PURPOSE
In vitro test for the quantitative determination of uric acid in serum, plasma and urine on Roche/Hitachi cobas c systems.

DIRECTED TO
All qualified laboratory personnel.

SCOPE
This SOP applies to uric acid levels analyzed on the Roche/Hitachi cobas c systems by the method listed in the test principal.

Test Principal: Enzymatic colorimetric test.
Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

\[
\text{Uricase} \\
\text{Uric acid} + 2 \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2
\]

In the presence of peroxidase, 4-aminophenazone is oxidized by hydrogen peroxide to a quinone-diimine dye.

\[
\text{Peroxidase} \\
2 \text{H}_2\text{O}_2 + \text{H}^+ + \text{TOOS}^4 + 4-\text{aminophenazone} \rightarrow \text{quinone-diimine dye} + 4 \text{H}_2\text{O}
\]

The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

POLICIES
CDH Chemistry laboratory analyzes QC material for each analyte at predetermined intervals. Patient results will NOT be released until appropriate quality control material has been analyzed and validated within the stated time frame for that test.

QUALITY CONTROL
For serum quality control, use Bio-Rad MultiQual Levels 1 and 3.
For urine quality control, use Bio-Rad Urine Chemistry Controls Levels 1 and 2.
The control intervals for this assay are once per shift, after a calibration and with each new reagent cassette.
If controls are out of range, refer to SOP CHM 3.2.3 Outline of Quality Control Decisions and Posting

SPECIMEN
Serum: SST or Plain Red Top tube.
Plasma: Li-heparin
Stability: Serum or Plasma, 5 days at 2-8 °C.
Urine: Assay as soon as possible. Add NaOH for a pH>8.0. Do not refrigerate.
Stability: Urine (upon NaOH addition, pH>8.0) 4 days at 15-25 °C.
REAGENTS, EQUIPMENT, AND SUPPLIES

<table>
<thead>
<tr>
<th>Roche cobas c</th>
<th>Bio-Rad MultiQual Levels 1 and 3</th>
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<tbody>
<tr>
<td>DI water</td>
<td>UA2 reagent packs</td>
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<tr>
<td>C.f.a.s. calibrator and DI water</td>
<td>2 Point calibration needed with every lot number change and when indicated by the QC results.</td>
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Reagent working solutions

R1- Phosphate buffer: 0.05 mol/L, pH 7.8; TOOS: 7 mmol/L; fatty alcohol polyglycol ether: 4.8 %; ascorbate oxidase (EC 1.10.3.3; zucchini) ≥ 83.5 µkat/L (25 °C); stabilizers

R2-Phosphate buffer: 0.1 mol/L, pH 7.8; potassium hexacyanoferrate (II): 0.3 mmol/L; 4-aminophenazone ≥ 3 mmol/L; uricase (EC 1.7.3.3; Arthrobacter protophormiae) ≥ 83.4 µkat/L (25 °C); peroxidase (POD) (EC 1.11.1.7; horseradish) ≥ 50 µkat/L (25 °C); stabilizers

Reagent packs are ready for use, labeled “UA2” and stored at refrigerated temperature. Load reagent onto cobas in usual manner per SOP CHM 3.3.1 Cobas 6000 Operating Procedure.

PROCEDURE

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<tr>
<th>STEP</th>
<th>Procedure</th>
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<tr>
<td>1.</td>
<td>Ensure there is enough reagent on the instrument for everything required; calibration, quality controls and patient testing. If insufficient, load additional reagent packs per SOP CHM 3.3.1 Cobas 6000 Operation or SOP CHM 3.3.2 COBAS 6000 Operating Procedure Second and Third Shift</td>
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<td>2.</td>
<td>Calibrate the analyte if necessary. This can be determined through the cobas instrument computer by checking the RECOMMENDED CALIBRATION box on the cobas. See SOP CHM 3.3.1 Cobas 6000 Operation or SOP CHM 3.3.2 COBAS 6000 Operating Procedure Second and Third Shift</td>
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<td>3.</td>
<td>Analyze QC material if required.</td>
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<td>4.</td>
<td>Obtain specimen, centrifuge and separate serum, plasma or urine.</td>
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<td>5.</td>
<td>Put specimen to be tested in proper rack for cobas 6000 and place on instrument and click START&gt; START. The instrument will read</td>
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the bar code and perform necessary testing.

6. Check LIS for results. Address any error messages and call any critical results following SOP CHM 3.9.2 Posting Results for Interfaced Chemistry Analyzers using SoftLab Computer System

7. Verify patient results if no issues or when all issues are resolved.

8. If one cobas is inoperable, use the other cobas for testing if possible. If BOTH cobas analyzers are out of service send STAT specimens to Holyoke hospital according to SOP CHM 3.7.1 Specimen Handling for Testing Sent to Holyoke Hospital. Call to be sure the tests are conducted at Holyoke Hospital before sending specimens. HH laboratory (413) 540-5073 and HH switch board (413) 536-5221. Routine specimens will be properly stored in the walk-in refrigerator or freezer until testing can be completed.

INTERPRETATION

Expected Value: Serum / Plasma: 2.4 – 7.0 mg/dL

24 hour Urine: 250 – 750 mg/ day

Measuring Range:

**Serum/plasma 0.2-25.0 mg/dL**

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2.5

**Urine: 2.2-275 mg/dL (131-16362 µmol/L)**

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2.5

**Clinical Significance:** Uric acid is the final product of purine metabolism in the human organism. Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.

The oxidation of uric acid provides the basis for two approaches to the quantitative determination of this purine metabolite. One approach is the
reduction of phosphotungstic acid in an alkaline solution to tungsten blue, which is measured photometrically. The method is, however, subject to interferences from drugs and reducing substances other than uric acid.

A second approach, described by Praetorius and Poulson, utilizes the enzyme uricase to oxidize uric acid; this method eliminates the interferences intrinsic to chemical oxidation. Uricase can be employed in methods that involve the UV measurement of the consumption of uric acid or in combination with other enzymes to provide a colorimetric assay.

Another method is the colorimetric method developed by Town et al. The sample is initially incubated with a reagent mixture containing ascorbate oxidase and a clearing system. In this test system it is important that any ascorbic acid present in the sample is eliminated in the preliminary reaction; this precludes any ascorbic acid interference with the subsequent POD indicator reaction. Upon addition of the starter reagent, oxidation of uric acid by uricase begins.

The Roche assay described here is a slight modification of the colorimetric method described above. In this reaction, the peroxide reacts in the presence of peroxidase (POD), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS), and 4-aminophenazone to form a quinone-diimine dye. The intensity of the red color formed is proportional to the uric acid concentration and is determined photometrically.

**ADDENDUM**

**Precautions and warnings:** For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines.

**Limitations and Interferences:** Criterion: Recovery within ± 10% of initial value at a uric acid concentration of 7 mg/dL (417 µmol/L)

**Serum/plasma**

Icterus: No significant interference up to an I-index of 40 (approximate conjugated and unconjugated bilirubin concentration: 684 µmol/L (40 mg/dL))

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L (1000 mg/dL))

Lipemia (Intralipid) No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Ascorbic acid < 0.17 mmol/L (< 3 mg/dL) does not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.
Exceptions: Calcium dobesilate causes artificially low uric acid results.

Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.

Exceptions: Calcium dobesilate, Levodopa and methyldopa can all cause artificially low uric acid results.

High homogentisic acid concentrations in urine samples lead to false results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

For additional information refer to SOP CHM 3.1.1 Specimen Requirements, Stability and Acceptability

REFERENCES

Roche cobas application sheet for uric Acid (UA2),2010-09;v7
### STANDARD OPERATING PROCEDURE
**URIC ACID**

**Cooley Dickinson Hospital Laboratory**
30 Locust Street
Northampton, MA 01061

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