

Urinalysis JAX_URI_001

Purpose

Urinalysis is performed to monitor renal function and will be performed as part of the adult phenotyping pipeline for KOMP2 mice. Samples will be analyzed using the Beckman AU680 autoanalyzer. Levels of urinary albumin, creatinine, magnesium and glucose are measured. The albumin to creatinine ratio (ACR) will be derived. A standard curve is run for microalbumin with each set of samples and used to derive an adjusted albumin value for use in the ACR.

Experimental Design

- **Minimum number of animals :** 7M + 7F
- **Age at test:** Week 13
- **Sex:** We would expect the results of this test to show sexual dimorphism

Equipment

1. Clinical chemistry analyzer
2. 1.5 mL Eppendorf tubes
3. Pipettes (200-1000 ul)

Procedure

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

Sample collection and preparation:

- a. Collect the appropriate volume of urine required (70ul), for the clinical chemistry analyser being used for assessment, in a microcentrifuge tube Time of day for collection is in the morning, starting no earlier than 07:30. If required volume is not obtained the first day, repeat collection on the next day and combine with Day 1 sample for same mouse.
- b. Keep whole urine samples on ice until analysis. If samples cannot be analysed immediately, keep them at 4°C until analysis.

c. Analysis of samples is optimally done on the day of collection. When not possible the samples can be stored at 2-8°C. If samples require storage for > 48 hours, freeze at -20 °C in single aliquots. All samples are allowed to come to room temperature prior to analysis.

d. Use samples undiluted or diluted to a ratio of 1:1 with deionised water if the volume is insufficient.

Notes

Urine collection is performed as a non-fasting procedure, always in the morning.

Dilution. Dilution of urine 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.

Metadata & examples

Metadata	Example
Equipment Name	ID of the machine used when more than 1 is used having same model and manufacturer Mickey Mouse, etc.
Equipment manufacturer	Manufacturer of the equipment. E.g. Olympus Diagnostics.
Equipment model	Model of the equipment. E.g. AU400, AU680
Anaesthesia used for urine collection	The drug used for anaesthesia during urine collection. E. g. Isoflurane. Or None.
Method of urine collection	Concise description of the method used for urine collection.
Samples kept on ice between collection and analysis	Yes/No.
Sample status	Indicate if the sample were frozen (analysis on the same day of collection not possible) Fresh/Frozen.

Urine dilution	Dilution is highly discouraged but if necessary indicate the extent. E.g. No/1:1.
ID of urine collection SOP	ID of the protocol followed for urine collection. Can be a center specific protocol. E
Date and time of urine collection	Time of day for collection is in the morning, starting no earlier than 07:30. E.g. Year
Date of measurement	The day of urine analysis. Year, month, day.
Experimenter ID	An ID of any format to be used coherently both inside the same procedure and for

Data QC

These are the kits for calibrators and QC that Jax uses:

Test	Au #	Test kit #	Calibrator Kit #	QC Kit #	Frequency
Creatinine	12. CRE	Beckman OSR6178	Beckman DR0091	BioRad 395X (2 levels)	Calibrator and Control day of a t
MicroAlbumin	27. MALB	Beckman OSR6167	Beckman ODR3024	BioRad 395X (2 levels)	Calibrator and Control day of a t
Magnesium	20.MG	Beckman OSR6789	Beckman DR0090	BioRad 395X (2 levels)	Calibrator and Control day of a t
Glucose	92.GLUX	Beckman OSR6121	Beckman DR0090	BioRad 395X (2 levels)	Calibrator and Control day of a t

To create a standard curve for calculating microalbumin:

Sigma Mouse Albumin (A3139-10MG) Dilution Procedure to create Standard Curve.

- 1) Reconstitute with 1 mL Reagent Grade H₂O
- 2) This Stock will = 1,000 mg/dL Microalbumin
- 3) Make a 2 X dilution of stock (resulting in new stock solution #2 of 500 mg/dL)
with Beckman Diluent 1 (PN# 442825)
- 4) Make a 10X dilution stock # 2 (to new stock #3 of 50 mg/dL)
- 5) Perform serial dilution 1:1 of stock #3 to obtain the following series of standards for MicroAlbumin:
 - 25 mg/dL
 - 12.5 mg/dL
 - 6.25 mg/dL
 - 3.125 mg/dL
 - 1.5625 mg/dL
 - 0 (Diluent Only)
- 6) Run standard samples on the AU680

Using the MicroAlbumin Regression template, insert the measured values for the standards and redraw the regression line. Update the value in the divisor box to reflect the value in front of x from the regression line. The formula is set to divide each measured sample value by the divisor to calculate the known concentration (mg/dL). Then input the creatinine concentration (mg/dL) and it will calculate and convert the ratio (mg alb/g creatinine).

Parameters and Metadata

microAlbumin (calculated) JAX_URI_001_001 | v1.1

simpleParameter

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

Unit Measured: mg/dl

Creatinine JAX_URI_002_001 | v1.1

simpleParameter

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

Unit Measured: mg/dl

Albumin to Creatinine ratio JAX_URI_003_001 | v1.2

simpleParameter

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

Unit Measured: mg/g

Magnesium JAX_URI_004_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: mg/dl

Glucose JAX_URI_005_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: mg/dl

Equipment name JAX_URI_006_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Equipment manufacturer JAX_URI_007_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: false

Is Annotated: false

Options: Beckman Coulter,

Equipment model JAX_URI_008_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: AU 680,

Anesthesia used for urine collection JAX_URI_009_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Method of urine collection JAX_URI_010_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Fasting prior to experiment JAX_URI_011_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: No,

Moved from Cage for Fasting JAX_URI_012_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: No,

Approximate period for Fasting JAX_URI_013_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: None,

Samples kept on ice between collection and analysis JAX_U

RI_014_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: Yes, No,

Sample status JAX_URI_015_001 | v1.0
procedureMetadata

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

Options: Fresh, Frozen,

Urine dilution JAX_URI_016_001 | v1.0
procedureMetadata

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

Options: Neat, 1:1, 1:2,

ID of Urine collection JAX_URI_017_001 | v1.0
procedureMetadata

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

Date and time of urine collection JAX_URI_018_001 | v1.1

procedureMetadata

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

Unit Measured: Date/time

Date of measurement JAX_URI_019_001 | v1.2

procedureMetadata

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

Unit Measured: Date

Experimenter ID JAX_URI_020_001 | v1.0

procedureMetadata

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