Practical Session 1 – Total Bilirubin

Learning Outcomes

- **To understand how Bilirubin serum levels are determined:**
  - **To Produce a calibration curve**
  - **To run serum controls**
- **To determine the Bilirubin result on an unknown sample**

Reagents and consumables	
Bilirubin reagent	Working reagent (3,5-dichlorophenyldiazonium tetrafluoroborate)
Bilirubin blanking reagent	To remove endogenous interference
DI Water	Spectrophotometer blank
Calibration standard	58µmol/l
Serum quality controls	Known Low, medium and high bilirubin levels
Spectrophotometer cuvettes	Use for spectrophotometer reaction wells and determining absorbance's
Pipette tips	Blue
Graph paper	To draw calibration curve

Before starting this practical, please ensure spectrophotometer has been switched on allowed to warm up for 15 minutes.

Exercise 1: Produce calibration curve

Task

You are required to produce a 2-point calibration curve using a known calibration standard. You will be required to draw this curve on the graph paper provided, to an appropriate scale, and determine you own suitable X and Y axis (Concentration v Absorbance).

Method

1. Into a spectrophotometer cuvette pipette 380 µl of DI water.
2. Into a second spectrophotometer cuvette pipette 380 µl of calibrator standard.
3. Add 2mls of reagent to both cuvettes above and mix.

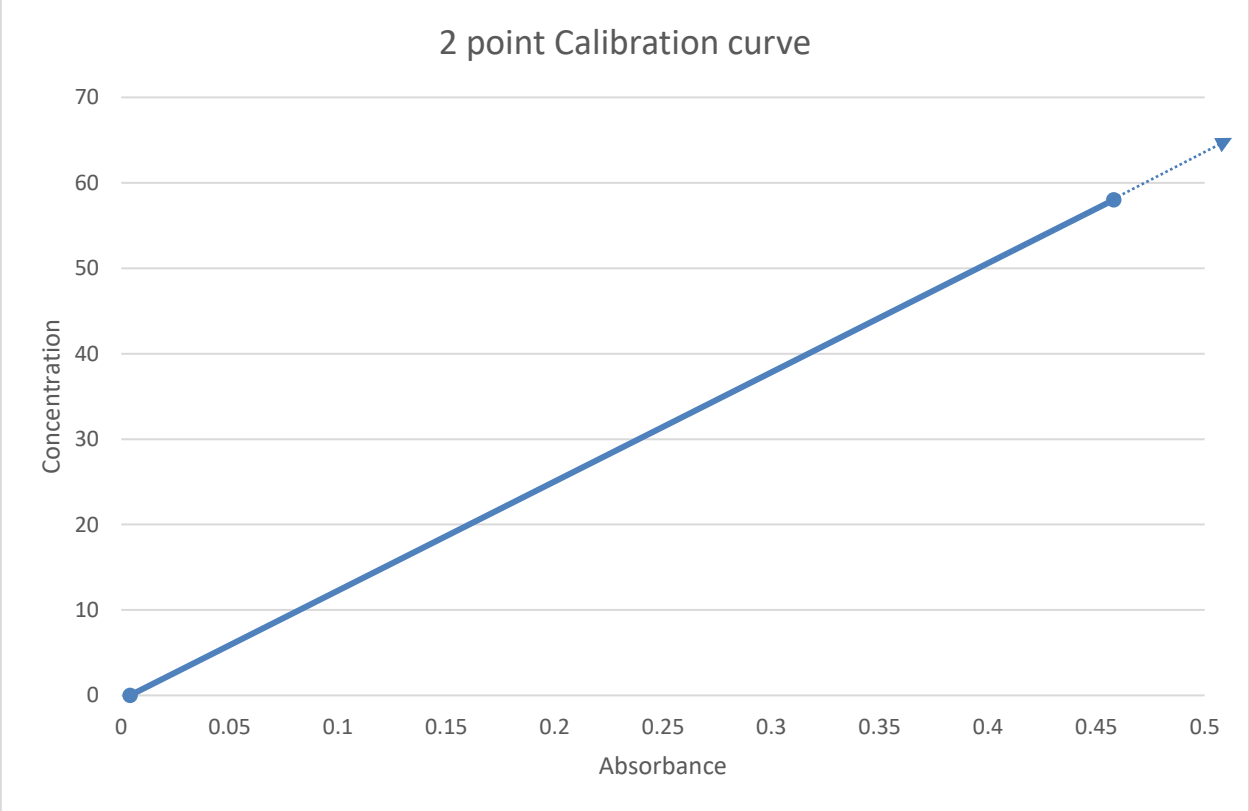
4. Incubate at room temperature for 10 minutes.
5. Measure the absorbance of each at 540nm (use DI as blank).
6. Plot both absorbance values on scaled calibration curve to produce a 2-point calibration.

Results

Record your absorbance results below

2-point calibration curve absorbance results			
Calibration point	Material	Concentration	Absorbance
1st	DI water	0 µmol/l	0.004
2nd	Calibrator	58µmol/l	0.458

Example curve:



Remember you will need to extrapolate the graph! (absorbance up to 1 and concentration up to 160).

Exercise 2: Quality control your standard curve

Task

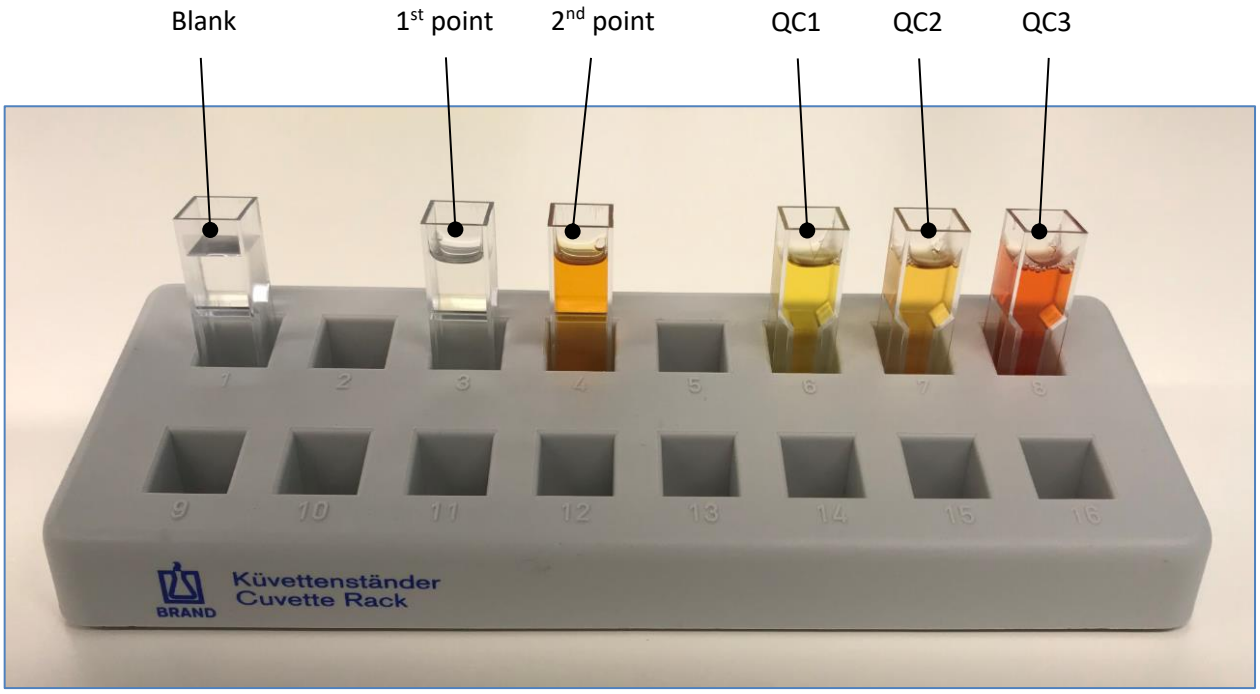
You are required to test they suitability of your calibration curve using the 3 known QC samples provided:

- Level 1 Low bilirubin sample
- Level 2 Medium bilirubin sample
- Level 3 High bilirubin sample

Method

Use the same method as for exercise 1 above (substituting DI water and calibrator for the 3 QC samples):

1. 380 µl of QC sample.
2. Add 2mls of reagent and mix.
3. Incubate for 10 minutes.
4. Measure absorbance at 540nm.
5. Record absorbance results for each QC in table below



Appearance of cuvettes after reagent incubation

Results

Record your QC absorbance results in the table below and then use this to determine the concentration of bilirubin in the 3 QC samples using your calibration curve:

QC Sample	Absorbance	Concentration
Level 1	0.112	
Level 2	0.233	
Level 3	0.954	

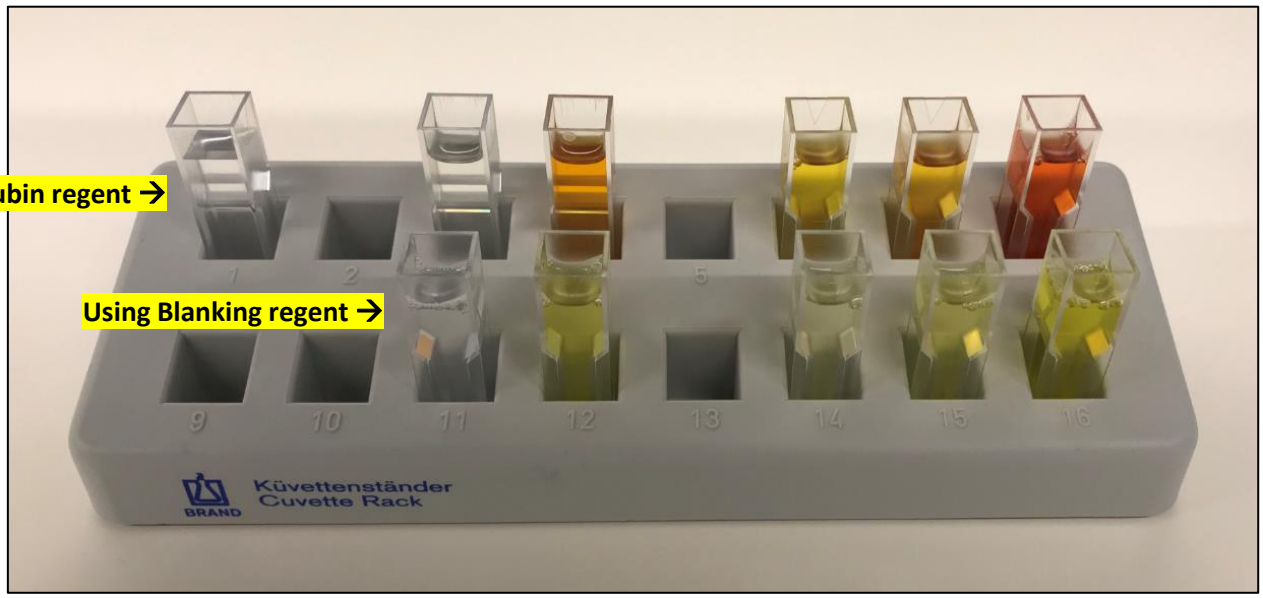
Compare you measured QC concentrations with the QC limits chart below

QC absorbance result			
QC Sample	Running Mean	SD	Results acceptable (Yes / No)
Level 1	13.8	0.4	
Level 2	37.6	0.7	
Level 3	140.3	2.4	

Exercise 3: Adjusting your standard curve for endogenous interference

Task

You are to repeat both previous exercises above (Standard curve and QC) but this time using **blanking reagent** (to factor in endogenous interference) instead of bilirubin reagent (same method volumes, incubation and spectrophotometer absorbance).



Results – Calibration curve

Record your results in the table below:

2-point calibration curve absorbance results			
Calibration point	Material	Concentration	Absorbance (2)
1st	DI water	0 µmol/l	0.001
2nd	Calibrator	58µmol/l	0.092

Subtract the absorbance's above (2) from your first set of absorbance results in the first calibration curve exercise (1) to produce a new calibration curve (use same graph scale as previous):

Readjusted absorbance results			
Calibration point	Absorbance (1)	Absorbance (2)	Final absorbance (new calibration curve)
1st			
2nd			

Again, subtract the absorbance's when using the blanking reagent (2) from the exercise using the bilirubin reagent (1) to determine the bilirubin concentration of the 3 QC samples using your new calibration curve.

QC Sample	Absorbance (1)	Absorbance (2)	Final QC absorbance	Final Concentration
Level 1		0.014		
Level 2		0.035		
Level 3		0.04		

Compare you measured QC concentrations with the QC limits chart – are the QC results acceptable? If not use 2 SD limits.

Exercise 4: determine the Bilirubin result on an unknown sample

QC Sample	Absorbance (1)	Absorbance (2)	Final QC absorbance	Final Concentration
Unknown patient sample	0.830	0.039		

Use second adjusted calibration curve