Determination of Creatinine

Related topics
Creatinine, clearance, kidney function, photometry, Jaffé’s reaction

Principle
Creatinine is generated as a metabolite of the muscle metabolism, in relation to the muscle mass, and is excreted with the urine. Increased creatinine levels are found in acute kidney failure, chronic kidney insufficiency and hypoperfusion of the kidneys. Analysis of the creatinine concentration is performed photometrically with Jaffé’s method. Creatinine is suitable as a clearance substance because creatinine is excreted through the kidneys at a rate of 99%. The creatinine clearance is determined on the basis of the creatinine concentration in the serum and 24h-urine, taking the quantity collected and the time of collection into account.

Material

<table>
<thead>
<tr>
<th>Student Basic Set</th>
<th>1 Disposable gloves, M, latex, 100 pcs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Microliter pipette 10 – 100 µl</td>
<td>46359-00</td>
</tr>
<tr>
<td>1 Microliter pipette 100 – 1000 µl</td>
<td>1 Semi-micro cuvettes</td>
</tr>
<tr>
<td>1 Graduated pipette 10 ml, scale division</td>
<td>35662-10</td>
</tr>
<tr>
<td>0.1 ml</td>
<td>1 Photometer</td>
</tr>
<tr>
<td>1 Pipette filler</td>
<td>35602-99</td>
</tr>
<tr>
<td>1 Digital stop watch</td>
<td>Chemicals</td>
</tr>
<tr>
<td>1 Laboratory beaker, 100 ml, PP</td>
<td>1 Water, distilled, 5 l</td>
</tr>
<tr>
<td>1 Laboratory pencil, waterproof</td>
<td>Additionally, the following reagents are required:</td>
</tr>
<tr>
<td>1 Cuvette rack</td>
<td>Control sample TruLab N</td>
</tr>
<tr>
<td>1 Pipette tips 2 - 200 µl in holders, 96 pcs.</td>
<td>Control sample kit TruLab P</td>
</tr>
<tr>
<td>1 Pipette tips 50 - 1000 µl in holders, 100 pcs.</td>
<td>Creatinine reagent with standard</td>
</tr>
</tbody>
</table>

Fig. 1: Material
Note: The reagents and control samples used for this experiment are listed in the Appendix. Please note! Always observe the preliminary remarks when performing this experiment.

Tasks
1. Determine the creatinine content in a serum-urine sample as well as in the control sample.
2. Calculate the associated deviation of the individual measured value of the control sample.
3. Calculate the creatinine clearance for your patient.

Procedure

Preparation of reagent
- Prepare working reagent: 4 parts R1 + 1 part R2
- Bring reagent to room temperature following the instructions in the package leaflet.

Material for the analysis
- Serum - Please refer to chapter “Pre-Analytics”: For the determination of the clearance, the serum withdrawal has to take place at the same time as the urine collection.
- The standard solution is ready for use.
- TruLab P or N is used as a control sample. It is lyophilised. Dissolve in distilled water following instructions in the package leaflet (refer to chapter with preliminary remarks).
- Urine: Dilute 1 + 49 with Aqua dest.
- (In the event that lower creatinine levels are to be expected in the urine, lower dilutions, e.g. 1 + 29, may be prepared.)
- Urine collection over 24h is required for the determination of the creatinine clearance (please refer to chapter “Pre-Analytics”).

Task 1: Determine the creatinine content in a serum sample and in a urine sample.
- Adjust the photometer to the wavelength of 490 nm (the thickness of the layer is 1 cm).
- The temperature should be 20 – 25°C.
- The measurement is performed against the reagent blank value.
- Mix each solution thoroughly.
In total, 5 samples are measured as follows:
- Reagent blank value
- Standard
- Control sample
- Patient serum sample
- Patient urine sample – Please note! Urine samples have to be diluted 1 : 49, please refer to section “Material for the examination”.

Fig. 2: Measuring workflow
Determination of Creatinine

Measure reagent blank value
- Switch on device: on/off
- Set wave length: 490 nm
- Pipette 50 µl of distilled water into a cuvette and place cuvette into measuring point.
- Pipette 1000 µl of creatinine reagent into this cuvette. Pipetting scheme.
  After 60 sec.: Press button “R”.
- After another 120 sec.: Press button “T”

Extinction differences may also occur in the measurement of the blank value. In this case, the corresponding value has to be subtracted from the following samples.

Measure samples
Please also refer to table “Pipetting scheme”. 
- Pipette 50 µl of the sample into a cuvette and place cuvette into measuring point.
- Add 1000 µl of creatinine reagent: Press button “R”
- After 60 sec, read extinction E1: Press button “T”
- After a further 120 sec, read extinction E2: Press button “T”
- Switch off device on/off

<table>
<thead>
<tr>
<th>Reagent blank value</th>
<th>Sample (standard/. control / patient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqua dest. 50 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample (standard/. control / patient)</td>
<td>50 µl</td>
</tr>
<tr>
<td>Creatinine reagent 1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

Basics
This test is used as diagnostic test to check the function of the kidneys. It is also applied in the follow-up of kidney diseases. Creatinine is generated in the course of the decomposition of creatinine, which is required for muscle contraction. It is produced in relation to the muscle mass and excreted with the urine. Increased creatinine levels are found in acute kidney failure, chronic kidney insufficiency and hypoperfusion of the kidneys. Low creatinine levels are not caused by any disorder.

The increase of the creatinine concentration in the serum will be proportional to the decrease of the quantity of creatinine which is excreted through the kidneys into the urine. However, it will only start to rise when the filtration rate of the kidney is limited to 50% of the standard rate (creatinine-blind range).

The calculation of the creatinine clearance is thus a possibility for the early detection of kidney disorders. The creatinine clearance in ml/min is defined as that quantity of blood plasma which is cleared of creatinine by the activity of the kidneys alone. It is thus a means to record the glomerular filtration rate GFR. The clinical relevance of creatinine clearance is the early detection of limited kidney function. In a healthy subject, creatinine is filtrated exclusively through the glomeruli; in the tubules however, it neither secreted nor reabsorbed. For this reason, creatinine is suited as a clearance substance.

Thus, both the creatinine concentration in 24 h urine and in the serum are required for the determination of the clearance. High creatinine levels in the serum will result in a low creatinine clearance value. The creatinine clearance thus declines with the severity of the functional disorder of the kidneys.

Further diagnostics: Multiple test strips, urine sediments, urea, proteins in the urine.
Determination of Creatinine

Test principle
In the Jaffé’s reaction introduced here, creatinine forms an orange-red colour complex with picrate in an alkaline solution. The dye generated in this manner is proportional to the creatinine concentration in the sample. Apart from creatinine, a multitude of other physiologically occurring metabolites and also drugs trigger a comparable colour reaction. These unspecific reactions, however, are slower to occur so that the chromogens produced and not corresponding to creatinine are of hardly any consequence in this measuring method. These pseudo-creatines (e.g. pyruvate, 2-ketoglutarate) will only react after approx. 10 minutes have passed.

Evaluation

Task 1: Determine the creatinine content in a serum sample and in a urine sample.

The creatinine content can be determined directly from \( \Delta E \) (or \( \Delta E - \Delta E_{\text{Blank value}} \)) of the samples in comparison to the standard sample, the concentration of which is known. When determining the creatinine content of the urine, the previous dilution has to be taken into account. In case of a 1+49 dilution of the urine, factor 50 is included in the formula, in case of a 1 + 29 urine dilution, factor 30 will be included.

\[
\text{Concentration}_{\text{Creatinine}_{\text{Serum}}}[\text{mg/dl}] = \frac{\Delta E_{\text{Sample}}}{\Delta E_{\text{Standard}}} \cdot \text{Concentration}_{\text{Standard}}
\]

\[
\text{Concentration}_{\text{Creatinine}_{\text{Urine}}}[\text{mg/dl}] = \frac{\Delta E_{\text{Sample}}}{\Delta E_{\text{Standard}}} \cdot \text{Concentration}_{\text{Standard}} \cdot 50
\]

Calculation example

In our exemplary test, we obtained the following values:

<table>
<thead>
<tr>
<th></th>
<th>Extinction E₁</th>
<th>Extinction E₂</th>
<th>( \Delta E )</th>
<th>Concentration [mg/dl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (Std)</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>2.0</td>
</tr>
<tr>
<td>Control sample (TruLab P)</td>
<td>0.18</td>
<td>0.22</td>
<td>0.04</td>
<td>8.0</td>
</tr>
<tr>
<td>Patient Serum</td>
<td>0.16</td>
<td>0.18</td>
<td>0.02</td>
<td>4.0</td>
</tr>
<tr>
<td>Patient Urine</td>
<td>0.01</td>
<td>0.03</td>
<td>0.02</td>
<td>200</td>
</tr>
</tbody>
</table>

\[
\text{Concentration}_{\text{Control}}[\text{mg/dl}] = \frac{\Delta E_{\text{Control}}}{\Delta E_{\text{Standard}}} \cdot \text{Concentration}_{\text{Standard}} = \frac{0.04}{0.01} \cdot 2.0 \text{ mg/dl} = 8.0 \text{ mg/dl}
\]

\[
\text{Concentration}_{\text{Patient}}[\text{mg/dl}] = \frac{\Delta E_{\text{Patient}}}{\Delta E_{\text{Standard}}} \cdot \text{Concentration}_{\text{Standard}} = \frac{0.04}{0.01} \cdot 2.0 \text{ mg/dl} = 8.0 \text{ mg/dl}
\]
**Reference values**

Creatinine in serum

Recommendation  
Adults: <1 mg/dl  
Creatinine clearance >98 ml/min

Reference values depend to a high degree on the respective method. Additional information on reference values: Please refer to chapter “Preliminary Remarks”.

**Task 2:** Calculate the deviation of the individual measured value of the control sample.

The deviation of the individual measured value of the control sample is used to verify whether errors occurred in the experiment (according to Guidelines of the Federal Medical Council on quality assurance in medical laboratory tests. Please observe statutory provisions in your country - see preliminary remarks).

The value of the control sample determined in the experiment is the so-called Actual Value. But we also know the true value of the control sample, called Target value. It is indicated in the package leaflet of the control serum (Trulab P or N). The following formula is used to calculate the deviation of the individual measured value of the control sample (abbr. KoPEM) from the determined actual value and the known target value:

\[
KoPEM[\%] = \frac{Target - Actual}{Target} \cdot 100
\]

Calculation example:

Concentration of control sample Actual: 8.0 mg/dl  
Concentration of control sample Target: 7.6 mg/dl

\[
KoPEM[\%] = \frac{7.6 - 8.0}{7.6} \cdot 100 = -5.2\%
\]

Following this, the deviation of the individual measured value of the control sample from the target value is compared to the limits permitted under general rules in the respective country. In the Federal Republic of Germany, this limit for creatinine is indicated as +/-11.5 % in Table B 1a RiliBÄK. [Guidelines of the German Federal Medical Council on quality assurance in medical laboratory tests] In our example, the individual measured value of the control sample fulfils this default. The patient may thus be assessed.

**Please note:** Please observe statutory provisions in your respective country!

Please refer to chapter “Preliminary Remarks”.

**Task 3:** Calculate the creatinine clearance for your patient.

As creatinine clearance is defined as that quantity of plasma, from which the creatinine is completely eliminated within one minute by kidney activity, one has to use the urine volume of one minute and not the total value of 24 h.
Determination of Creatinine

The calculation of clearance $C$ is performed with the following formula:

$$C = \frac{U \cdot V}{P}$$

$C =$ Clearance (plasma volume in ml/min)
$P =$ Concentration of creatinine in the serum mg/dl
$U =$ Concentration of creatinine in the urine mg/dl
$V =$ Urine volume in ml, in relation to 1 minute.

Calculation example for above patient (collected volume: 2000 ml in 24 hours = 1440 minutes)

$$C = \frac{200 \text{mg/dl} \cdot 2000 \text{ml}}{4 \text{mg/dl} \cdot 1440 \text{min}} = 69 \text{ml/min}$$

Questions

- What is the name of the determination method?  
  *Jaffé’s method.*

- Why is observing the incubation time so important for the determination?  
  *Apart from creatinine, a multitude of other physiologically occurring metabolites and also drugs trigger a comparable colour reaction. These unspecific reactions, however, are slower to occur, so that the chromogens produced and not corresponding to creatinine are of hardly any consequence in this measuring method. These pseudo-creatinines (e.g. pyruvate, 2-ketoglutarate) will only react after approx. 10 minutes have passed.*

- Why is creatinine suitable for determining clearance?  
  *Because creatinine is an endogenous metabolite, 99 % of which is excreted.*

- Which test material is used for clearance determination?  
  *Serum and 24h-urine.*
Lab Report

Task 1:
Enter your data from task 1 in the following table.

<table>
<thead>
<tr>
<th></th>
<th>Extinction $E_1$</th>
<th>Extinction $E_2$</th>
<th>$\Delta E = \frac{E_2-E_1}{E_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kit TruLab P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Task 2:

Result of the deviation of the individual measured value of the control sample:

_____________________________________________________________________________

Task 3:

Creatinine clearance of patient

_____________________________________________________________________________

Questions

- What is the name of the determination method?

_____________________________________________________________________________

- Why is observing the incubation time so important for the determination?

_____________________________________________________________________________

_____________________________________________________________________________

_____________________________________________________________________________

_____________________________________________________________________________
- Why is creatinine suitable for determining clearance?

- Which test material is used for clearance determination?