

# Clinical Chemistry HRWLLA\_CBC\_003

## Purpose

Clinical chemistry determines biochemical parameters in plasma including enzymatic activity, specific substrates and electrolytes.

Ontological description: MP:0001545 – blood physiology abnormalities.

## Experimental Design

- **Minimum number of animals** : 7M + 7F
- **Age at test**: Week 59
- **Sex**: We would expect the results of this test to show sexual dimorphism

## Equipment

1. Clinical chemistry analyser
2. Vortex
3. Refrigerated centrifuge
4. Eppendorf tubes
5. Pipettes (200-1000  $\mu$ l)

## Procedure

Set up the clinical chemistry analyser and perform QC analyses of the control reagents in accordance with the equipment guidelines.

### Sample collection and preparation:

- a. Collect the appropriate volume of blood required (160-200  $\mu$ l of plasma), for the clinical chemistry analyser being used for assessment, in gel tube containing lithium Heparin with the relevant blood collection procedure (see IMPC protocol Blood collection by retro-orbital puncture). Time of day for collection is in the morning, starting no earlier than 07:30.
- b. Keep whole blood samples in a bag on wet ice until centrifugation. Centrifuge for 10 minutes at 5000 x g in a refrigerated centrifuge set at 8°C. If plasma samples cannot be analysed immediately, keep them in the fridge until analysis.
- c. Analysis of samples is optimally done on the day of collection. When not possible the plasma samples can be stored at 2-8°C. If samples require storage for > 48 hours,

freeze plasma at -20 °C in single aliquots. All samples are allowed to come to room temperature prior to analysis.

- d. Use plasma samples undiluted or diluted to a ratio of 1:2 with deionised water if the volume is insufficient.
- e. Plasma samples that were frozen or stored in the fridge should be vortexed briefly and centrifuged again at ~5000 x g for 2-3 minutes immediately prior to analysis. If necessary, remove fibrin clots using a wooden applicator.

### Analysis:

Samples that produce results that lie outside the linear range for a specific assay have to be re-tested. In some cases it may be necessary to dilute samples with water to bring test results into range.

## Notes

Blood collection for Clinical Chemistry and Hematology is usually performed as a non-fasting, terminal procedure but can be performed as a non-terminal procedure under certain circumstances. Mice from the terminal procedure may be used for subsequent gross pathology and other procedures included in terminal assessments. Whole blood (for Hematology) and plasma (for Clinical Chemistry) require different collection tubes so two independent samples are required from each mouse.

The information about the date of the experiment, that is the date when the measurement is performed, is an important parameter which is to be submitted in the Experiment xml file (dateOfExperiment="2013-02-28").

**Dilution.** Dilution of blood is highly discouraged, but is allowed when the total necessary amount is not obtained. If dilution is necessary then the assays should be done in one run.

**Hemolysis.** Two fields currently exist to capture metadata information about the hemolysis status in the clinical chemistry plasma samples. The first is the LIH Hemolysis severity score which can only be performed by clinics who run one of the Beckman Coulter AU-series of analysers. Such clinics are encouraged to capture and submit the hemolysis score of the LIH test in this field. Clinics who do not have an AU analyser are encouraged to use the second /alternative field which is simply titled Hemolysis. Simply enter "slight", "moderate", or "marked" based on whether the sample is visibly haemolysed or not. Provision of this information is not compulsory and it is suggested that any clinic completes at least one field or the other (not both).

## Data QC

1. Plasma samples must be free of Fibrin clots in order to be analysed.
2. Badly haemolysed samples should be discarded.
3. Each morning, all parameters are tested with control sera (see ESLIM\_015\_001\_Annex\_3: Controls for biochemistry on AU400). Some parameters are tested with control serum level 1 (Beckman Coulter System Reagent, ODC0003)

and control serum level 2 (Beckman Coulter System Reagent, ODC0004), which consists of lyophilised human plasma with a normal and a pathological concentration. Other parameters are tested with specific controls from other suppliers.

4. Controls are thawed and vortexed before utilisation and loaded according to the analyser's display. Control values must lie within the acceptable range indicated by the manufacturer, otherwise the specific tests must be recalibrated and specific measurements repeated. Controls can be stored in 200l aliquots at -20°C for up to 1 week.

## Metadata and examples

| Metadata                                            | Example                                                                                                                                                   |
|-----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Equipment ID                                        | ID of the machine used when more than 1 is used having same model and manufacturer. E.g. machine 1, machine 2, machine Minnie, machine Mickey Mouse, etc. |
| Equipment manufacturer                              | Manufacturer of the equipment. E.g. Olympus Diagnostics.                                                                                                  |
| Equipment model                                     | Model of the equipment. E.g. AU400                                                                                                                        |
| Blood collection tubes                              | The tubes used for blood collection. E.g. Sarstedt Li-Heparin gel tubes or Kabe Labortechnik Lithium heparin coated tubes.                                |
| Anaesthesia used for blood collection               | The drug used for anaesthesia during blood collection. E.g. Isoflurane.                                                                                   |
| Method of blood collection                          | Concise description of the method used for blood collection. E.g. retro-orbital puncture.                                                                 |
| Anticoagulant                                       | Anticoagulant drug used for blood collection. E.g. Li-Heparin.                                                                                            |
| Samples kept on ice between collection and analysis | Yes/No.                                                                                                                                                   |
|                                                     |                                                                                                                                                           |

|                                                            |                                                                                                                                                                                          |
|------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Storage temperature from blood collection till measurement | E.g. 2°C                                                                                                                                                                                 |
| Sample status                                              | Indicate if the sample were frozen (analysis on the same day of collection not possible) or fresh (analysis on the same day of collection). E.g Fresh/Frozen.                            |
| Plasma dilution                                            | Dilution is highly discouraged but if necessary indicate here. E.g. “No dilution” or 1:2. Note that results submitted to DCC are assumed to be already corrected for any dilutions made. |
| ID of blood collection SOP                                 | ID of the protocol followed for blood collection. Can be a center specific protocol. E.g. ESLIM_024_001.                                                                                 |
| Date and time of blood collection                          | Time of day for collection is in the morning, starting no earlier than 07:30. E.g. Year, month, day, time.                                                                               |
| Date of measurement                                        | The day of blood analysis. Year, month, day.                                                                                                                                             |
| Hemolysis status                                           | If no AU analyser score is provided, indicate here the gauged degree of hemolysis. E.g. slight/moderate/marked.                                                                          |
| Blood collection experimenter ID                           | An ID of any format to be used coherently both inside the same procedure and for all procedures indicating the experimenter who collected the blood. E.g. Harw_001, or 1/2/3.            |
| Blood analysis experimenter ID                             | An ID of any format to be used coherently both inside the same procedure and for all procedures indicating the experimenter who analyzed the blood. E.g. Harw_001, or 1/2/3.             |
| Date equipment last calibrated                             | Most recent date in which the equipment (or any part of) used in the procedure was subject to a calibration event.                                                                       |
| Date and time of sacrifice                                 | The date and time when the mouse is sacrificed.                                                                                                                                          |

# Parameters and Metadata

## Phosphorus HRWLLA\_CBC\_010\_001 | v1.6

simpleParameter

Req. Analysis: false      Req. Upload: true      Is Annotated: true

Unit Measured: mg/dl

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## Anticoagulant HRWLLA\_CBC\_038\_001 | v1.1

procedureMetadata

Req. Analysis: false      Req. Upload: true      Is Annotated: false

Options: Heparine, Sodium Heparin, No, Lithium Heparin,

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## Total cholesterol HRWLLA\_CBC\_015\_001 | v1.4

simpleParameter

Req. Analysis: false      Req. Upload: true      Is Annotated: true

Unit Measured: mg/dl

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**Creatinine** HRWLLA\_CBC\_005\_001 | v1.5

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: mg/dl

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**Blood analysis experimenter ID** HRWLLA\_CBC\_051\_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

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**Blood collection experimenter ID** HRWLLA\_CBC\_049\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

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**Chloride** HRWLLA\_CBC\_003\_001 | v1.4

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mmol/l

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## Alpha-amylase HRWLLA\_CBC\_023\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: U/l

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## Hemolysis status HRWLLA\_CBC\_048\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Options: None, Slight, Moderate, Marked,

---

## Difficult bleed HRWLLA\_CBC\_055\_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Options: Yes, No,

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## Creatine kinase HRWLLA\_CBC\_028\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: U/l

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## Glycosilated hemoglobin A1c (HbA1c) HRWLLA\_CBC\_052\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## Calcium HRWLLA\_CBC\_009\_001 | v1.5

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: mg/dl

---

## Date and time of blood collection HRWLLA\_CBC\_046\_001 | v1.2

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

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## Magnesium

HRWLLA\_CBC\_054\_001 | v1.5

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mg/dl

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## Glucose

HRWLLA\_CBC\_018\_001 | v1.5

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: mg/dl

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## Glycerol

HRWLLA\_CBC\_027\_001 | v1.4

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mmol/l

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## Alkaline phosphatase HRWLLA\_CBC\_014\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: U/l

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## Reagent manufacturer HRWLLA\_CBC\_059\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: false

Is Annotated: false

Options: Microgenics, Wako and Sekisui, Beckman Coulter,

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## Thyroxine HRWLLA\_CBC\_053\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: ug/dl

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## Anesthesia used for blood collection HRWLLA\_CBC\_036\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

**Options:**

Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg)/ Antipamezole (Antisedan, 1mg/kg),

Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg),

Injection narcosis with Ketamine (137mg/kg)/Xylazine (6.6mg/kg),

Injection narcosis with Sodium Pentobarbital (Euthatal),

Injection narcosis with Sodium Pentobarbital (Somnopentyl), No,

Gas anaesthesia with Isoflurane,

Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg),

Injection narcosis with Sodium Pentobarbital (Pentobarb, 0.1ml),

Injection narcosis with Tribromoethanol (Avertin),

Injection narcosis with Ketamine (100mg/kg)/ Xylazine (10mg/kg )/Antipamezole (Antisedan, 1mg/kg),

Topical local anaesthesia with EMLA,

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**Ferritin** HRWLLA\_CBC\_030\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: ng/ml

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**Storage temperature from blood collection till measurement** HRWLLA\_CBC\_041\_001 | v1.3

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: C

Options: 18-22, 4, -80, 2,

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**Fructosamine** HRWLLA\_CBC\_020\_001 | v1.2

simpleParameter

Req. Analysis: false      Req. Upload: false      Is Annotated: true

Unit Measured: umol/l

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**Albumin** HRWLLA\_CBC\_007\_001 | v1.2

simpleParameter

Req. Analysis: false      Req. Upload: true      Is Annotated: true

Unit Measured: g/l

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**Triglycerides** HRWLLA\_CBC\_017\_001 | v1.4

simpleParameter

Req. Analysis: false      Req. Upload: true      Is Annotated: true

Unit Measured: mg/dl

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**Cholesterol ratio** HRWLLA\_CBC\_058\_001 | v1.0

simpleParameter

Req. Analysis: false      Req. Upload: false      Is Annotated: true

Derivation: div('HRWLLA\_CBC\_015\_001', 'HRWLLA\_CBC\_016\_001')

**Sample type** HRWLLA\_CBC\_056\_001 | v1.0

procedureMetadata

Req. Analysis: true      Req. Upload: true      Is Annotated: false

Options: Plasma, Serum,

**Equipment ID** HRWLLA\_CBC\_033\_001 | v1.0

procedureMetadata

Req. Analysis: false      Req. Upload: true      Is Annotated: false

**Date equipment last calibrated** HRWLLA\_CBC\_050\_001 | v1.2

procedureMetadata

Req. Analysis: false      Req. Upload: false      Is Annotated: false

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## C-reactive protein

HRWLLA\_CBC\_032\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mg/l

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## Samples kept on ice between collection and analysis

HRWL

LA\_CBC\_042\_001 | v1.1

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: Yes, No,

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## Transferrin

HRWLLA\_CBC\_031\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mg/dl

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## Lipase HRWLLA\_CBC\_021\_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: U/l

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## Sample dilution HRWLLA\_CBC\_044\_001 | v1.2

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Options: 1:3, 1:5, Neat serum, 1:2, Yes (by Equipment, automatically), 1:4, Neat plasma, Varies,

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## HDL-cholesterol HRWLLA\_CBC\_016\_001 | v1.4

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: mg/dl

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## Uric acid HRWLLA\_CBC\_029\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: umol/l

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## Total protein

HRWLLA\_CBC\_006\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: g/l

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## Fasting

HRWLLA\_CBC\_057\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: Four hours before bleeding, No, Sixteen hours before bleeding,

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## Sample status

HRWLLA\_CBC\_043\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Options: Fresh and frozen, Frozen, Fresh,

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## UIBC (unsaturated iron binding capacity) HRWLLA\_CBC\_024\_001

| v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: umol/l

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## Iron HRWLLA\_CBC\_011\_001 | v1.5

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mg/dl

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## Lactate dehydrogenase HRWLLA\_CBC\_022\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: U/l

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**Alanine aminotransferase** HRWLLA\_CBC\_013\_001 | v1.2

simpleParameter

Req. Analysis: false      Req. Upload: true      Is Annotated: true

Unit Measured: U/l

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**Sodium** HRWLLA\_CBC\_001\_001 | v1.3

simpleParameter

Req. Analysis: false      Req. Upload: false      Is Annotated: true

Unit Measured: mmol/l

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**Potassium** HRWLLA\_CBC\_002\_001 | v1.3

simpleParameter

Req. Analysis: false      Req. Upload: false      Is Annotated: true

Unit Measured: mmol/l

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**LDL-cholesterol** HRWLLA\_CBC\_025\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mg/dl

**Aspartate aminotransferase** HRWLLA\_CBC\_012\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: U/l

**Equipment manufacturer** HRWLLA\_CBC\_034\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: Roche, Beckman Coulter, JEOL (Siemens), Olympus Diagnostics, Cobas, Hitachi,

**Method of blood collection** HRWLLA\_CBC\_037\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: Tail vein, Cardiac puncture, Jugular vein, Heart puncture, Retro-orbital puncture,

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## ID of blood collection SOP HRWLLA\_CBC\_045\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Options: RIKENMPP\_004a\_003, sop.inv.019, ESLIM\_024\_001, sop.inv.063, PHENO\_CBC,

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## Equipment model HRWLLA\_CBC\_035\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: AU 680, Integra 400 Plus, JCA-BM6070, UniCel 600 Pro, Hitachi 917, AU 400, 7020, AU 480, JCA-BM2250 (Advia 2400), DxC AU 700, Cobas,

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## Date and time of sacrifice HRWLLA\_CBC\_040\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

---

## Urea (Blood Urea Nitrogen - BUN) HRWLLA\_CBC\_004\_001 | v1.5

simpleParameter

**Req. Analysis:** false

**Req. Upload:** true

**Is Annotated:** true

**Unit Measured:** mg/dl

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## Total bilirubin HRWLLA\_CBC\_008\_001 | v1.4

simpleParameter

**Req. Analysis:** false

**Req. Upload:** true

**Is Annotated:** true

**Unit Measured:** mg/dl

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## Blood collection tubes HRWLLA\_CBC\_039\_001 | v1.1

procedureMetadata

**Req. Analysis:** false

**Req. Upload:** false

**Is Annotated:** false

**Options:** Eppendorf 1.7ml, Sarstedt Li-Heparin gel tubes,  
Kabe Labortechnik Lithium heparin coated tubes,  
TERUMO CAPIJECT Lithium heparin coated tubes,  
BD Microtainer Lithium Heparin/PST Gel Blood Tube, BD Microtainer Lithium Heparin Tube,  
Kabe Labortechnik 1000ul Lithium Heparin, Greiner MiniCollect Lithium Heparin 1ml,

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## Free fatty acids HRWLLA\_CBC\_026\_001 | v1.4

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: mmol/l

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**LIH (Hemolysis Severity - available on AU analysers)** HRWL

LA\_CBC\_019\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

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