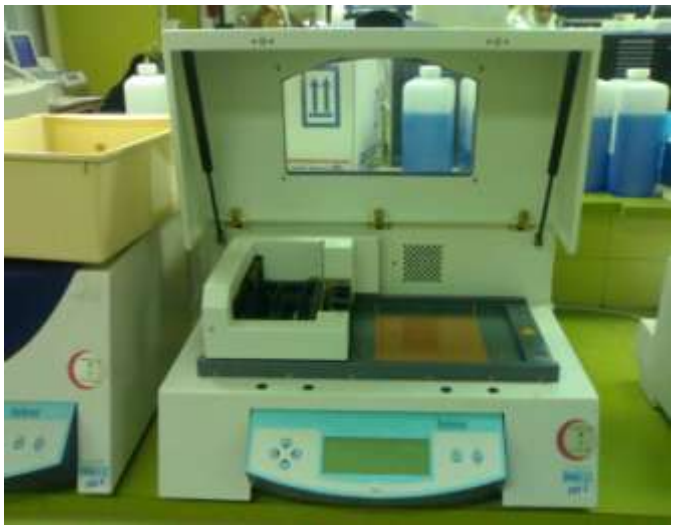
Main Composition of Make Electrophoresis are: SPIFE Alkaline Hb gel, Acid Blue Stain Concentrate, Hemoglobin Lyzing agent, Destine Solution Concentrate and Other Kit components.

Sample Preparation:

1. Take 200µl of whole blood with 1ml of normal saline in glass tube, mix well.
2. Centrifugation
3. Remove supernatant and add a further 1ml of normal saline and mix well.
4. Repeat steps 2&3 x2.
3. Remove supernatant, take sediment.
4. Add lysing agent [60µl of lyse with 15 µl of sample (wash cell)]
5. Put the sample after added lysing in the fridge for 1 hour.
6. Take 38µl of sample, and then put in wells A, B and C.
7. Put the wells in the machine, and then put applicator position PIFE comb in position 2, 8 and 16.



8. Put the Rep. prep. On left side of the chamber, put the gel, then remove cover, put anode [inside] and cathode [outside]



9. Make dry of gel by use filter paper. Then, Close the chamber.
10. Turn ON from at the back of machine, Select the Alkaline-Hb test program and Following the prompts apply the samples and perform the electrophoresis.
11. At the end of the electrophoresis, open the chamber and remove both gel block using the Gel Block Remover.
12. STAINNIG:

[Preparation of stain]

Each bottle concentrated Acid Blue stain. Dilute the contents of the vial in 700 ml of purified water. Stir overnight band filter before use. Store the stain in a tightly closed bottle.

Put the stain in container, put the gel in the stain for 4 min.. Then, put the gel in the destain solution to come clear blue. Then, incubation for 2 hours.

Note: Destain Solution Preparation:

1. Take 20 ml of destain solution.
2. Add 1000 ml of distilled water.
3. Mix, then, Save until utilize.
4. Finally, read gel by program in the computer.

Pre-Marriage testing

Complete Blood Count [CBC]

Seckling test

Hemoglobin electrophoresis Alkaline.

Erythrocyte Sedimentation Rate [ESR]

Blood collected into an anticoagulant is long graduated tube held in a vertical position. The red cells settle to the bottom leaving a layer of plasma above. The height of the column of plasma after 1 hour indicates the sedimentation rate of the erythrocyte.

Method:

1. Fill the test tube containing 0.2 ml of sodium citrate up to graduation mark with blood.
2. Mix immediately by gently capsizing three to four time.
3. Insert the pipette through the pierceable stopper. The blood will automatically rise to zero.
4. It is absolutely essential that the pipette makes firm contact with the bottom of the tube.
5. Wait 1 hour, then note the height of the column of plasma in mm graduation starting from the 0 mark at the top of the tube.

Note: Normal range: Men 1-10 mm/1hr

Women 3-14 mm/hr

BCT MACHINE

This machine used in measure of blood coagulation specific Prothrombin Time [PT], Activated Partial Thromboplastin Time [APTT], and INR.

The control;

1. Before start the control should be following;

I. Select the routine, then user wash cycle, and then appear list consist

Normal reins Intensive washing Disinfection

II. Then select the Disinfection ⟶ OK
After the end, select the Intensive washing ⟶ OK
After the end, select the Normal reins ⟶ OK
After end wash cycle, make the control of PT and APTT,

2. Before the start should be preparation of control solution by:
Take control powder + 1ml of distilled water, then mix well
After end of preparation of control.
3. Select the control, then measure contro , then PT, and PTT then close.

Preparation of sample

a. Take 4.5 ml blood then put on 0.5 ml trisodium citrate, then mix [Up down]
b. Centrifugation for 5 min at 1500-2000 rpm.

Entering the sample in the machine

1. Start from position 4, after the finish entering the sample closing the chamber, then from screen, select the Rotor.
2. Rotor ID [127], press OK.
3. Enter the lab number and name.
4. Select the testes, after the finish entering the details of the sample press Load, and then press close.
5. Result will be printed automatically.

Diagnostica stago coagulometer [ST4]

This the equipments used in blood coagulation test.

Prothrombin time [PT]

Method

a. Take 4.5 ml blood then put on 0.5 ml trisodium citrate, then mix [Up down]
b. Centrifugation for 5 min at 1500-2000 rpm.
c. Take 50µlof plasma
d. Incubate then add 100µlof PT reagent and start the time
e. Result will be in see.

Activated Partial Thromboplastin Time [APTT]

a. Take 4.5 ml blood then put on 0.5 ml trisodium citrate, then mix [Up down]
b. Centrifugation for 5 min at 1500-2000 rpm.
c. Take 50µl of plasma with 50µl APPT reagent.
d. Incubate at 37 °C for 180 sec.
e. Add 50µl of calcium chloride and start the time.
f. Result will be in see.

Fibrin Degradation Product [FDP]

a. Take 4.5 ml blood then put on 0.5 ml trisodium citrate, then mix [Up down]
b. Centrifugation for 5 min at 1500-2000 rpm.
c. Take 20µlof plasma put on the test slide. Add 20µlof the latex reagent.
d. Promptly mix the suspension the rotate the slide gently for 3 minutes.
e. Precisely 3 minutes after mixing. Check for agglution

Note*; normal value < 0.20 µg/ ml*

Third Section

BIOCHEMISTRY DEPARTMENT

Type of Tests tube

Red [plain tube]
Green [lithium heparin]
Gray [contain glycolytic inhibitor for glucose]
Gold tube [serum separating tube[SST]]
Lavender tube[K-EDTA tube]
TYPE of SAMPLE
Serum, plasma or urine

Tests Available;

A. Routine tests

1. Blood glucose [BG]
2. Blood urea nitrogen[BUN]
3. Serum Creatinine
4. Potassium [K+]
5. Sodium [Na+]

B. Liver function tests

1. Serum glutamate pyrovate transmenase [SGPT]
2. Serum glutamate oxaloacetat transmenase [SGOT]
3. Alkaline phosphatase [ALP]
4. Total bilirubin [TB]
5. Direct bilirubin [DB]
6. Lactate dehydrogenase [LDH]
7. Total protein [TP]
8. Albumin [Alb]
9. γ- Glutamin transmenase [GGT]

C.Cardic enzymes

1. Creatinkinase [CK]
2. Creatinkinase-Muscle and brain
3. Lactate dehydrogenase [LDH]
4. Serum glutamate oxaloacetat transmenase [SGOT]

D. Lipid Profile;

1. Chlestrol [Chol.]

2. Triglycerides [TG]
3. High density lipoprotein [HDL]
4. Low density lipoprotein [LDL]

Other tests

1. Uric Acid [UA]
2. Calcium [Ca].
3. Inorganic phosphorus [P]
4. Amylase [Amy]
5. Chloride [Cl]
6. Magnesium [Mg]
7. Carbon dioxide [CO_2]
8. Iron [Fe]
9. Albumin / Globulin [A/G]
10. Creatinine clearance [Crea. Clear.]
11. 24° Urine Protein
12. Plasma ammonia [Should be send sample to laboratory in ice].
13. Hemoglobin A/c[HBA1C]

[All tests measurement by kite in equipments]

Equipments;

1. LABOFUGE 200 centrifuge.
2. LABOFUGE 400 centrifuge.
3. ABL 710
4. SYNCRON CX3
5. HITACHI 902
6. COBAS [INTEGRA]

1. LABOFUGE 200 centrifuge.
2. LABOFUGE 400 centrifuge

The function of two machines is separate fluid to two components [supernatant + sediment] for example blood to plasma or serum and cells [Plasma is containing the clotting factor but serum not contain clotting factor].

3. *ABL 710 [For Blood Gases Measurement]*

1. Make sure that the analyzer is in the READYCMODE.
2. Lift the syringe inlet flap.
3. Place the syringe tip into the inlet.

4. Select the desired mode by pressing the appropriate touch-key.
5. Then press the [Start] touch-key.
6. If the selected parameters screen appears, select or deselect the desired parameters and press the [Aspirate] touch-key.
7. Aspiration of the sample begins.
8. The Patient identification Screen appears.
9. Enter patient identification data using the screen keyboard.
10. When the close inlet prompt appears on the screen, remove the syringe and close the inlet flap.
11. When the measurement is finished the patient Result screen automatically appears.

4. SYNCRON CX3

General Description;

Is a microprocessor- controlled random access biochemistry analyzer which measures the concentration of sodium, potassium, chloride, carbon dioxide, glucose, urea nitrogen, creatinine, creatinine clearance, total calcium and total protein in biological fluids.

Load Reagent

1. Press MASTER SCREEN key.
2. Press F2 reagent load.
3. Move cursor down to reagents to be loaded.
4. Press select key to highlight reagent to be loaded.
5. Press F1 load then MASTER SCREEN.

Prime

1. Press MASTER SCREENB key.
2. Press 1 then ENTER.
3. Press F3, and then press F1.
4. Enter number of primes desired, press ENTER.

Calibration

1. From MASTER SCREEN press F4.
2. Move cursor and select chemistries to be calibrated.
3. Press F1, then sector number.
4. Go back to MASTER SCREEN then press START.

Run the Control

1. From the MASTER SCREEN press F1.
2. Press F1, and then choose SECTOR number.
3. Press F3, and then enter.
4. Highlight and select the chemistries to be run.
5. Press F8 to program another sample.

Program and run sample

1. Centrifugation of blood, separate of serum [plasma], [if sample is blood but if sample is C.S.F. or urine no need this step.]
2. Put the serum [plasma] in the special cup, then enter the cup in SECTOR.
3. From the MASTER SCREEN press F1, then F1, to choose SECTOR number.
4. Type SAMPLE I.D. and press ENTER.
5. Press ENTER, then press SELECT to highlight chemistries to be run.

6. Press F8 to program a new sample.
7. Press MASTER SCREEN, then START.
8. Result will be printed automatically.

Creatinine clearance

You can measurement by the kit in the *SYNCRON CX3* machine.

Type of sample:

You need two samples are blood serum and timed urine

Method of measure

1. Give the patent measuring container.
2. You said for the patent: Start the collection urine from morning e.g. [8.00 am] to next day in the same time [8.00 am].
3. Draw from the patent blood sample. Then, Put in the plane tube.
4. Read the volume of patent urine. Then, record the volume on the request.
5. Separation the blood sample by centrifuge.
6. Take the serum. Then, put in the cup.
7. Enter the sample in machine.
8. Select crea. test. Then running of sample.
9. After that take crea. result. Then, make the following;
   a. Take small quantity of the urine. Then, put in the cup.
   b. Enter the cup in the sector. Then, enter the sector in theCX3 machine.
   c. Press F1 then F1.
      Enter the sector number and cup number.
      Enter the ID sample.
      Select the crea. test.
      Press enter. Then, change type of sample by press F1.
      [Select timed urine].
      Press enter. Then, appear list contain

1. Volume of urine [ml]

2. Time of collection [hours].
3. Value of crea. in the blood [mg/dl]
4. Surface area [no need in].
10. Press Enter. Then master screen.
11. Press start.

Note: After finish the run automatically result prented**.**

5. HITACHI 902

GENRAL DESCRIPTION;

The HITACH902 system is automatic Biochemistry analyzer which measure Liver function, renal function, lipid profile, cardiac enzymes, blood sugar, C.S.F. sugar, serum amylase and other tests in biological fluid.

Control

1. Check the reagent by the reagent print [press OK, then reagent print]
2. a. Press Home.
   b. press maint.
   c. press incub. Wash.
   d. press start.
3. a. Press Page up.
   b. press Home.

c. press maint.
d. press wash.
e. press start.

4. a. press Home.
b. press maint.
c. press control[1 and 2 select all biochemistry tests except [CK-MB] but 3 select CK-MB only then, press accept.
d. press Page up, then OK.
e. press control, then press page up.
f. Press start.

If result of control is good therefore not need calibration but if result of control is not good therefore need calibration.

Calibration Method

a. press Home.
b. press maint.
c. press calbration. Then, select the bioch. Test. Next press accept.
d. press Easy Mode.
e. Press OK.
f. press calibration. Then, press page up.
g. Press start.

Enter and run the sample

1. Centrifugation of blood, separate of serum [plasma], [if sample is blood but if sample is C.S.F. or urine no need this step.].
2. Put the serum [plasma] in the special cup, and then enter the cup
in the special position in machine.
3. Press I.D., enter, and then press I.D. number.
4. Press page up, then select tests, then accept.
5. If lamp is light [no press start] but if lamp is non light [press start].
6. Result will be printed automatically.

COBAS [INTEGRA]

COBAS system is automatic biochemistry analyzer which measure Routine tests, Liver function, renal function, Lipid profile, Cardiac enzymes, C.S.F. Sugar, plasma ammonia, serum amylase and other tests in biological fluid.

Control

Before make the control of this machine should be following

1. Change the activator every day.

Serum + heparin ⟶ Activator

2. Load the activator in the position.
3. Appear of list contain anything change. Then make label on activator.

Next, go to Service Action recommended.
Then, press Begun of day, Next, select all by Shift + ↓
Then, press perform.
Note: Should be the machine in state of start but not in state of Restart.
After finish the prim you can make of control.

Control

1. Press Quality control. Then, press All.
2. Press AMM-N then, press Save. Next Go to [AMM-P, PNCK, PNUP, PPHL and PPUP] by the same method one by one last press start.

Note: Check on all the control is work or not by press on worklist.

Method of clear for Rack in the machine

1. Take the rack out the machine.
2. Go to order then, tools Next, purgeorder then, Yes.

But clear of Result by go to order then, tools Next, Resultorder then, Yes.

Enter and run the sample

1. Centrifugation of blood, separate of serum [plasma]. [If sample is blood but if sample is C.S.F. or urine no need this step].
2. Put the serum [plasma] in the special cup, and then enter the cup in the special position in machine.
3. From the screen go to Sample, then I.D, OK, then appear square contain [Name, age and six] Press OK.
4. Select the position of sample and type of sample [serum, plasma or urine]
5. Select the tests, then press Save.
6. Press start.
7. After finish the run, Result will be printed automatically.

Fourth Section

MICROSCOPY DEPARTMENT

Type of sample

Urine, Stool, or Semen.

Urine

Routine urine analysis Pregnancy test

Routine urine analysis

1. Physical examination volume, color, Appearance, odor, and specific gravity by refractometer.
2. Chemical examination by strips;

Blood, Urobilinogen, Bilirubin, Protein, Nitrate, Ketones, pH and Glucose.

3. Microscopic examination

1. Transfer the sample to glass tube.
2. Centrifuge at 2000-30000 rpm for 3 min.
3. Remove the supernatant fluid.
4. Resuspend the sediment by gently tapping the bottom of the tube.
5. Take 1 drop of sediment and put on glass slide and cover with coverglass.
6. Use central, subdued, light, and easily obtained by lowering condenser of most microscopes for routine study.
7. Examine entire coverglass area with low-power lens [16mm]; casts are most easily found in this way.
8. Examine for details and identification of sdtructures 10 low-power [10x] with subdued, count the casts and report.

Examine 10 high-power lens [40x], Report numerical values of RBC, WBC and tubular cells.

Pregnancy test ⟶ By strips.

Stool Analysis

Examination

1. Physical examination
   Color, Consistency, PH and Mucous
2. Chemical examination
   Occult blood and bile.
3. Microscopic examination
   Protozoa [E. histolytica, E.colim G. lamblia, T. hominis and C. mesnili.

Semen analysis

Microscopic Characteristics
Pus cells, epithelial cells, Parasite/Ova, Sperm morphology,
Sperm count and Motility:

	One hour	**Tow hours**	**Three hours**
Motile	%	%	%
Sluggish	%	%	%
Non motile	%	%	%

Fifth Section

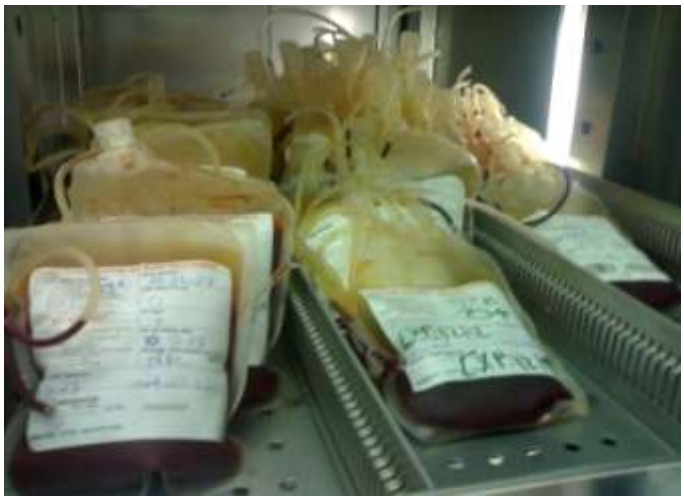
BLOOD BANK DEPARTMENT

Blood Donation

It is a vital section of the laboratory. This section is incharge of receiving/ collecting blood from donors, processing it into its component, blood typing, screening for malaria parasite and forwarding sample to Serology Department for additional screening tests.

Blood Donation Method

1. Take donor's personal identification [Name, Address and Age]
2. Ask for clearance from the physician that donor is fit to donate blood. [Blood pressure, temperature, body weiht and blood type are checked].
3. Registare the whole data in Registration book.
4. Prepare the blood bag-with complete details [Data of collection, date of Expiry, Blood type and Serial Number]
5. Let the donor rest for at least 20 min. on the chair.
6. Ready for bleeding/extraction.
7. Do not leave the donor take another rest for some time after extraction.
8. Let the donor take another rest for some time after extraction.
9. Prepare blood sample for serology test.
10. Write Laboratory number on the blood sample tube.



Forward and Reverse ABO Typing
[Tube method]

I. Forward Typing [Direct] Procedure

1. Label tube anti-A, anti-b and Anti-A,B.
2. Add one drops of a 3-5% RBC suspension to the tubes labeled anti-A, anti B and anti-A, B.
3. Add two drops of each reagent into the appropriately labelrd test tubes.
4. Gently shake the tubes in order to mix thoroughly.
5. Incubate foi5 min.
6. Centrifuge for 1min. at 1800rpm.
7. Gently shake the tubes in arder to resupend the cells.
8. Read under microscope for agglutination and grade the degree of agglutination observed.

II. Reverse Typing [Indirect] Procedure

1. Label tubes A_1, B and O.
2. Prepare a 5% suspension of red cells A_1, B and O in saline.
3. Place one drops of each cell suspension into the appropriately labeled test tubes.
4. Add 2 drops of the patient's serum into each tube and mix.
5. Centrifuge for 1 min. at 1800 rpm.
6. Gently shake the tube s in order to resuspend the cells.
7. Read macroscopically for agglutination and grade the degree of agglutination observed.

II. Suggestive for Rh D Negative

1. 1 drop of 2-4 % suspension of test cells, add 2 drops of anti-D.
2. Mix and incubate at 37°C for 45 min.
3. Look for agglutination.
4. If test is Positive, record the sample as D positive.
5. If test is Negative i.e. no agglutination, wash the cells 3-4 times with saline and decant the last wash completely.
6. Add 2 drops of AHG and mix gently.
7. Centrifuge at 1000 rpm for 1min.

8. Resuspend the cell button gently, look for agglutination and record the results.

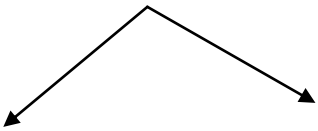
CROSSMATCH

The crossmatch is an in-vitro procedure to determine serologic compatibility between the donor's red cell and the recipient's serum. This is accomplished by adding a suspension of donor's cells to the recipient's serum and observing for agglutination and / or hemolysis in the various phases.

Method

1. Place 2 drops of recipient's serum in a properly labeled test tube (1 tube for each unit to be crossmatched.
2. Add 1 drop of 3-4% saline suspension of washed donor red cells. Mix.
3. Spin and observe for agglutination. Read and record results.
4. Add 2 drops of Bovine Albumin [22% solution].
5. Incubate at 37°C for 15-20 min.
6. Centrifuge immediately upon completion of incubation. Examine for hemolysis Dislodge the cell button and observe for agglutination macroscopically. Record results.
7. Wash 4 times with saline.
8. Add 2 drops of anti-human globulin and mix.
9. Centrifuge. Examine macroscopically with agglutination viewer for agglutination. Record results.
10. To all negative tests, add 1 drop of IgG sensitized red cells. Centrifuge and examine for agglutination. If no Agglutination is present, test must be repeated.

Coombs testes



Indirect coomb's Direct Antiglobulin coomb's

Method Indirect coomb's Test

1. To 4 drops of patient's serum, add 2 drops of washed O+ RBC.
2. Mix- centrifuge- mix.
3. Incubate for 20-30 min. at 37°C.
4. Wash 4 times with normal saline solution.
5. Decant final wash completely.
6. Add Coomb's sera 2-4 drops.
7. Mix-centrifuge-Mix.
8. Incubate for 20-30 min. at 37°C.
9. Observe for agglutination.

Method Direct Antiglobulin [coomb's] Test [DAT]

1. Put 1 drop of 2-4% cell suspension to be tested in a clean labelled glass tube.
2. Wash the cells 3 times with saline.
3. Decent the final wash completely.
4. Add 1-2 drops of AHG reagent.
5. Mix and centrifuge at 1000 rpm for 1min.
6. Gently shake the tube and read the result.
7. If the test result is NEGATIVE [no agglutination], add 1drp of control cells.
8. Mix and centrifuge at 1000 rpm for 1min. and look for agglutination. If no agglutination is seen, the result is invalid. Repeat the test procedure.

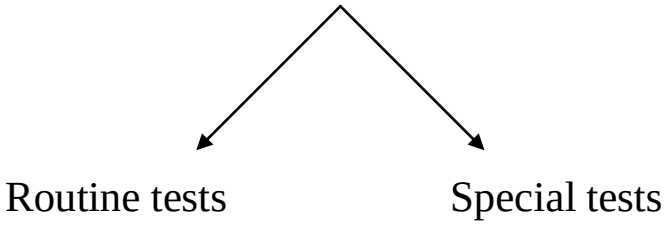
Sixth Section

SEROLOGY DEPARTMENT

Types of sample.

Serum or plasma.

Type of tests



Routine tests

1. ASO titer [Anti-Striptolysine O].
2. Rheumatoid factor.
3. C. Reactive Protein
4. Toxoplasmosis
5. VDRL.
6. Brucella; Abortus and Melitensis
7. Widal test;
   a. Salmonella typhi H,O.
   b. Salmonella Paratyphi A-H, O
   c. Salmonella Paratyphi B-H, O
   d. Salmonella Paratyphi C-H, O

Special tests

1. HIV
2. HBc
3. HCV
4. HbsAg
5. Anti-HBs
6. HTLV1+2

List of Equipment's;

1. Rotator.
2. Micropipettes - difrferent sizes.
3. Washer.
4. Incubator.
5. Reader.

First [Routine tests]

ASO / C-reactive Protein [CRP] LATEX TESTS

The ASO / CRP reagent Kit is based on an immunological reaction between antisera bound to biologically inert latex particles and ASO/CRP in the test specimen. When serum containing ASO/ CRP is mixed with the latex reagent, visible agglutination occurs.

Rheumatoid Factor [RF] LATEX TESTS

Th RF reagent Kit is based on an immunological reaction between human IgG bound to biologically inert latex particles and Rheumatoid factor in the test specimen. When serum containing is Rheumatoid factor mixed with the latex reagent, visible agglutination occurs.

Wdial and Weil- FELIX tests

Plasmatec staind antigen suspensions may be used to identify and quantities specific antibodies in human sera following infection with certain Salmonellae and Brucellae pathogens. When serum containing is Salmonellae or Brucellae mixed with the latex reagent, visible agglutination occurs.

Toxoplasmosis

Th Toxoplasma reagent Kit is based on an immunological reaction between human IgM bound to biologically inert latex particles and Toxoplasma in the test specimen. When serum containing is Toxoplasma mixed with the latex reagent, visible agglutination occurs.

RPR [VDRAL] reditest

Rapid test for the qualitative and quantitative detection of syphilis in serum or plasma.

The RPR antigen is a cardiolipin suspension containg charcoal microparticles. This antigen detects an antibody, Present in the serum of syphilitic patients. When a specimen contain regains, a flocculation of the antigen is produced, which coagglutinate with the charcoal microparticles giving back clumps of different size depending of the regain titer. With nonreactive specimens, no reaction will take place and a homogenous grey colour will be maintained.

Second Special tests

Main principles of all Special tests are based on Antibody-Antigen interaction.

Determination of all tests by kit [Method depend on partnership].

For example Method of HIV test

1. Make centrifugation of blood, then separate serum.
2. Take 25 µl of conjugate1 [R6].
3. Add 75µl of sample[plasma or serum] [start put of position F1], negative control[R3][put in position A1] ,CUTOFF [R4] [put in B1, C1 and D1] or positive control[R5][put in E1].
4. Incubate at 37°C for 1 hour.
5. Wash [3 times]
6. Add 100µl from conjagate2 [R7a + R 7b].
7. Incubate at room temp. for 30 min.
8. Wash [5 times].
9. Add 80 µl from stopping solution [R10].
10. Read after 4 min. from adding stopping solution. [by reader machine].