**Piccolo Xpress Chemistry Analyser Operation**

### The Piccolo Xpress chemistry analyser provides quantatative determinations of Alanine aminotransferase (ALT), Albumin, Alkaline phosphatase (ALP), Amylase, Aspartate aminotransferase (AST), C-reactive protein (CRP),Calcium, Creatinine,Gamma glutamyltransferase (GGT), glucose, total protein,urea and urric acid in lithium heparin whole blood or serum

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# Hazards and Precautions.

## Procedure Risk Assessment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Hazard** | **Risk** | **Assessment of Risk** | **Overall Procedural Risk** |
| **Chemical** | *Minor injury* | *Possible* | *Medium* | *Medium*  |
| **Biological**  | *Death* | *Rare* | *Low* |
| **Physical** | *Minor Injury* | *Unlikely* | *Low* |
| **Room** | *Death* | *Rare* | *Low* |
| **Mechanical** | *N/A* | *N/A* | *N/A* |

##  Chemical

*Please note – only chemicals which present a risk when used in this procedure need to be included in this table.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Chemical Risk** | **Precautions** | **Storage and Discard Requirements** | **1st Aid Measures** | **MSDS reference**(Q-pulse) |
| *Skin irritanthttp://www.hse.gov.uk/chemical-classification/images/pictogram-gallery/irritant.giflyophylised chemical beads enclosed in a plastic rotor*Chemicals used in rotor:D-MannitolPolyethylene glycol, 8000 Dextran, 70 USP Tris(hydroxymethyl)amino Polyethylene glycol, 3400 Polyethylene glycol, 2000 Sodium Chloride POPSO, Disodium salt Sodium Thiocyanate L-Aspartic Acid Lithium Hydroxide, Tris, HCL | Wear gloves when handling rotor | Store in fridge and discard into a sharpsafe™ | Fush exposed skin with copious amounts of water for at least 15 minutes  | POCT-SDS4 |
|  |  |  |  |  |

## Biological Hazard

|  |  |  |
| --- | --- | --- |
| **Biological Risk** | **Precautions** | **Precautions** |
| There is a risk of contact with blood borne viruses and other infective agents when handling patient samples and quality control | Wear Gloves when handling samples and quality control |  |

## Physical Hazard

*Please note – this includes any manual handling and VDU risk assessments required and need to be included in this table.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Physical Risk** | **Precautions** | **1st Aid Measures** | ***Any pertinent other*** ***Reference material***   |
| *Electrical* | Place the analyzer on a level surface that is free of hair, dust, and other contaminants. Do notplace the analyzer near a sunny window or any other heat source. |  |  |

## Room Risk Assessment (if required)

*Please note – this includes any room which had special requirements for example dark rooms, CL3 laboratories.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Room Risk** | **Precautions** | **1st Aid Measures** | ***Any pertinent other*** ***Reference material***   |
|  |  |  |  |
|  |  |  |  |

# CONTENT

# Purpose of the examination

The Piccolo Xpress chemistry system consists of a portable analyzer and disposable single-use reagent discs. Each reagent disc contains all the reagents needed to perform a panel of tests on a single sample. The Scottish Organ Retrieval Team transports the analyser to the donor hospital and the results are used to detemine the suitability of organs for transplant by analysing blood samples every 30 minutes during Normothermic Regional Perfusion.

# Principle and method of the procedure used for examinations

The operator introduces a heparinized whole blood sample (or heparinized plasma, serum, or control) into the reagent disc. The reagent disc contains a diluent and test-specific reagent beads.

The operator then places the disc in the Piccolo Xpress chemistry analyser and enters the appropriate identification numbers.

The analyzer automatically performs the remainder of the testing protocol.

The reagent disc spins and whole blood is separated into plasma and blood cells. During this time, the disc is heated to 37 ºC (98.6 ºF). Precisely measured quantities of plasma and diluent enter the mixing chamber and are mixed together. Through centrifugal and capillary forces, the diluted plasma is distributed to cuvettes on the perimeter of the disc. Reagent beads in the cuvettes are dissolved by the diluted plasma. This solution is thoroughly mixed and the resulting chemical reactions are monitored photometrically by the analyser. Optical signals generated by the chemical reactions are used to calculate analyte concentrations. Calibration data specific for the chemistries in each disc are provided to the analyzer by the bar code printed on the barcode ring

1. Alanine Aminotransferase (ALT)

ALT catalyses the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyses the conversion of pyruvate to lactate. Concomitantly NADH is oxidised to NAD+

L-Alanine + α-ketoglutarate → L-Glutamate + Pyruvate

Pyruvate + NADH + H+ → Lactate + NAD+

The rate of change of the absorbance difference between 340nm and 405nm is due to the conversion of NADH to NAD+ and is directly proportional to the amount of ALT present in the sample

1. Albumin

Bromocresol purple (BCP) when bound with albumin, changes colour from a yellow to blue colour. The absorbance maximum changes with the colour shift.

BCP + Albumin → BCP-Albumin Complex

Bound albumin is proportional to the concentration of albumin in the sample. This is an endpoint reaction that is measured as absorbance at 600nm.

1. Alkaline Phosphatase (ALP)

Alkaline phosphatase hydrolyses *p-*Nitrophenyl phosphate in a metal – ion buffer and forms *p*-nitrophenol and phosphate

*p-*Nitrophenyl Phosphate → *p-*Nitrophenol + phosphate

The amount of ALP in the sample is proportional to the rate of increase in absorbance between 405nm and 500nm

1. Amylase (AMY)

The substrate, 2-chloro-*p*-nitrophenly-α-D-maltotrioside (CNPG3), reacts with α-amylase in the patient sample, releasing 2-chloro-*p-*nitrophenol (CNP). The release of CNP creates a change in colour.

CNPG3 → CNP + D- Maltotrioside

The reaction is measured bichromatically at 405nm and 500nm. The change in absorbance due to the formation of CNP is directly proportional to α-amylase activity in the sample

1. Aspartate Aminotransferase (AST)

AST catalyses the reaction of L-aspartate and α-ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidised to NAD+ by the catalyst MDH

L-aspartate + α-ketoglutarate → Oxaloacetate + L-glutamate

Oxaloacetate + NADH + H+ → Malate + NAD+

The rate of absorbance change at 340nm / 405mn caused by the conversion of NADH to NAD+ is directly proportional to the amount of AST present in the sample

1. Calcium (Ca)

Calcium in the patient sample binds with arsenaso 111 to form a calcium dye complex

Ca + arsenazo 111 → Ca arsenazo 111 complex

The endpoint reaction is monitored at 405nm, 467nm and 600nm. The amount of calcium in the sample is proportional to the absorbence

1. Creatinine (CRE)

Creatinine amidohydrolase hydrolyses creatinine to creatine. A second enzyme, creatine amidinohydrolsae, catalyses the formation of sarcosine from creatine. Sarcosine oxidase causes the oxidation of sarcosine to glycine, formaldehyde and hydrogen peroxide (H2O2). Peroxidase catalyses the reaction among hydrogen peroxide, 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) and 4-aminoantipyrine (4-AAP) into a red quinoneimine dye. Sodium ferrocyanide and ascorbate oxidase are added to the reaction mixture to minimise the potential interference of bilirubin and ascorbic acid, respectively.

Creatinine + H2O → Creatine

Creatine + H2O → Sarcosine + Urea

Sarcosine + H2O + O2 → Glycine + Formaldehyde + H2O2

H2O2 + TBHBA + 4-AAP → Red Quinoneimine dye + H2O

Two cuvettes are used to determine the concentration of creatinine in the sample. Endogenous creatine is measured in the blank cuvette which is subtracted from the combined endogenous creatine and the creatine formed from the enzyme reactions in the test cuvette. Once the endogenous creatine is eliminated from the calculations, the concentration of creatinine is proportional to the intensity of the red colour produces. The endpoint reaction is measured as the difference in absorbance between 550nm and 600 nm.

1. Gamma Glutamyltransferase (GGT)

The addition of sample containing GGT to the substrates L-ˠ-glutamyl-3-carboxy-4-nitroaniline and glycylglycine (gly-gly) causes the formation of L-ˠ-glutamyl-glycylglicine (glu-gly-gly) and 3-carboxy-4-nitroaniline

L-ˠ-glutamyl-3-carboxy-4-nitroanilide + gly-gly → glu-gly-gly + 3-carboxy-4-nitroaniline

The absorbance of this rate reaction is measured at 405nm. The production of 3-carboxy-4-nitroaniline is directly proportional to the GGT activity in the sample.

1. Glucose (GLU)

The reaction of glucose with adenosine triphosphate (ATP), catalysed br hexokinase (HK) produces glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyses the reaction of 6-G-P into 6-phosphogluconate and the reduction of nicotinamide adenine dinucleotide (NAD+) to NADH.

Glucose + ATP → G-6-P + ADP

G-6-P + NAD+ → 6-phosphogluconate + NADH + H+

The absorbance is measured bichromatically at 340nm and 850nm. The production of NADH is directly proportional to the amount of glucose present in the sample.

1. Total Bilirubin (TBIL)

Bilirubin is oxidised by bilirubin oxidase into biliverdin

Bilirubin + O2 → Biliverdin + H2O

Bilirubin is quantitated as the difference in absorbance between 467nm and 550nm. The initial absorbance of this endpoint reaction is determined from the bilirubin blank cuvette and the final absorbance is obtained from the bilirubin test cuvette. The amount of bilirubin in the sample is proportional to the difference between the initial and final absorbance measurement.

1. Total protein (TP)

The protein solution is treated with cupric (Cu11) ions in a strong alkaline medium. Sodium potassium tartrate and potassium iodide are added to prevent the precipitation of copper hydroxide and the auto- reduction of copper respectively. The Cu11 ions react with peptide bonds between the carbonyl oxygen and amide nitrogen atoms to form a coloured Cu-protein complex

Total protein + Cu11 → Cu-protein complex

The amount of total protein in the sample is directly proportional to the absorbance of the Cu-protein complex. The total protein test is an endpoint reaction and the absorbance is measured as the difference in absorbance between 550nm and 850nm.

1. Blood Urea Nitrogen (BUN)

In the coupled-enzyme reaction, urease hydrolyses urea into ammonia and carbon dioxide. Upon combining ammonia with α-ketoglutarate and reduced nicotinamide adenine dinucleotide (NADH), the enzyme glutamate dehydrogenase (GLDH) oxidises NADH to NAD+

Urea + H20 → 2NH3 + CO2

NH3 + α-ketoglutarate + NADH → L-Glutamate + H20 + NAD+

The rate of change of the absorbance difference between 340nm and 405nm is caused by the conversion of NADH to NAD+ and is directly proportional to the amount of urea present in the sample.

1. Uric Acid (UA)

Uricase catalyses the oxidation of uric acid to allantoin and hydrogen peroxide. Peroxidase catalyses the reaction among the hydrogen peroxide (H2O2), 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzenesulphonic acid (DHBSA) into a red quinoneimine dye. Sodium ferrocyanide and ascorbate oxidase are added to the reaction to minimise the potential interference of bilirubin and ascorbic acid.

Uric acid + O2 + H2O → Allantoin + CO2 + H2O2

H2O2 + 4-AAP + DHBSA → Quinoneimine dye + H2O

# c) Performance characteristics (see 5.5.1.2 and 5.5.1.3)

# Type of sample (e.g. plasma, serum, urine)

The Piccolo Xpress chemistry analyzer accepts **lithium-heparinised** whole blood, plasma, or serum samples

# e) Patient preparation

# f) Type of container and additives

Withdraw blood from sampling line into a plain plastic sterile syringe. Decant into a sterile container and follow the steps for running a sample below.

*.*

# g) Required equipment and reagents

**Operational Materials Catalog Number**

Pipette tips, disposable, pack of 96 Abaxis #500-9007

Mini pipette, 100 ml, gray Abaxis #500-9006

Internal printer paper rolls, box of 6 Abaxis #1100-4410

Fan filter, covered Abaxis #1987-0009

 Piccolo® Reagent Disc – General Chemistry 13

 400-1029 (single); 400-0029 (10 pack); 400-0029-4 (4 pack)

# h) Environmental and safety controls

Refer to the various MSDS and kit inserts for instructions on the storage, handling and disposal of the material.

POCT-EXT6

POCT-SDS4

POCT-SDS5

# i) Calibration procedures (metrological traceability)

Refer to Piccolo traceability: ref POCT-EXT6

# j) Procedural steps

**Running a patient sample**

Turn on the analyser by pressing the Power button on the front of the analyser

The analyser turns on then performs a self test

If the analyser needs time to warm the disc chamber to operating temperature the display shows ‘warming’



When the analyser reaches operating temperature it displays ‘analyse’



The reagent discs can be used straight from the fridge. The discs can remain in the sealed pouch at room temperature for a cummulative period of up to 48 hours

Check for tears and punctures in unopened foil pouch, use disk within 20 minutes of opening

 Fill the sample chamber.

1. Using the Piccolo 100 μl volume pipette, firmly attach a new tip to the end of the pipette.

2. With your index finger or thumb, push the pipette button to the stop position and hold it down for sample pickup.

3. Immerse the tip 2–3 mm below the surface of the sample as shown below



1. Slowly release the button to pick up the sample, pause then remove the pipette from the sample tube.
2. Make sure there are no air bubbles or air gaps in the pipette tip
3. Place the pipette tip into the discs sample chamber at 45° so that the entire sample flows into the sample chamber, the tip should touch the sample chamber



1. Push the plunger down with a slow, continuous motion. Take care not to overfill the sample chamber
2. Keep the the pipette plunger pressed down until the pipette tip is removed from the sample chamber
3. Discard the pipette tip into a sharpsafe™
4. Carry the prepared disc to the analyser. Hold the disc by it’s edges and keep level to avoid spills
5. Place the disc in the recessed area in the drawer
6. Select **close**. The analyser closes the drawer
7. Select the sample type from patient or control
8. Enter an ID number for the sample
9. Then select done
10. The analyser checks the disc type and begins processing the sample
11. When the sample processing is complete the results will be printed automatically
12. Select **open** to open the disc drawer
13. Remove the disc from the drawer and discard into a sharpsafe™
14. When finished select **close** to close the drawer and return the analyser to standby mode

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# k) Quality control procedures

The control material used is Randox Abaxis chemistry controls level 1 and 2, which are supplied lyophilised.

To reconstitute:

1. Carefully pipette 1ml diluent into the serum vial 

Push the pipette button to the first stop and hold down. Release slowly to pick up diluent. Push the pipette slowly to the second stop to dispense diluent into the serum vial.

1. Close the serum vial and invert gently several times 
2. Allow to stand for 30 minutes before use. Ensure contents are completely dissolved by swirling gently. Avoid formation of foam. Do not shake. 

 **STORAGE AND STABILITY**

OPENED: Store refrigerated (+2ºC to +8ºC). Reconstituted serum is stable for 8 hours at +15ºC to +25ºC or 7 days at +2ºC to +8ºC and 1 month when frozen once at -20ºC . Only the required amount of product should be removed. After use, any residual product should NOT BE RETURNED to the original vial.

UNOPENED: Store refrigerated (+2ºC to +8ºC). Stable to expiration date printed on individual vials.

Quality controls (level 1 and 2) are run before the instrument is used at the donor hospital

**To run a QC**

 1. In the home screen, select ‘analyse’ to open the disc drawer



2. Select the control type to use by using the up and down arrow keys



Using the reconstituted QC material analyse following steps 1 – 20 in running a patient sample

Once complete check the printout to make sure that the results are within the target range

# l) Interferences (e.g. lipaemia, haemolysis, bilirubinemia, drugs) and cross reactions

Any results that are affected by >10% interference from haemolysis, lipaemia or icterus are suppressed and HEM, LIP or ICT respectively are printed on the printout in place of the result.

For a complete list of interfering substances, please refer to the General Chemistry 13 package insert POCT-EXT6

# m) Principle of procedure for calculating results including, where relevant, the measurement uncertainty of measured quantity values

Not applicable

# n) Biological reference intervals or clinical decision values

Albumin 35 – 55 g/L

Alkaline Phosphatase 42 – 141 U/L

Alanine Aminotransferase 10 – 47 U/L

Aspartate Aminotransferase 11- 38 U/L

Amylase 14 – 97 U/L

Urea 2.5 – 7.9 mmol/L

Calcium 2.00 – 2.58 mmol/L

Creatinine 53 – 106 µmol/L

Gamma Glutamyltransferase 5 – 65 U/L

Glucose 4.1 – 6.6

Bilirubin 3 – 27 µmol/L

Total Protein 64 – 81 g/L

Uric Acid 2.2 – 8.0 mg/DL

# o) Reportable interval of examination results

Albumin 10 – 65 g/L

Alkaline Phosphatase 5 – 2400 U/L

Alanine Aminotransferase 5 – 2000 U/L

Aspartate Aminotransferase 5 – 2000 U/L

Amylase 5 – 4000 U/L

Urea 0.7 – 64.3 mmol/L

Calcium 1 – 4 mmol/L

Creatinine 18 – 1768 µmol/L

Gamma Glutamyltransferase 5 – 3000 U/L

Glucose 0.56 – 38.9 mmol/L

Bilirubin 1.7 – 513 µmol/L

Total Protein 20 – 140 g/L

Uric Acid 1-15 mg/dL

# p) Instructions for determining quantitative results when a result is not within the measurement interval

When a result is outwith the measurement interval, a sample must be sent to the laboratory for confirmation

# q) Alert/Critical values, where appropriate

# r) Laboratory clinical interpretation

Not applicable

# s) Potential sources of variation

Incorrect sample collection or collection into a tube with EDTA, fluoride oxalate or citrate as an anticoagulant will interfere with test results

# t) references

Piccolo Xpress User Operator’s Manual POCT-EXT7

General Chemistry 13 Package insert POCT-EXT6

Piccolo Chemistry 13 SDS POCT-SDS4

Piccolo QC SDS POCT-SDS5