Accuster Training Module

This training module provides a comprehensive overview of essential practices in Accuster Diagnostic Labs, enhancing your skills to deliver accurate diagnostic services effectively.

About Us

Accuster Technologies is a research-based manufacturing organization operating in the field of In-Vitro Diagnostics (IVD). We are diligently working to address last-mile care for people in the remotest locations across the country.

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GENERAL TERMS

CALIBRATION: Calibration is the process of evaluating and adjusting the precision and accuracy of the mobile lab. calibration is used to fix the linearity of the mobile lab and re-standard the analyser.

QUALITY CONTROL (L1, L2) : QC is part of the quality management system by which laboratories assure that the performance of biochemical analyser and validity of Analyser. The goal of QC is to detect, evaluate and correct errors due to test system failure, environmental conditions, or operator performance, before patient results are reported.

QUALITY CONTROL PREPARATION :

Quality control needs to be handled carefully, just like patient samples. Quality control is performed just like any other test; according to the procedures of each test.

To get the Quality control ready, take a control bottle and add 5.0 mL of distilled or deionized water. Then, put the cap back on and allow this product to stand for 20 minutes. Give it a swirl every now and then.

Before you take a sample, gently swirl the bottle a few times to make sure everything is mixed well. After you're done using it, put the cap back on right away and store it properly.

C1, C2, C3 And L1 , L2 : We can perform quality control in our system in two ways. When we have only two quality controls, we check accuracy using L1 and L2. However, if we have three quality controls, we use the C1, C2, and C3 modes for quality control in the analyzer.

LINEARITY : The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples within a given range. This is why to check the proportionality of the result with concentrations, it is necessary to justify the linearity between LOWER Concentration & UPPER Concentration.

CENTRIFUGE : Works at 6000 rpm . centrifuge used to separate fluid, based on density . In the lab mostly we used a centrifuge to separate serum from blood.

INCUBATOR : Incubator is used to maintain temperature and incubation of sample (37℃). We can adjust the temperature of this incubator , the temperature ranges from 25℃ to 45℃ . Alarm system is also available for time management and result accuracy .

ANALYZER : Analyser is working on Beer & Lambert's Law (the absorbance of a solution is directly proportional to the conc. of the absorbing material present in the solution and path length) and working with 340λ to 639λ wavelength.

SATELLITE CONTROL: The satellite control module operates on GSM frequencies of 850, 900, 1800, and 1900 MHz, utilizing a FLASH SIM800C (24Mbit) and 32Mbit RAM. It sends data once daily upon device startup, taking approximately 1.5 minutes to complete the process, during which the device should not

be powered off. The data transmission consists of 4 or 5 messages *"**Sending ctrl Reading, Sending Daily Data Counter, Sending factor Test Counter, Sending Daily calibr Counter, Sending Phy location**"* indicated on the display as various notifications. The device stores information regarding test samples and quality control (QC) applications in its memory and sends this data to **report.accuster.com** upon subsequent startup. The geographical location of the device is sent to the portal as latitude and longitude coordinates, and financial and QC controls are implemented to track resources and maintain product quality standards. Additionally, signal strength is categorized from marginal to excellent, providing insights into connectivity status.

KEY POINTS:-

1. Two types of test are performed on analyser, Accurate all (end point) and kinetic test . In End point, we measure product concentration after completing the reaction and after a specific time (specific incubation time).
2. In a kinetic test , the progress of the reaction is continuously measured by an analyzer as substrates (Reagent + sample reaction) are converted into products with respect to time and temperature. Temperature and time should be constant.
3. Creatinine is the only test that is a kinetic test but we performed in **ACCURATE ALL** mode. In creatinine, sampling after incubation the reaction will start simultaneously, maintaining the proper 37℃ out the cuvette one by one while sampling.
4. Read the handling of reagents in literature, mostly reagents are stored at 2℃ to 8℃,while sampling keeps the reagent 20 to 25 min at room temperature.

ACCURATE ALL

1.) SUGAR TEST

Test	Normal Range	Child	Critical value
Glucose fasting	74-100	60 - 100	low <45.0 high >500
Post prandial Sugar	70-140		**Newborn** low <30, high >130
Glucose Random	60-140		

CALIBRATION (Erba Reagent)				
	1x	**2x**	**4x**	**6x**
Reagent	500µl	500µl	500µl	500µl
Standard	5µl	10µl	20µl	30µl
Solution Conc.	100 mg/dl	200 mg/dl	400 mg/dl	600 mg/dl
Mix well and incubate for 15 min at 37℃ or 25 min at RT				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl reagents in 4 cuvette
- Add glucose standard in glucose reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20µl in 3rd cuvette 30µl in 4th cuvette
- Incubate all cuvette in incubator at 37℃ for 15 min
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x, put the cuvette on an analyzer one by one and set the calibration.
- press 5 to show the details
- for exit from the calibration mode press “Del” key

TEST PROCEDURE: ***(GOD-POD method)***

- take 500µl reagent in cuvette
- Add 5µl patient serum mix it well and incubate at 37℃ for 10 min
- after incubation press 1 and select Accurate all to test and select test (Glucose)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

1. Fasting sugar -10 to 12 hrs of fasting must be required .
2. PP sugar-sample will be taken after fasting sugar, patient eats food and just after 2 hrs of the food time taken countdown the time to take the sample again for pp.
3. Random-there is no specific time required for taking the sample .

2.)UREA TEST(Ure)

Normal range	male	Female	Critical value
Urea	19 - 45	13 - 40	>80 except dialysis
BUN	8 - 23	8-23	

CALIBRATION (Beacon)				
	1x	**2x**	**4X**	**6X**
Reagent 1	500µl	500µl	500µl	500µl
standard	5 µl	10µl	20µl	30µl
Mix well and incubate for 3 min at 37℃ or 5 min at RT				
Reagent 2	500µl	500µl	500µl	500µl
Sol^{n} conc	40 mg/dl	80 mg/dl	160 mg/dl	240 mg/dl
Mix well and incubate for 5 min at 37℃ or 10 min at RT				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl reagents 1 in 4 cuvette
- Add Urea standard in reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20µl in 3rd cuvette 30µl in 4th cuvette
- Incubate all cuvette in incubator at 37℃ for 3min
- Now add 500ul reagent 2 in all 4 cuvettes, mix well and incubate for 5 min at 37℃
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE (*BERTHELOT METHOD)*

- 500µl reagent1 in cuvette
- Add 5µl patient serum mix it well and incubate at 37℃ for 3 min
- Now add 500µl reagent 2 in the cuvette, mix it well and incubate at 37℃ for 5 min.
- After incubation press 1 and select Accurate all to test and select test (Urea)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

3.) URIC ACID (Uri)

Normal Range	**Male**	**Female**
Uric Acid	3.5 to 7.2	2.6 to 6.0

CALIBRATION (Erba Reagent)				
	1X	**2X**	**4X**	**6X**
Reagent	500µl	500µl	500µl	500µl
standard	10µl	20µl	40µl	60µl
Sol^{n} Conc.	6 mg/dl	12 mg/dl	24 mg/dl	36 mg/dl
Mix well and incubate at 37℃ for 10 min. or 15 min. at RT				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl reagents in 4 cuvette
- Add glucose standard in glucose reagent according to table, for ex. 10µl standard in first cuvette, 20µl in 2nd cuvette, 40µl in 3rd cuvette 60µl in 4th cuvette
- Incubate all cuvette in incubator at 37℃ for 10 min
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x, put the cuvette on an analyzer one by one and set the calibration.
- press 5 to show the details
- for exit from the calibration mode press “Del” key

TEST PROCEDURE *(Trinder Method)*

- Take 500µl reagent in cuvette
- Add 10µl patient serum mix it well and incubate at 37℃ for 10 min
- After incubation press 1 and select Accurate all to test and select test (Uric acid)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

4) CREATININE (CrE) (End Point)

CREATININE (Cogent Reagent)	
Step 1) Make supernatant for Sample test	
Sample	2 ml
P.A.R	200 µl
Mix well and Centrifuge at 3000 - 4000 rpm for 5 min.	

Step 2) To perform calibration				
	1x	**2x**	**4x**	**6x**
P.A.R. (L1)	500 µl	500 µl	500 µl	500 µl
Creatinine Std.	50 µl	100 µl	200 µl	300 µl
Buffer reagent	50 µl	50 µl	50 µl	50 µl
Sol. Conc.	2 mg/dl	4 mg/dl	8 mg/dl	12 mg/dl
Mix well and incubate for 20 min at RT				

STEP 1) PREPARE SUPERNATANT FOR TEST

- Take 200 µl sample in cuvette
- Add 2 ml P.A.R. Reagent and mix well
- After centrifuge at 3000-4000 rpm for 5 min , take supernatant for further use
- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl P.A.R. reagents in 4 cuvette
- Add standard in reagent according to table, for ex.50 µl standard in first cuvette, 100µl in 2nd cuvette, 200 ul in 3rd cuvette 300ul in 4th cuvette
- Incubate all cuvette in incubator at RT for 20 min
- press 1 to select “Accurate all”
- Now press zero (0) for calibration

- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x, 2x, 4x and 6x put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE:

- take 50 µl buffer reagent in cuvette
- Add 1.1 serum supernatant mix it well and incubate at RT for 20 min
- After incubation press 1 and select Accurate all to test and select test (CrE)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

5) CREATININE TEST(CRK)

Normal Range	Male	Female	Child	critical value
Creatinine	0.7 to 1.3	0.6 to 1.1	0.3 to 0.7	>5.0 except dialysis

CALIBRATION (Erba Reagent)	
Reagent, R1 (Picric Acid)	250ul
Reagent, R2 (Alkaline reagent)	250ul
Mix and incubate for 10 min. at 37℃	
Standard	50ul
Mix well and take reading	

CALIBRATION PROCEDURE

- Take 250 µl of Reagent R1 in the cuvette and then add 250 µl of reagent R2 in the cuvette
- Place it in incubator at 37℃ for 10 min
- now start the analyser , press 1 to select "Accurate all"
- Press 5 to select creatinine test (kinetic test)
- Now press "NEW" to ask standard and then press "0" zero to ask for zero reference
- Now put the Distilled Water cuvette into the analyser and press "enter" to set Zero reference
- For calibration, take 50 µl standard in pipette and take one cuvette from incubator in which reagent are incubated , and put index finger on enter button
- Press "Enter" and mix the 50 µl Std ,mix the reagent well and put it into the analyser at lag time within 20 sec.
- Calibration is complete now, STD Conc. should be 2 mg/dl

NOTE: We can perform calibration of this test with normal method , ("0" → "000" →2 →1, select test)

TEST PROCEDURE: (***JAFFE'S METHOD INITIAL RATE)***

- Take 250 µl of R1 reagent in the cuvette and then add 250 µl of R2 reagent in the cuvette and mix it well
- Place it in an incubator at 37℃ for 10 min.
- Now press 1 to select Accurate All and then press 5 to select the test (CRK)

- Take 50 µl sample in the micropipette
- We will only take out one cuvette from the incubator and close the incubator to maintain the temperature of the cuvette inside the incubator. Then, **we will hold the cuvette using the thumb and index finger of the left hand and place the smallest finger on the enter button. T**hen, we will use the thumb of the right hand to press the pipette's press button. Now, we need to mix the sample into the reagent and press “ENTER” at the same time. **Mixing the sample and pressing enter should occur simultaneously.**
- After 60 sec result will be shown on screen

Fig.) The correct way to hold a cuvette and add a sample , along with pressing the enter button is demonstrated (All kinetic test)

NOTE:

1. Make sure not to remove the cuvette box from the incubator while performing the Creatinine test because temperature maintenance plays an important role. Take out the cuvettes one by one from the incubator and recap the incubator to maintain the temperature.
2. Time plays an important role in creatinine, there is “lag” time & “Reading time”. lag time is 20 sec , during this we need to mix our sample & place the cuvette inside the analyzer within 20 sec. The Reading time will start at 60 sec for the result. Something we need to understand “ the reading time and the reaction of our sample should start at the same time after then press “Enter” button and mix the sample at the same time.

6.)PHOSPHORUS(Pho)

Normal Range	Male	Female	Child	Critical Range
Phosphorus	2.5 to 4.5	2.5 to 4.5	4.0 to 6.5	< 1.1

CALIBRATION (Coral)				
	1X	**2X**	**4X**	**6X**
Reagent L1	250µl	250µl	250µl	250µl
Reagent L2	250µl	250µl	250µl	250µl
Standard	25µl	50µl	100µl	150 µl
C.R.	250µl	250µl	250µl	250µl
Sol Conc.	5 mg/dl	10 mg/dl	20 mg/dl	30 mg/dl
Mix well and incubate for 5 min at 37℃				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 250µl reagents L1 in 4 cuvette and then add 250µl Reagent L2 and mix it well
- Add Phosphorus standard in reagent according to table, for ex. 25µl standard in first cuvette, 50µl in 2nd cuvette, 100µl in 3rd cuvette 150µl in 4th cuvette
- Now add Coloring Reagent 250 µl in cuvette
- Incubate all cuvette in incubator at 37℃ for 5 min
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details and for exit from the calibration mode press “Del” key

TEST PROCEDURE *(Mod. GOMORRI'S METHOD)*

- Take 250µl L1 Reagent and then add 250 µl L2 reagent mix it well
- Now add 25µl serum sample in cuvette
- Add 250 µl Coloring reagent mix it well and incubate at 37℃ for 5 min
- After incubation press 1 and select Accurate all to test and select test (Pho)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

After the sample taken performs the test before 1 hour ,extra time will affect the result .Repeat the collection if 1 hour has passed.

7) POTASSIUM (K+)

Potassium	**Male**	**Female**	**Critical range**
Normal Range	3.5 to 5.5	3.8 to 5.2	<2.8 >6.0

CALIBRATION (Coral Reagent)				
	1x	2/3x	1/2x	1/3
Reagent	500µl	750 µl	500 µl	750 µl
Standard	10 µl	10 µl	5 µl	5 µl
Sol. Conc.	5 mMol/l	3.33 mMol/l	2.5 mM/l	1.6 mMol/l
Mix well and wait for 2 min at RT				
NOTE: *Serum should be collected as soon as possible after centrifugation*				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl, 750 µl, 500 µl, 750 µl reagents in 4 cuvette respectively
- Add Potassium Standard standard in reagent according to table, for ex. 10µl standard in first cuvette, 10µl in 2nd cuvette, 5µl in 3rd cuvette 5µl in 4th cuvette
- Incubate all cuvette in incubator at RT for 2 min
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE:

- Take 500µl reagent in cuvette
- Add 10µl patient serum mix it well and incubate at RT for 2 min
- After incubation press 1 and select Accurate all to test and select test (Potassium)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

After the sample taken performs the test before 1 hour ,extra time will affect the result .Repeat the collection if 1 hour has passed.

8)SODIUM (Na+)

Sodium	Male	Female	Critical range
Normal Range	135 to 155	135 to 146	<120 >160

CALIBRATION : Calibration and Test performed in two steps

Step. 1 To prepare supernatant of standard and Sample		
	Standard	**Test**
Precipitating Reagent L1	500 µl	500 µl
Standard	10 µl	
Serum		10 µl
Mix well & centrifuge for 5 min . take supernatant for further use		

Step 2.) Calibration & testing (Coral Reagent)			
	Blank	**Standard**	**Test**
Acid L2	500 µl	500 µl	500 µl
Supernatant		10 µl	10µl
Precipitating Reagent	20µl		
coloring reagent L3	50 µl	50 µl	50 µl
Mix well and incubate for 10 min at 37℃			

Step 1) How to prepare Supernatant of standard and the sample

- Take 500µl Precipitating Reagent in two cuvette
- Add 10 µl standard in one cuvette and 10 µl serum in another cuvette separately
- Centrifuge for at least 5 min and collect the supernatant

Step 2) CALIBRATION PROCEDURE

- take 500 µl "Acid L2" in two cuvette separately , one is for blank and another is for standard
- Add 10 ul standard supernatant in standard cuvette and 10 µl precipitating reagent in Blank cuvette
- Now add 50 µl "coloring reagent" in both cuvette mix and incubate for 10 min at 37℃
- Press 1 to select "Accurate all"
- Press 8 to select Sodium test
- Now press "NEW" to ask standard and then press "0" zero to ask for zero reference
- Now put the Blank cuvette into the analyser and press "enter" to set Zero reference
- Now put the standard cuvette in the analyser and press enter
- Calibration is complete now we can perform the test.

NOTE: ***We can perform calibration of this test with normal method , ("0" → "000" →2 →1, select test)***

TEST PROCEDURE (*COLORIMETRIC METHOD)*

- Take 500 µl "Acid L2" in cuvette
- Add 10 µl serum supernatant in cuvette
- Now add 50 µl "coloring reagent" in cuvette mix and incubate for 10 min at 37℃
- Press 1 to select "Accurate all"
- Press 8 to select Sodium test
- Now put the cuvette into the analyser and enter the label no of patient and press enter and take reading of the sample

PRECAUTION

After the sample taken performs the test before 1 hour ,extra time will affect the result .Repeat the collection if 1 hour has passed.

9) CHLORIDE(Cl^-)

Chloride	Male	Female	Critical range
Normal range	98 to 106	98 to 106	<80.0 >115.0

CALIBRATION (coral reagent)				
	1x	**2/3x**	**1/2x**	**1/3x**
Reagent	1000 µl	1500 µl	1000 µl	1500 µl
Standard	10 µl	10 µl	5 µl	5 µl
Sol. Conc.	100 meq/l	66 meq/l	50 meq/l	33.3 meq/l
Read immediately without incubation				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 1000 µl, 1500 µl, 1000 µl, 1500 µl reagents in 4 cuvette respectively
- Add Potassium Standard standard in reagent according to table, for ex. 10µl standard in first cuvette, 10µl in 2nd cuvette, 5µl in 3rd cuvette 5µl in 4th cuvette, mix well and read immediately without incubation
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE: ***(THIOCYANATE METHOD)***

- Take 1000µl reagent in cuvette
- Add 10µl patient serum mix it well and read immediately without incubation
- Press 1 and select Accurate all to test and select test (Chloride)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

After the sample taken performs the test before 1 hour ,extra time will affect the result .Repeat the collection if 1 hour has passed.

10.)MAGNESIUM (Mg)

Magnesium	Male	Female	Child	Critical range
Normal Range	1.3 to 2.5	1.3 to 2.5	1.5 to 2.0	<1.4 , >4.7

CALIBRATION (coral reagent)				
	1x	**2x**	**4x**	**6x**
Reagent L1	250 µl	250 µl	250 µl	250 µl
Reagent L2	250 µl	250 µl	250 µl	250 µl
Standard	5 µl	10 µl	20 µl	30 µl
Sol. Conc.	2 meq/l	4 meq/l	8 meq/l	12 meq/l
Mix well and incubate for 2 min. at 37°C				

CALIBRATION PROCEDURE

- Take 250 µl of Reagent L1 in the cuvette and then add 250 µl of reagent L2 in the cuvette
- Add Magnesium Standard standard in reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20ul in 3rd cuvette 30 in 4th cuvette, mix well and read immediately without incubation
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x on screen, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE: ***(CALMAGITE METHOD)***

- Take 250 µl of R1 reagent in the cuvette and then add 250 µl of R2 reagent in the cuvette and mix it well
- Place it in an incubator at 37℃ for 10 min.
- Now press 1 to select Accurate All and then press 10 to select the test(Mg)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

After the sample taken performs the test before 1 hour ,extra time will affect the result .Repeat the collection if 1 hour has passed.

11) CALCIUM (Ca)

Calcium	**Male**	**Female**	**Critical range**
Normal Range	8.7 to 11.0	8.7 to 11.0	<6.0 >13.0

CALIBRATION (coral reagent)				
	1x	**2/3x**	**1/2x**	**1/3x**
Reagent L1	250 µl	375 µl	250 µl	375 µl
Reagent L2	250 µl	375 µl	250 µl	375 µl
Standard	10 µl	10 µl	5 µl	5 µl
Sol. Conc.	10 mg/dl	6.6 mg/dl	5 mg/dl	3.3 mg/dl
mix well and incubate for 2 min at RT				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 250µl, 375 µl, 250 µl, 375µl reagents in 4 cuvette respectively
- Add standard in reagent according to table, for ex. 10µl standard in first cuvette, 10µl in 2nd cuvette, 5µl in 3rd cuvette 5ul in 4th cuvette
- Incubate all cuvette at RT for 2 min
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE: ***(OCPC METHOD)***

- Take 250µl reagent L1 in cuvette then add 250 ul reagent L2
- Add 10µl patient serum mix it well and incubate at RT for 2 min
- After incubation press 1 and select Accurate all to test and select test (Calcium)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

After the sample taken performs the test before 1 hour ,extra time will affect the result .Repeat the collection if 1 hour has passed.

12) HEMOGLOBIN (Hb)

Hemoglobin	**Male**	**Female**	**Child**	**Critical range**
Normal Range	12.0 to 18.0	12.0 to 16.0	Newborn : 16.0 to 25.0 Infants: 11.0 to 14.0 Children upto 10 yr : 12.0 to 16.0	<7.0 >23

CALIBRATION (RFCL Reagent)				
	1x	2/3x	1/2x	1/3x
Reagent	……	200 µl	300 µl	400 µl
Standard	500 µl	400 µl	300 µl	200 µl
Sol. Conc.	15 g/dl	10 g/dl	7.5 g/dl	5 g/dl
Mix well and wait for 3 min				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 200µl, 300µl, 400µl in 2nd, 3rd and 4th cuvette respectively, leave 1st cuvette blank
- Add standard in reagent according to table, for ex. 500µl standard in first cuvette, 400µl in 2nd cuvette, 300ul in 3rd cuvette 200 µl in 4th cuvette
- Incubate all cuvette in at RT for 3 min
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDUR*E: (CYANMETHEMOGLOBIN METHOD)*

- Take 2000 µl reagent in cuvette
- Add 8µl patient blood mix it well and incubate at RT for 3 min
- After incubation press 1 and select Accurate all to test and select test (Hb)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

Don’t mix the reagent & blood with bare hand used gloves.

13)CHOLESTEROL (Ch)

Cholesterol	**Male**	**Female**	**Child**	**Critical Range**
Normal Range	<200	<200	<170	**Adult** :200- 239 mg/dl, **Child** : 170 to 199

CALIBRATION (Erba Reagent)				
	0.5x	**1x**	**2x**	**3x**
Reagent	500 µl	500 µl	500 µl	500 µl
Standard	5 µl	10 µl	20 µl	30 µl
Sol. Conc.	100 mg/dl	200 mg/dl	400 mg/dl	600 mg/dl
Mix well and incubate for 10 min at 37℃ or 15 min at RT				
37℃ save standard Conc. 100 mg/dl				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl reagents in 4 cuvette
- Add standard in reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20µl in 3rd cuvette 30µl in 4th cuvette
- Incubate all cuvette in incubator at 37℃ for 10 min
- Now start the analyzer, there is 2 option, press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 0.5x, 1x, 2x and 3x put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE: ***(ENZYMATIC COLORIMETRIC TEST)***

- Take 500µl reagent in cuvette
- Add 10 µl patient serum mix it well and incubate at 37℃ for 10 min
- After incubation press 1 and select Accurate all to test and select test (Ch)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

fasting is necessary while sampling without fasting the reading will come higher.

14) TRIGLYCERIDE (Tg)

Triglyceride	**Expected values**
Normal range	<161
High	161 to 199
Hypertriglyceridemic	200 to 499
Critical Range	> 499

CALIBRATION (Coral Reagent)				
	1x	**2x**	**4x**	**6x**
Reagent L1	400 µl	400µl	400µl	400µl
Reagent L2	100	100	100	100
Standard	5µl	10µl	20µl	30µl
Sol. Conc.	200 mg/dl	400 mg/dl	800 mg/dl	1200 mg/dl
Mix well and incubate for 10 min at 37°C				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 400µl reagents L1 in 4 cuvette and then add 100µl L2 Reagent mix them properly
- Add standard in reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20µl in 3rd cuvette 30µl in 4th cuvette
- Incubate all cuvette in incubator at 37°C for 10 min
- Now start the analyzer, there is 2 option, press 1 to select "Accurate all"
- Now press zero (0) for calibration
- press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x, 2x, 4x and 6x put the cuvette on an analyzer one by one and set the calibration.

- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE: (*GPO - PAP METHOD)*

- Take 400µl reagent L1 and then add 100µl Reagent L2 in cuvette mix them properly
- Add 10 µl patient serum mix it well and incubate at 37℃ for 10 min
- After incubation press 1 and select Accurate all to test and select test (Ch)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

PRECAUTION:

fasting is necessary while sampling without fasting the reading will come higher.

15)HDL CHOLESTEROL (HD)

Cholesterol	**Male**	**Female**
Normal Range	30 to 65 mg/dl	35 to 80 mg/dl

CALIBRATION (Erba Reagent)	
Step 1) Make supernatant for Sample test	
Serum	125 µl
HDL Reagent	250 µl
Mix well and wait for 10 min. , Centrifuge at 3000 - 4000 rpm for 5 min.	

Step 2) To perform calibration				
	1x	**2x**	**4x**	**6x**
Cholesterol Reagent	500 µl	500 µl	500 µl	500 µl
HDL Std.	25 µl	50 µl	100 µl	150 µl
Sol. Conc.	25 mg/dl	50 mg/dl	100 mg/dl	150 mg/dl
Mix well and incubate for 10 min at 37℃, Result multiples by 3 or use factor 3.				

STEP 1) PREPARE SUPERNATANT FOR TEST

- Take 125 µl serum in cuvette
- Add 250 µl HDL Reagent, mix and wait for 10 min
- After 10 min centrifuge at 3000-4000 rpm for 5 min , take supernatant for further use

STEP 2) TO PERFORM CALIBRATION PROCESS

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl cholesterol reagents in 4 cuvette
- Add HDL standard in reagent according to table, for ex.25µl standard in first cuvette, 50µl in 2nd cuvette, 100ul in 3rd cuvette 150ul in 4th cuvette
- Incubate all cuvette in incubator at 37℃ for 10 min
- Now start the analyzer, there is 2 option, press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x, 2x, 4x and 6x put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE: (*PEG / CHOD - METHOD)*

- take 500ul Cholesterol reagent in cuvette
- Add 25 ul serum supernatant mix it well and incubate at 37℃ for 10 min
- After incubation press 1 and select Accurate all to test and select test (HD)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample, and then you have to multiply the result by 3 or you can set factor (3) directly into the analyzer.

PRECAUTION:

fasting is necessary while sampling without fasting the reading will come higher.

16) LDLc…..LDL (Calculative Test)

LDL	Male	Female
Normal Range	130 to 160	130 to 160

PROCESS : ***Select test then enter the reading of Cholesterol, Triglyceride and HDL Then press "ENTER" and read the Result of LDL***

17) ALKALINE PHOSPHATE (AP)

Alkaline Phosphate	Male	female	Child
Normal Range	53 to 128 u/l	42 to 96 u/l	54 to 369

CALIBRATION (Coral Reagent)					
	1x	**2x**	**4x**	**6x**	**Test**
Distilled water	500 µl	500 µl	500 µl	500 µl	500 µl
Reagent L1	500 µl	500 µl	500 µl	500 µl	500 µl
Reagent L2	50 µl	50 µl	50 µl	50 µl	50 µl
Mix well and incubate at 37℃ for 3 min.					
Standard	25 µl	50 µl	100 µl	150 µl	……
serum					25 µl
Mix well & incubate at 37℃ for 15 min.					
Reagent L3	500 µl	500 µl	500 µl	500 µl	500 µl
Mix well and Read					
Sol. Conc.	71.4 mg/dl	142.8 mg/dl	285.6 mg/dl	428.4 mg/dl	

NOTE: Check the Factor should be 7.14 mg/dl, (1 K.A= 7.14 mg/dl)

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl Distilled water in 4 cuvette
- Add 500 reagent L1 in all 4 cuvette
- Add 50 µl reagent L2 in all cuvette, mix well & incubate at 37℃ for 3 min.
- Add standard in reagent according to table, for ex. 25µl standard in first cuvette, 50µl in 2nd cuvette, 100 µl in 3rd cuvette 150 µl in 4th cuvette
- Incubate all cuvette in incubator at 37°C for 15 min
- Add 500 µl of reagent L3 in cuvette , mix well and Read
- Press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x, 2x, 4x and 6x put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE: (*Mod. KIND & KINGS METHOD)*

- Take 500µl Distilled water in cuvette
- Add 500 reagent L1
- Add 50 µl reagent L2 in all cuvette, mix well & incubate at 37℃ for 3 min.
- Add 25 µl patient serum mix it well and incubate at 37℃ for 15 min
- After incubation press 1 and select Accurate all to test and select test (AP)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

18) SGOT (End point)

SGOT	Male	Female
Normal Range	0-35	0-31

Calibration and Testing procedure (Medsource Reagent)					
	1x	**2.5x**	**5x**	**20x**	**TEST**
Substrate	250 µl	250 µl	250 µl	250 µl	250 µl
Sample					10 ul
Calibrator diluted	10 µl	25 µl	50 µl	200 µl	
incubate for 60 min. at 37°C					
color Reagent	250 µl	250 µl	250 µl	250 µl	250 µl
incubate for 20 min at 37°C					
Alkaline Reagent Diluted	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ul
Sol. Conc.	40 u/l	100 u/l	200 u/l	800 u/l	unknown

STEP 1) PREPARATION OF STANDARD (1:4 dilution)

- Take 125 µl of Calibrator and add 375 ul distilled water(standard Conc. should be 40 u/l

STEP 2) PREPARE DILUTED ALKALINE REAGENT

- Dilute the Alkaline Reagent 10 times, i.e., to prepare 10 ml of solution, Take 1 ml of Alkaline Reagent and 9 ml of distilled water and mix it well.

CALIBRATION PROCESS

- Take 4 Cuvette in cuvette box for calibration process
- Add calibrator (diluted) in substrate according to table, for ex. 10 µl standard in first cuvette, 25 µl in 2nd cuvette, 50 µl in 3rd cuvette 200 µl in 4th cuvette
- Incubate all cuvette in incubator at 37°C for 60 min
- Add 250 µl of coloring reagent in cuvette , mix well and incubate for 20 min at 37°C
- Add 1.5 ml of alkaline reagent (diluted) in all cuvette, mix well and read
- Press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x, 2.5, 4x, and 20x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TESTING PROCEDURE (*DNPH METHOD)*

- Take 250 ul substrate in cuvette
- Add 10 ul sample , mix and incubate for 60 min at 37°C
- After incubation add 250 ul coloring reagent an incubate for 20 min at 37°C
- Add 1.5 ml of Alkaline Reagent mix and read
- Press 1 and select Accurate all to test and select test (SGOT)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

19) SGPT (End Point)

SGPT	Male	Female
Normal Range	0-45	0-34

Calibration and Testing procedure (Medsource Reagent)					
	1x	**2.5x**	**5x**	**20x**	**TEST**
Substrate	250 µl	250 µl	250 µl	250 µl	250 µl
Sample					10 ul
Calibrator diluted	10 µl	25 µl	50 µl	200 µl	
incubate for 30 min. at 37°C					
color Reagent	250 µl	250 µl	250 µl	250 µl	250 µl
incubate for 20 min at 37°C					
Alkaline Reagent Diluted	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ul
Sol. Conc. (unit/liter)	42.5 u/l	106 u/l	212.5 u/l	850 u/l	unknown

STEP 1) PREPARATION OF STANDARD (1:4 dilution)

Take 125 ul of Calibrator and add 375 ul distilled water(standard Conc. should be 42.5 u/l

STEP 2) PREPARE DILUTED ALKALINE REAGENT

Dilute the Alkaline Reagent 10 times, i.e., to prepare 10 ml of solution, Take 1 ml of Alkaline Reagent and 9 ml of distilled water and mix it well.

CALIBRATION PROCESS

- Take 4 Cuvette in cuvette box for calibration process
- Add calibrator (diluted) in substrate according to table, for ex. 10 µl standard in first cuvette, 25 µl in 2nd cuvette, 50 µl in 3rd cuvette 200 µl in 4th cuvette
- Incubate all cuvette in incubator at 37°C for 30 min
- Add 250 µl of coloring reagent in cuvette , mix well and incubate for 20 min at 37°C
- Add 1.5 ml of alkaline reagent (diluted) in all cuvette, mix well and read
- Press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x, 2.5, 4x, and 20x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TESTING PROCEDURE *(DNPH METHOD)*

- Take 250 µl substrate in cuvette
- Add 10 µl sample , mix and incubate for 60 min at 37°C
- After incubation add 250 µl coloring reagent an incubate for 20 min at 37°C
- Add 1.5 ml of Alkaline Reagent mix and read
- Press 1 and select Accurate all to test and select test (SGOT)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

20)TOTAL PROTEIN (TP)

Total Protein	Male	Female	Child	Critical
Normal Range	6.4 to 8.3	6.4 to 8.3	5.1 to 7.3 g/dl	<4.0 >9.0

CALIBRATION (Erba Reagent)				
	1x	**2x**	**4x**	**6x**
Reagent	500µl	500µl	500µl	500µl
Standard	10µl	20µl	40µl	60µl
Solution Conc.	6 g/dl	12 g/dl	24 g/dl	36 g/dl
Mix well and incubate for 10 min at 37℃ or 15 min at RT				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl reagents in 4 cuvette
- Add standard in reagent according to table, for ex. 10µl standard in first cuvette, 20µl in 2nd cuvette, 40 µl in 3rd cuvette 60 µl in 4th cuvette
- Incubate all cuvette in incubator at 37℃ for 10 min
- Press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE: ***(COLORIMETRIC TEST - BIURET METHOD)***

- Take 500 µl reagent in cuvette
- Add 10 µl patient serum mix it well and incubate at 37℃ for 10 min
- After incubation press 1 and select Accurate all to test and select test (TP)
- Now put the cuvette into analyzer and enter the label number of patient

- Press enter key and take the reading of the sample

21) ALBUMIN (Ab)

Albumin	**Male**	**Female**	**Child**
Normal Range	3.5 To 5.2	3.5 To 5.2	3.2 to 4.5 g/dl

CALIBRATION (Erba Reagent)				
	1x	**2x**	**4x**	**6x**
Reagent	500µl	500µl	500µl	500µl
Standard	5µl	10µl	20µl	30µl
Solution Conc.	3.6 g/dl	7.2 g/dl	14.4 g/dl	21.6 g/dl
Mix well and incubate for 2 min at RT				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl reagents in 4 cuvette
- Add glucose standard in glucose reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20 µl in 3rd cuvette 30ul in 4th cuvette
- Incubate all cuvette in incubator at RT for 2 min
- Press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE: (*BCG METHOD)*

- Take 500 µl reagent in cuvette
- Add 5 µl patient serum mix it well and incubate at RT for 2 min
- After incubation press 1 and select Accurate all to test and select test (Ab)
- Now put the cuvette into analyzer and enter the label number of patient

- Press enter key and take the reading of the sample

22)TOTAL BILIRUBIN (TB)

Total Bilirubin	**Male**	**Female**	**Critical Range**
Normal Range	0.2 to 1.0	0.2 to 1.0	>15.0

CALIBRATION (Erba Reagent)					
To prepare working reagent : 500 ul total Bilirubin reagent (R1) + 10 ul Nitrite reagent (R3)					
	1x	**2x**	**3x**	**4x**	**TEST**
Distilled water	400 µl	300 µl	200 µl	100 µl	(WR)500 µl
standard	100 µl	200 µl	300 µl	400 µl	
serum					25 µl
Sol. Conc.	1.3 mg/dl	2.6 mg/dl	3.9 mg/dl	5.2 mg/dl	
Mix well and incubate for 5 min at 37℃ or 10 min at RT					

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 400 µl in 1st, 300 µl in 2nd, 200 µl in 3rd and 100 µl in 4th cuvette
- Add standard in reagent according to table, for ex. 100 µl standard in first cuvette, 200 µl in 2nd cuvette, 300 µl in 3rd cuvette 400 µl in 4th cuvette
- Incubate all cuvette in at 37℃ for 5 min
- Press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE ***(DIAZO METHOD)***

- Take 500 µl of R1reagent in cuvette
- Add 10 µl Nitrite Reagent , mix well (working reagent ready to further use)
- Now add 25 µl serum , mix well and incubate at 37℃ for 5 min
- After incubation press 1 and select Accurate all to test and select test (TB)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

22)TOTAL BILIRUBIN (TB)

Total Bilirubin	**Male**	**Female**	**Critical Range**
Normal Range	0.2 to 1.0	0.2 to 1.0	>15.0

CALIBRATION (Coral)					
To prepare working reagent : 500 ul total Bilirubin reagent (R1) + 25 ul Nitrite reagent (R3)					
	1x	**2x**	**3x**	**4x**	**TEST**
Distilled water	400 µl	300 µl	200 µl	100 µl	(WR)500 µl
standard	100 µl	200 µl	300 µl	400 µl	
serum					50 µl
Sol. Conc.	1.3 mg/dl	2.6 mg/dl	3.9 mg/dl	5.2 mg/dl	
Mix well and incubate for 5 min at 37℃ or 10 min at RT					

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 400 µl in 1st, 300 µl in 2nd, 200 µl in 3rd and 100 µl in 4th cuvette
- Add standard in reagent according to table, for ex. 100 µl standard in first cuvette, 200 µl in 2nd cuvette, 300 ul in 3rd cuvette 400 ul in 4th cuvette
- Incubate all cuvette in at 37℃ for 5 min
- Press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE (*Mod. Jendrassik & Grof method)*

- Take 500 µl of R1reagent in cuvette
- Add 25 µl Nitrite Reagent , mix well (working reagent ready to further use)
- Now add 50 ul serum , mix well and incubate at 37℃ for 5 min
- After incubation press 1 and select Accurate all to test and select test (TB)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

23)DIRECT BILIRUBIN (DB)

Direct Bilirubin	**Male**	**Female**
Normal Range	0.2	0.2

CALIBRATION (Erba reagent)					
To prepare working reagent : 500 ul Direct Bilirubin reagent (R1) + 5 ul Nitrite reagent (R3)					
	1x	**2x**	**3x**	**4x**	**TEST**
Distilled water	400 µl	300 µl	200 µl	100 µl	(WR)500 µl
standard	100 µl	200 µl	300 µl	400 µl	
serum					25 µl
Sol. Conc.	0.4 mg/dl	0.8 mg/dl	1.6 mg/dl	2.4 mg/dl	
Mix well and incubate for 5 min at 37℃ or 10 min at RT					

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 400 µl in 1st, 300 µl in 2nd, 200 µl in 3rd and 100 µl in 4th cuvette
- Add standard in reagent according to table, for ex. 100 µl standard in first cuvette, 200 µl in 2nd cuvette, 300 ul in 3rd cuvette 400 ul in 4th cuvette
- Incubate all cuvette in at 37℃ for 5 min
- Press 1 to select “Accurate all”
- Now press zero (0) for calibration
- Press (000) it’s a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press “Del” key

TEST PROCEDURE *(DIAZO METHOD*)

- Take 500 µl of R1reagent in cuvette
- Add 5 ul Nitrite Reagent , mix well (working reagent ready to further use)
- Now add 25 µl serum , mix well and incubate at 37℃ for 5 min
- After incubation press 1 and select Accurate all to test and select test (DB)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

23)DIRECT BILIRUBIN (DB)

Direct Bilirubin	**Male**	**Female**
Normal Range	0.2	0.2

CALIBRATION (Coral Reagent)					
To prepare working reagent : 500 ul Direct Bilirubin reagent (R1) + 25 ul Nitrite reagent (R3)					
	1x	**2x**	**3x**	**4x**	**TEST**
Distilled water	400 µl	300 µl	200 µl	100 µl	(WR)500 µl
standard	100 µl	200 µl	300 µl	400 µl	
serum					50 µl
Sol. Conc.	0.4 mg/dl	0.8 mg/dl	1.6 mg/dl	2.4 mg/dl	
Mix well and incubate for 5 min at 37℃ or 10 min at RT					

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 400 ul in 1st, 300 ul in 2nd, 200 ul in 3rd and 100 ul in 4th cuvette
- Add standard in reagent according to table, for ex. 100 µl standard in first cuvette, 200 µl in 2nd cuvette, 300 ul in 3rd cuvette 400 ul in 4th cuvette
- Incubate all cuvette in at 37℃ for 5 min
- Press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2/3x, 1/2x, 1/3x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE (*Mod. Jendrassik & Grof method)*

- Take 500 ul of R1reagent in cuvette
- Add 25 ul Nitrite Reagent , mix well (working reagent ready to further use)
- Now add 50 ul serum , mix well and incubate at 37℃ for 5 min
- After incubation press 1 and select Accurate all to test and select test (DB)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

24) VLDL (VD) Calculative Test

VLDL = Triglyceride (TG) / 5

VLDL	**Male**	**Female**
Normal Range	0-30	0-30

25) GLOBULIN (Gb) Calculative test

Globulin = Total protein - Albumin

26) A:G Ratio

27) HDL:LDL

29) INDIRECT BILIRUBIN (IB) Calculative Test

Indirect Bilirubin = Total Bilirubin - Direct Bilirubin

30) R.B.C. = Hb/3

31)PCV(PC) = Hb×3

32)MCV(MV) = PCV × 10 / RBC

33)MCH (MH) = Hb × 10 / RBC

34)MCHC(MC) = Hb × 100 / PCV

35)PHOSPHOROUS (PH) (UV Method)

Phosphorous	**Male**	**Female**	**Child**
Normal Range	2.5 to 5.0	2.5 to 5.0	4.0 to 6.5

CALIBRATION (coral reagent) UV Method				
	1x	**2x**	**4x**	**6x**
Reagent L1	400 µl	400 µl	400 µl	400 µl
Reagent L2	100 µl	100 µl	100 µl	100 µl
Standard	5 µl	10 µl	20 µl	30 µl
Sol. Conc.	5 mg/dl	10 mg/dl	20 mg/dl	30 mg/dl
Mix well and incubate for 5 min. at RT				
NOTE : This test should be perform on "ACCUKINE" Mode				

CALIBRATION PROCEDURE

- Take 400 µl of Reagent L1 in the cuvette and then add 100 µl of reagent L2 in the cuvette
- Add Standard standard in reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20ul in 3rd cuvette 30 in 4th cuvette, mix well and read immediately without incubation
- press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x on screen, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE:

- Take 250 µl of R1 reagent in the cuvette and then add 250 µl of R2 reagent in the cuvette and mix it well
- Place it in an incubator at RT for 5 min.
- Now press 1 to select Accurate All and then press 10 to select the test(PH)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

36) CRP (CR)

CRP	**Male**	**Female**
Normal Range	0 - 6 mg/dl	0 - 6 mg/dl

CALIBRATION & TESTING PROCEDURE (Erba Reagent)		
	Standard	**Test**
Reagent R1	400 µl	400 µl
Reagent R2	100 µl	100 µl
Mix well and incubate for 15 min at 37℃		
Standard	10 µl	
Sample		10 µl
Mix well and Read as soon as possible		

CALIBRATION PROCEDURE

- Take 400 µl of R1Reagent in the cuvette and then add 100 ul of R2 reagent in the cuvette
- Place it in incubator at 37℃ for 15 min
- Now start the analyser , press 1 to select “Accurate all”
- Press 36 to select CRP test
- Now press “NEW” to ask standard and then press “0” zero to ask for zero reference
- Now put the Distilled Water cuvette into the analyser and press “enter” to set Zero reference
- For calibration, take 10 µl standard in pipette and take one cuvette from incubator in which reagent are incubated , and put index finger on enter button
- Press “Enter” and mix the 10 µl Std at the same time, mix the reagent well and put it into the analyser

NOTE: We can perform calibration of this test with normal method , ("0" → "000" →2 →1, select test)

TEST PROCEDURE: (*T-LTX METOD)*

- Take 400 µl of R1 reagent in the cuvette and then add 100 µl of R2 reagent in the cuvette and mix it well
- Place it in an incubator at 37℃ for 15 min.
- Now press 1 to select Accurate All and then select the test (CR)
- Take 10 µl sample in the micropipette
- Take one cuvette from incubator in which reagent are incubated, and put the index finger on enter button
- Press 'Enter" and mix the 10 ul sample in the reagent at the same time , mix the sample well and put it into the analyser, after 120 sec result will be shown on screen

37) MICRO PROTEIN (uP)

Micro Protein	Male	Female
Normal Range	10 to 140	10 to 140

CALIBRATION AND TESTING PROCEDURE (Erba Reagent)				
STEP 1) Take urine sample in test tube and centrifuge it for 5 min, Take supernatant for further use				
	1X	**2X**	**4X**	**6X**
Reagent	500 µl	500 µl	500 µl	500 µl
standard	5 µl	10 µl	20 µl	30 µl
Urine supernatant				5 ul
Sol^n Conc.	100 mg/dl	200 mg/dl	400 mg/dl	600 mg/dl
Mix well and incubate at 37℃ for 10 min. or 15 min. at RT				

CALIBRATION PROCEDURE

- Take 4 Cuvette in cuvette box for calibration process
- Add 500µl reagents in 4 cuvette
- Add standard in reagent according to table, for ex. 5µl standard in first cuvette, 10µl in 2nd cuvette, 20 µl in 3rd cuvette 30 µl in 4th cuvette
- Incubate all cuvette in incubator at 37℃ for 10 min
- Press 1 to select "Accurate all"
- Now press zero (0) for calibration
- Press (000) it's a calibration code after that press 2 and then press 1 for calibration and then select test that we want to calibrate
- Now it shows zero reference, take reading of Distilled water for zero reference.
- Now it shows standard 1x. 2x, 4x, 6x, put the cuvette on an analyzer one by one and set the calibration.
- Press 5 to show the details
- For exit from the calibration mode press "Del" key

TEST PROCEDURE (*PYROGALLOL RED METHOD)*

- Take 500 µl reagent in cuvette
- Add 5 µl urine supernatant mix it well and incubate at 37℃ for 10 min
- After incubation press 1 and select Accurate all to test and select test (uP)
- Now put the cuvette into analyzer and enter the label number of patient
- Press enter key and take the reading of the sample

ACCUKINE

1)SGOT

SGOT	Male	Female
Normal Range	0 to 35	0 to 31

TESTING PROCEDURE *(IFFCC Method)*

- Press 2 for “accukine
- ”Then put distilled water into analyser and press enter, wait for 120 sec to set the Temperature (temp. should be 37℃)
- Press 1 to select SGOT test
- Now press “Enter” key and enter the label no in the analyzer
- Take test cuvette and add 500 µl Reagent in it
- Press ‘Enter” and mix the 50 µl sample in the reagent with lag time 60 sec. , mix well and put it into the analyser, after 240 sec result will be shown on screen

2)SGPT

SGPT	Male	Female
Normal Range	0 - 45	0 - 34

TESTING PROCEDURE *(IFCC Method)*

- Press 2 for “accukine”
- Then put distilled water into analyser and press enter, wait for 120 sec to set the Temperature (temp. should be 37℃)
- press 1 to select SGOT test
- Now press “Enter” key and enter the label no in the analyzer
- Take test cuvette and add 500 ul Reagent in it
- Press ‘Enter” and mix the 50 ul sample in the reagent at the same time , mix the sample well and put it into the analyser, after 240 sec result will be shown on screen

3)UREA

Normal range	male	Female	Critical value
Urea	19 - 45	13 - 40	>80 except dialysis
BUN	8 - 23	8-23	

CALIBRATION & TESTING PROCEDURE(Erba Reagent)		
	Standard	**Testing**
Reagent	500 µl	500 µl
Standard	10 µl	
Sample		10 µl
mix and Read (without incubation)		

CALIBRATION PROCEDURE

- Press 2 for “accukine”
- Then put distilled water into analyser and press enter, wait for 120 sec to set the Temperature (temp. should be 37℃)
- Take 500 ul Reagent in cuvette
- Add 10 ul standard , mix and put it into the analyser
- Now select test
- press “NEW”
- To select the calibration press ‘READ” key and mix 10 µl standard at the same time and put it into the analyser, after 80 sec reading will complete and calibration set.

TESTING PROCEDURE (*Initial Rate)*

- Press 2 for “accukine”
- Then put distilled water into analyser and press enter, wait for 120 sec to set the Temperature (temp. should be 37℃)
- Press 1 to select urea test
- Now press “Enter” key and enter the label no in the analyzer
- Take test cuvette and add 500 µl Reagent in it
- Press ‘Enter” and mix the 10 µl sample in the reagent at the same time , mix the sample well and put it into the analyser, after 80 sec result will be shown on screen

MICROSCOPIC EXAMINATION

INTRODUCTION

You are aware that the primary function of red blood cells (RBCs) is to transport oxygen to the body's cells, whereas white blood cells (WBCs) are part of our immune system that protect us from infectious agents and the primary function of Platelets is to prevent and stop bleeding , they clump together to form a clot that helps stop bleeding. The Neubauer chamber will be used in this exercise to count total RBC and WBC and Platelets . The number of cells per cubic millimeter of blood is the most commonly used unit of measurement for blood cell counts.

General Feature of the Neubauer Chamber

The Neubauer's chamber is a rectangular indentation in a thick glass slide with a grid of perpendicular lines, the rectangular chamber has a precision volume chamber. The Neubauer chamber has ruled area of total 9 square mm and the depth is 0.1 mm. When the coverslip is placed on the counting chamber, the space between the cover glass and the base of the grooved area measures 0.1 mm in depth.

RBC & Platelets Counting area: The red blood cells and Platelets are counted in the 5 squares of the Central square (divided into 25 squares, each of which is divided into 16 squares). These include four corner squares and one central square of the Large square.

WBC Counting area: The four large squares located at the corners of Neubauer's Chamber are used for white blood cell count.

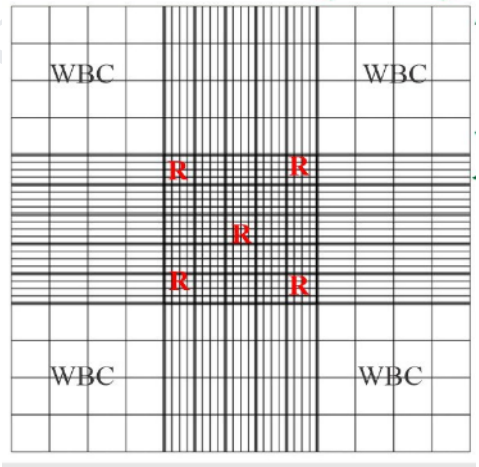

Fig. Neubauer's Chamber

TOTAL RBC COUNT

PRINCIPLE

The blood specimen contains a large number of red blood cells. The counting of such large numbers under the microscope is almost impossible. Therefore, the RBC count is performed with the help of a hemocytometer. The methodology involves the accurate dilution of blood specimens (200 times)with the RBC diluting fluid for which preferably Hayem's fluid is used as it is isotonic to blood. The total number of Red blood cells is then calculated by the number of blood cells counted in Neubauer's chamber and multiplying by dilution factor.

Normal values of human Red Blood cells

Men –4.8-5.5 million/mm^3, Women –4.5-5 million/mm^3

RBC Diluting Fluid (Hayem's fluid)

- Sodium Chloride : 0.5g (maintain osmolarity, so that RBC maintain their shape and size)
- Sodium Sulfate : 2.5g (Prevent aggregation of RBC)
- Mercuric Chloride : 0.25 (Act as a preservative, antifungal antibacterial)

PROCEDURE:
Dilution 1:200

- Take 1990 µl diluting fluid in eppendorf
- Add 10 µl of blood and mix well
- Incubate at RT for 5 min
- Take 10-30 diluting blood sample in pipette and charge the neubauer chamber carefully (don't overflow)
- Rest for 2 min.
- Count rbc under microscope

Calculation of total RBC count

The formula for RBCs count is = number of cells counted (N) × Dilution factor / Area x Depth
Where,
Number of Red blood cells (N)= ?
Dilution factor = 200
Area of 5 small squares =5/25 i.e. 1/5sq. mm in length
Depth of the chamber = 1/10 mm = 0.1 mm
Total RBCs/ mm3 = N X 200 / (1/5 X 0.1) = N X 200 X 50
= **N X 10,000 million/mm3**

PLATELETS COUNTS (Thrombocytes)

150,000 – 450,000 cells are present per cubic millimeter (mm^3) of blood

PROCEDURE 1:100

- Take 1980 µl diluting fluid in eppendorf
- Add 20 µl of blood and mix well
- Incubate at RT for 5 min
- Take 10-30 diluting blood sample in pipette and charge the neubauer chamber carefully (don't overflow)
- Rest for 2 min
- Count platelets under microscope

Calculation of total platelets count

The formula for Platelets count is = number of cells counted (N) × Dilution factor / Area x Depth

Where,

Number of Platelets (N)= ?

Dilution factor = 100

Area of 5 small squares =5/25 i.e. 1/5sq. mm in length

Depth of the chamber = 1/10 mm = 0.1 mm

Total platelets/mm^3 **= N X 1000 million/mm^3**

TOTAL LEUKOCYTE COUNT (TLC) (Leukocyte)

PRINCIPLE

Principle of WBC count is the same as of the RBC count in the human blood. The diluted blood suspension is placed in the Neubauer's chamber and the cells are counted under the microscope. Since the number of WBC in blood samples is much lower than the RBCs, the dilution factor is much lesser (1: 20) than that of RBC (1:200).

Normal reference range of WBC = 4500-11,000/ mm^3

WBC FLUID (Turk's Fluid)

- Glacial acetic acid : 2 ml(destruction of red blood cells)
- Gentian violet stain : 1 ml (stains the nuclei of leucocytes)
- NaCl : 0.9 g
- Distilled water : 97 ml (acts as a solvent)

PROCEDURE

- Take 380 µl diluting fluid in eppendorf
- Add 20 µl of blood and mix well
- Incubate at RT for 5 min
- Take 10-30 ul diluting blood sample in pipette and charge the neubauer chamber carefully (don't overflow)
- Rest for 2 min
- Count WBC under microscope

Calculation of total WBC count

Area of 4 WBC squares = 4 x 1 = $4mm^3$

Volume of 4 WBC square = 4 x 1/10 = 4/10 mm^3

Dilution factor = 1:20 (20)

Let us consider WBC cells in 4/10 mm^3 volume of diluted blood = n

Therefore, cells in 1 mm^3 volume of diluted blood = n x 10/4 cells in 1 mm^3 volume of undiluted blood

= nx10/4 x20 = n x 50 mm^3 **[N x 50]**

DIFFERENTIAL LEUKOCYTE COUNT (DLC)

It is white blood cell count for each type separately to determine the ratio of each type,

The importance of this test:

- Confirm automatic examination of allergic infections.
- Diagnosis of malignant blood disorder.

PROCEDURE

- Prepare the blood film by field stain
- Choosing the Field on slide (R1, R2)
- Microscopic examination (40x) and record the no. of all WBC types that observed.

FIELD STAIN

Field's stain is a Romanowsky-based staining technique used for staining of blood smear.

FIELD STAIN [A] (Give color to Nucleus)

- Methylene blue (analytical grade) = 1.6 grams.
- Azur = 1 gram.
- Disodium dihydrogen phosphate anhydrous = 10 grams.
- Potassium dihydrogen phosphate anhydrous = 12.5 grams.
- Distilled water = 1000 mL.

FIELD STAIN [B] (Give color to the Cytoplasm)

- Eosin(yellow water soluble) powder = 2.0 grams.
- Disodium dihydrogen phosphate anhydrous = 10 grams.
- Potassium dihydrogen phosphate anhydrous = 12.5 grams.
- Distilled water = 1000 mL.

METHYLENE BLUE (smear fixing)

PROCEDURE OF FIELD'S STAINING METHOD

- Make a perfect smear on glass slide
- Dry the smear
- Fixing the smear in "Methanol", dip into methanol 5 to 6 times or 5-6 sec and dry the slide
- Dip fixed smear to field stain B (Red stain) for 5 to 6 times
- wash with distilled water

- Now dip smear to Field stain A for 5 sec or 5 to 6 times and then wash with distilled water
- dry the slide
- observe the slide under microscope

Ziehl Neelsen Acid-fast staining (TB)

The ZN staining method is used to differentiate bacteria into acid fast groups and non-acid fast groups. This method is used to detect Mycobacterium

Carbol fuchsin

- Basic fuchsin : 10g
- Absolute alcohol : 100 ml
- Phenol : 50 g
- Distilled water : 900 ml

25% sulphuric acid (H2SO4)

- Conc. H2SO4 : 250 ml
- Distilled water : 750 ml

0. 1 % Methylene blue

- Methylene blue (BDH) : 0. 5 g
- Distilled water : 500 ml

Collection of sample and slide preparation :

Collection of Sputum collection, selection of the purulent portion for smear preparation and making smear is critical for good quality of smears.

Size: Take a purulent portion of sputum and prepare 2 - 3 cm length X 1 - 2 cm wide or 3 X 2 cm (100-150 fields to be counted in one length) smear in the center of the slide.

Evenness: Firmly make smear perpendicular to the slide (move in small concentric circles or coil like patterns).

Thickness: Place the slides on the piece of printed-paper. If letters cannot be read it is too thick. Allow the smear to air dry completely at room temperature. After air drying, fix the slide by passing it on the flame 3-4 times .

PROCEDURE

1. Prepare the smear: Make a smear of the sample culture on a clean, sterile microscopic slide.
2. Cover with carbolfuchsin: Submerge the smear in a drop of carbolfuchsin dye.
3. Heat: Use an alcohol lamp to heat the smear until steam rises,Heat each slide slowly until it is steaming. Do not boil. Maintain steaming for five minutes by using intermittent heat.
4. Wait: Let the smear stand for 5 minutes.

5. Wash: Gently wash the smear with flowing tap water.
6. Add acid: Add 25% sulfuric acid and leave it for 2–3 minutes.
7. Repeat: Repeat steps 5–6 until the smear appears pink.
8. Wash again: Wash the smear with water.
9. Add blue: Flood the smear with methylene blue dye and leave it for 30 seconds.
10. Wash again: Wash the smear with water.
11. Dry: Let the smear air dry.
12. Examine: Examine the stain under the oil immersion lens.

NOTE : Rinse the slide thoroughly with water. Drain excess water from the slide. Allow the smear to air dry. Do not heat or use blotting paper.

To avoid infection and contamination and keeping Hygiene in mind we designed the AccuTB slide.

1. Always wear personal protective gear like gloves and masks while handling sputum cups and slides
2. The AccuTB slide is designed with an in-built sink which eliminates the chances of splashing of reagents during testing and helps save lab technicians from being in contact with Tuberculosis.
3. The biomedical liquid waste is stored in the waste container and discarded in drainage with the application of Hypochlorite.
4. Disinfect the plastics in covered containers with 5% bleaching powder solution at least for one hour.
5. Dispose of the sputum cups and slide them into covered containers with 5% Sodium Hypochlorite solution for at least one hour.
6. 5% phenol use after disinfecting the working area and laboratory materials leave for

Advantages of the AccuTb slide

1. The AccuTB slide device is a portable Z-N staining testing machine for Tuberculosis. (It can be easily fit into the Labike)
2. The AccuTB slide is that the device can perform 8 slides at a time.
3. The time of testing reduces in the AccuTB slide. (45 minutes were used to perform the testing)
4. As the slide holding dimensions are fixed the chances of contamination minimize.
5. Low maintenance is required for the device.
6. Easy to operate.

ZN staining grading (RNTCP)	**Reporting**	**Grading**	**Field after examination**
>10 AFB per oil immersion fields	Positive	3+	20
1-10 AFB per oil immersion fields	Positive	2+	50
10-99 AFB per 100 oil immersion fields	Positive	1+	100
1-9 AFB/ per 100 oil immersion fields	Positive,scanty	Record exact number seen	200
No AFB per 100 oil immersion fields	Negative	0	100

Thank You !

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