Glucose GOD FS*
Diagnostic reagent for quantitative in vitro determination of glucose in serum or plasma on photometric systems

Order Information
Cat. No. Kit size
1 2550 99 10 021 R 5 x 25 ml + 1 x 3 ml Standard
1 2550 99 10 026 R 6 x 100 ml
1 2550 99 10 023 R 1 x 1000 ml
1 2550 99 10 030 6 x 3 ml Standard

Summary [1,2]
Measurement of glucose concentration in serum or plasma is mainly used in diagnosis and monitoring of treatment in diabetes mellitus. Other applications are the detection of neonatal hypoglycemia, the exclusion of pancreatic islet cell carcinoma as well as the evaluation of carbohydrate metabolism in various diseases.

Method
"GOD-PAP": enzymatic photometric test

Principle
Determination of glucose after enzymatic oxidation by glucose oxidase. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder’s reaction) [3].

\[
\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2
\]

\[
2 H_2O_2 + 4-\text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4 H_2O
\]

Reagents
Components and Concentrations in the Test
Reagent:
- Phosphate buffer pH 7.5 250 mmol/l
- Phenol 5 mmol/l
- 4-Aminoantipyrine 0.5 mmol/l
- Glucose oxidase (GOD) \( \geq 15 \text{ kU/l} \)
- Peroxidase (POD) \( \geq 1 \text{ kU/l} \)

Standard:
- 100 mg/dl (5.55 mmol/l)

Storage Instructions and Reagent Stability
The reagent is stable up to the end of the indicated month of expiry, if stored at 2–8 °C, protected from light and contamination is avoided. Do not freeze the reagents!

Note: It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 at 546 nm. The standard is stable up to the end of the indicated month of expiry, if stored at 2–25 °C.

Warnings and Precautions
1. The reagent contains sodium azide (0.95 g/l) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Take the necessary precautions for the use of laboratory reagents.

Waste Management
Please refer to local legal requirements.

Reagent Preparation
Reagent and standard are ready-to-use.

Materials required but not provided
NaCl solution 9 g/l.
General laboratory equipment.

Specimen
Serum, heparin plasma or EDTA plasma. Separate at the latest 1h after blood collection from cellular contents.
Stability after addition of a glycolytic inhibitor (NaF, KF):
- 1 day at 20–25 °C
- 7 days at 4–8 °C
Discard contaminated specimens.

Assay Procedure
Application sheets for automated systems are available on request.

- Wavelength 500 nm, Hg 546 nm
- Optical path 1 cm
- Temperature 20–25 °C / 37 °C
- Measurement Against reagent blank

Calculation
With standard or calibrator.

\[
\text{Glucose [mg/dl] = \frac{\Delta A \text{Sample} \times \text{Conc. Std / Cal [mg/dl]}}{\Delta A \text{ Std / Cal}}}
\]

Conversion factor
Glucose [mg/dl] \( \times 0.05551 = \) Glucose [mmol/l]

Calibrators and Controls
For the calibration of automated photometric systems the DiaSys TruCal U calibrator is recommended. For internal quality control DiaSys TruLab N and P controls should be assayed with each batch of samples.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCal U</td>
<td>5 9100 99 10 063</td>
</tr>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 062</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 061</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 062</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 061</td>
</tr>
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</table>
Performance Characteristics

Measuring range
The test has been developed to determine glucose concentrations within a measuring range from 1 – 400 mg/dl. When values exceed this range samples should be diluted 1 + 4 with NaCl solution (9 g/l) and the result multiplied by 5.

Specificity / Interferences
No interference was observed by ascorbic acid up to 15 mg/dl, bilirubin up to 40 mg/dl, hemoglobin up to 200 mg/dl and lipemia up to 2,000 mg/dl triglycerides.

Sensitivity / Limit of Detection
The lower limit of detection is 1 mg/dl.

Precision (at 37°C)

<table>
<thead>
<tr>
<th>Intra-assay precision n = 20</th>
<th>Mean [mg/dl]</th>
<th>SD [mg/dl]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>64.2</td>
<td>1.12</td>
<td>1.74</td>
</tr>
<tr>
<td>Sample 2</td>
<td>122</td>
<td>1.57</td>
<td>1.28</td>
</tr>
<tr>
<td>Sample 3</td>
<td>296</td>
<td>4.41</td>
<td>1.49</td>
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<table>
<thead>
<tr>
<th>Inter-assay precision n = 20</th>
<th>Mean [mg/dl]</th>
<th>SD [mg/dl]</th>
<th>CV [%]</th>
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<tbody>
<tr>
<td>Sample 1</td>
<td>92.5</td>
<td>1.10</td>
<td>1.19</td>
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<tr>
<td>Sample 2</td>
<td>121</td>
<td>1.02</td>
<td>0.84</td>
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<tr>
<td>Sample 3</td>
<td>292</td>
<td>2.01</td>
<td>0.69</td>
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</tbody>
</table>

Method Comparison
A comparison between DiaSys Glucose FS (y) and a commercially available test (x) using 78 samples gave following results: y = 1.00 x + 1.00 mg/dl; r= 0.996.

Reference Range [1]

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<tr>
<th></th>
<th>[mg/dl]</th>
<th>[mmol/l]</th>
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<tr>
<td>Newborns:</td>
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<tr>
<td>Cord blood</td>
<td>63 - 158</td>
<td>3.5 - 8.8</td>
</tr>
<tr>
<td>1 h</td>
<td>36 - 99</td>
<td>2.0 - 5.5</td>
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<tr>
<td>2 h</td>
<td>36 - 89</td>
<td>2.2 - 4.9</td>
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<tr>
<td>5 – 14 h</td>
<td>34 – 77</td>
<td>1.9 – 4.3</td>
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<tr>
<td>10 – 28 h</td>
<td>46 – 81</td>
<td>2.6 – 4.5</td>
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<tr>
<td>44 – 52 h</td>
<td>48 – 79</td>
<td>2.7 – 4.4</td>
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<tr>
<td>Children (fasting):</td>
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<tr>
<td>1 – 6 years</td>
<td>74 – 127</td>
<td>4.1 – 7.0</td>
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<tr>
<td>7 – 19 years</td>
<td>70 – 106</td>
<td>3.9 – 5.9</td>
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<tr>
<td>Adults (fasting):</td>
<td></td>
<td></td>
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<tr>
<td>Serum / plasma</td>
<td>70 – 115</td>
<td>3.9 – 6.4</td>
</tr>
</tbody>
</table>

Literature

Manufacturer
DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany
INTENDED USE
For the quantitative determination of total protein concentration in human serum.

INTRODUCTION
Serum protein is involved in the maintenance of normal distribution of water between blood and tissues through osmotic pressure. Low protein is primarily caused by malnutrition, impaired synthesis, loss (as by hemorrhage), or excessive protein catabolism. Elevated protein levels are caused mainly by dehydration.¹

The determination of total protein in serum makes use of the Biuret color reaction, known since 1878. Past attempts to stabilize the cupric ions in the alkaline reagent were unsuccessful until the addition of sodium potassium tartrate as a complexing agent². The present method for quantitative determination of total protein in serum is based on the method proposed by the American Association for Clinical Chemistry³ (AACC) and National Committee for Clinical Laboratory Standards (NCCLS).⁴

PRINCIPLE
The enzymatic reaction sequence employed in the assay of Total Protein is as follows:

\[
\text{Alkaline} \quad \text{Protein} + \text{Cu}^{++} \rightarrow \text{Cu-Protein Complex}\]

Protein in serum forms a blue colored complex when reacted with cupric ions in an alkaline solution. The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration.

REAGENT COMPOSITION
1. Total Protein Reagent: Sodium Hydroxide 600 mM, Copper Sulfate 12 mM, Sodium Potassium Tartrate 32 mM, Potassium Iodide 30 mM, and non-reactive ingredients.
2. Total Protein Standard: Bovine Albumin Ft. V with preservative 5.0 g/dl.

REAGENT STORAGE AND STABILITY
Store Total protein reagent at room temperature (18 - 25°C). Store Total protein standard at refrigerator (2 - 8°C).

REAGENT DETERIORATION
The reagent should be discarded if there is turbidity, or the presence of a black precipitate, which indicates reagent deterioration. The reagent should be a clear, pale blue solution.

WARNINGS AND PRECAUTIONS
1. For in vitro diagnostic use. CAUTION: In vitro diagnostic reagents may be hazardous. Handle in accordance with good laboratory procedures which dictate avoiding ingestion, and eye or skin contact.

2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The reagent contains sodium hydroxide that is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.

SPECIMEN COLLECTION
1. Test specimens should be serum and free from hemolysis.
2. Gross hemolysis will cause elevated results because of the released hemoglobin as well as the increase in background color.
3. Lipemic sera cause elevated results and should be run with a serum blank.
   a. Place 1.0 ml 0.9% saline in test tube.
   b. Add 0.02 ml (20 µl) sample.
   c. Zero spectrophotometer with 0.9% saline.
   d. Read and record absorbance of serum blank.
   e. Subtract blank absorbance from test absorbance.
   f. Calculate as usual.
4. Samples with bromsulfophthalein (BSP) will result in falsely elevated results.⁵

Protein in serum is stable for one (1) week at room temperature (15 - 30°C) and for at least one (1) month refrigerated (2 - 8°C) when guarded against evaporation. Alternate Volumes = 50 µl sample to 3 ml reagent.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices (3 ml, 50 µl)
2. Timer
3. Test tube and rack
4. Spectrophotometer

GENERAL INSTRUCTIONS
The reagent for Total Protein is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

AUTOMATED PROCEDURE
Refer to appropriate application manual available.

MANUAL PROCEDURE
1. Label tubes as Blank, Standard, Controls, Patients, etc.
2. Pipette 3.0 ml of reagent into each tube.
3. Add 0.05 ml (50 µl) of standard and patients to appropriate tubes and mix by inversion.
4. Let the tubes stand at room temperature (15 - 30°C) for ten (10) minutes.
5. Set spectrophotometer at 540 nm and zero instrument with the reagent blank. (Wavelength range: 500 – 550 nm).
6. Read and record absorbance of each tube.

* TC MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.
NOTE:
1. Final color is stable for sixty-minutes at room temperature.
2. Serums with values above 15.0 g/dl should be diluted 1:1 with 0.9% saline, re-run, and the final answer multiplied by two (2).
3. ALTERNATE VOLUMES: 20µl (0.02 ml) sample to 1.0 ml reagent. Calculations remain the same.

LIMITATIONS
1. The reagent is linear to 15.0 g/dl. Samples with values above 15.0 g/dl should be diluted 1:1 with 0.9% saline, re-run, and the result multiplied by two (2).
2. The Biuret procedure is not sensitive at low ranges (< 1 g/dl). Do not use for urine or spinal fluid.

CALCULATIONS
Abs. Of Unknown \times \text{ Conc. Of Standard} = \text{Total Protein (g/dl)}

Example:
Absorbance of unknown \quad = \quad 0.350
Absorbance of standard \quad = \quad 0.400
Concentration of standard \quad = \quad 5 \text{ g/dl}

\[
\frac{0.350}{0.400} \times 5 = 4.38 \text{ g/dl}
\]

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established total protein values may be routinely used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES
6.2 - 8.5 g/dl
1. The effect of posture, when blood is drawn, varies with the individual but recumbent values are usually lower than mandatory. Differences may be as much as 1.2 g/dl.
2. It is strongly recommended that each laboratory establish its own range of expected values.

PERFORMANCE CHARACTERISTICS
1. **Linearity:** 1.0 - 15.0 g/dl
2. **Comparison:** A comparison study when performed between this procedure and another procedure based on the same principle resulted in a correlation coefficient of 0.95 with a regression equation of \( y = 0.86 + 1.02. \)
3. **Precision:**

<table>
<thead>
<tr>
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<th>Within Run</th>
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</thead>
<tbody>
<tr>
<td>Mean (g/dl)</td>
<td>S.D.</td>
<td>C.V. %</td>
<td></td>
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<tr>
<td>6.8</td>
<td>0.12</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>0.08</td>
<td>2.1</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Run-to-Run</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Mean (g/dl)</td>
<td>S.D.</td>
<td>C.V. %</td>
<td></td>
</tr>
<tr>
<td>6.8</td>
<td>0.17</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>0.14</td>
<td>3.7</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES
**ORDER INFORMATION**

**REF** | **Kit size**
---|---
GA4201 00 | 10x50 ml
KL2012 00 | 8x20 ml
BK2012 00 | 2x60 ml

**INDICATION**

Albumin concentration is an index of liver synthetic activity. While its increase is typically caused by a hemoconcentration (in vivo for dehydration, in vitro for sample evaporation or protracted stasis by tourniquet), causes of hypoaalbuminemia are numerous: proteins loss (nephritic syndrome, burn, proteins-dispersing enteropathy), increased turnover (catabolic state, glucocorticoids), decreased protein uptake (malnutrition, low protein diets) and, liver disease. In particular, in chronic hepatitis its decrease is proportional to the progression to cirrhosis, representing a maker of prognosis and metabolic decompensation.

Plasmatic concentration is lower in newborn (2.4-4.4 g/dl). Within the first week values of the adults are reached (3.5-5 g/dl); then its production increases up to 4.5-5.4 g/dl at an age of six years and remains unchanged during adolescence. No significant difference is found among male and female.

**METHOD PRINCIPLE**

In citrate buffer albumin forms with green bromocresol (BCG) a coloured compound with a colour intensity proportional to the albumin concentration present in the sample.

**COMPOSITION**

**REAGENT A (liquid):**
- Citrate buffer 7.5 mmol/l
- BCG ≥ 150 µmol/l
- Sodium azide 0.05%

**STANDARD (liquid):**
- 1x3 ml
- Albumin 4 g/dl
- Sodium azide 0.05%

Verified against NIST reference standard.

**ANCILLARY EQUIPMENT**

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution 9 g/l

**SAMPLES**

Serum or plasma (heparin or EDTA). Stable one month at 2-8 °C, or one week at 15-25 °C.

**INTERNAL QUALITY CONTROL**

It is recommended to use commercial Quality Control sera with known Albumin concentration to check the correspondence of the obtained results with those expected and validate the data.

**ANALYTICAL PROCEDURE**

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>5 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>5 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes at room temperature (20-25 °C). Read the absorbance (A) of the standard and samples at 546 (520-570) nm against blank. Colour is stable for 60 minutes.

**Note:**

Reaction volumes can be proportionally modified.

**CALCULATION OF RESULTS**

Utilize the following formula:

\[
\text{Albumin, g/dl} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 4
\]

Values in g/dl can be modified to obtain g/l multiplying the results x 10.

**REFERENCE VALUES**

3.5 ÷ 5 g/dl

Each laboratory should establish reference ranges for its own patients population.

**ANALYTICAL PERFORMANCES**

**Precision**

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different Albumin concentrations. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (g/dl)</th>
<th>SD</th>
<th>%CV</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>4.6</td>
<td>0.03</td>
<td>0.7</td>
<td>0.14</td>
<td>3.0</td>
</tr>
<tr>
<td>Serum 2</td>
<td>4.0</td>
<td>0.02</td>
<td>0.5</td>
<td>0.14</td>
<td>3.5</td>
</tr>
<tr>
<td>Serum 3</td>
<td>3.3</td>
<td>0.02</td>
<td>0.6</td>
<td>0.10</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**Linearity**

The assay is linear up to 7 g/dl.

**Sensitivity**

Test sensitivity of the method, in terms of limit of detection, is 0.2 g/dl.

**Correlation**

A correlation study comparing the present method with a commercial one gave the following results:

\[
y = 0.624x + 1.616 \text{ g/dl} \quad r = 0.9691
\]
Interferences
In case of clear haemolysis or lipemia it is recommended the execution of a blank sample: mix 1 ml of physiological and 10 µl of sample, read absorbance against distilled water and subtract it to the absorbance value measured in the test. Hemoglobin up to 20 mg/dl doesn’t interfere.

PRECAUTIONS IN USE
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

REFERENCES
TECO DIAGNOSTICS
1268 N. Lakeview Ave.
Anaheim, CA 92807
1-800-222-9880

CREATININE TEST
KINETIC METHOD
TC-MATRIX

INTENDED USE
For the quantitative determination of creatinine concentration in serum or plasma on TC Matrix analyzers.

SUMMARY AND EXPLANATION OF THE TEST
Creatinine is excreted as a waste product by the kidneys. Increased serum creatinine levels usually indicate impairment of renal function. Creatinine appears in the glomerular filtrate and is not reabsorbed by the tubule. Hence, any condition that reduces the glomerular filtration rate will result in the increase of creatinine concentration in plasma. Since the excrete rate of creatinine is relatively constant and since its production rate is not influenced by protein catabolism or other external factors. The concentration of creatinine in serum is, therefore, a good measure of renal glomerular function. Creatinine Reagent is used to measure the creatinine concentration by a modified rate Jaffe method. In the reaction, creatinine combines with picric acid in an alkaline solution to form a creatinine-picrate complex.

The TC Matrix System automatically proportions the appropriate sample and reagent volumes into the cuvette. The system monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of creatinine in the sample and is used by the TC Matrix System to calculate and express the creatinine concentration.

Alkali
Creatinine + picric acid ———————→ Creatinine-picrate
(reddish colored complex)

REAGENT CONTENTS:
Each kit contains: Four Creatinine Reagent 1(4×40 ml)
Four Creatinine Reagent 1(4×8 ml)
Instruction Insert.

REAGENT PREPARATION
No preparation is required.

REAGENT COMPOSITION
Picric Acid: 8.1 mmol/L
Buffered to pH:>13.3
Also non-reactive chemicals for optimal system performance.

REAGENT STORAGE AND STABILITY
Teco Creatinine Reagent stored unopened at room temperature is stable until the expiration date showed on the bottle label. Once opened, Teco Creatinine Reagent is stable for 30 days, unless the expiration date is exceeded.
DO NOT FREEZE.

SPECIMEN COLLECTION AND HANDLING
1. The test can be performed on serum, plasma. For serum, blood is drawn into a tube which does not contain anticoagulant and allow clotting. The serum is them separated from the clot. A maximum limit of two hours from the time of collection is recommended.
2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum and plasma should be stored at 2°C to 8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
3. For plasma, add whole blood directly into a tube containing anticoagulant. Acceptable anticoagulants are listed in the “LIMITATIONS” section.

CALIBRATION
1. Calibrator required: TECO MULTI Calibrator.
2. The system must have a valid calibration in memory before controls or patient samples can be run.
3. The TC Matrix system will automatically perform checks on the calibration and produce data at the end of calibration.

Note: Refer to the Beckman SYNCHRON manual for further instructions on calibrating the instrument

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT
TECO MULTI Calibrator
At least two levels of control material.

LIMITATIONS
1. The anticoagulants Ammonium Heparin, EDTA, Lithium Heparin, Potassium Oxalate, Sodium Fluoride, Sodium Citrate and Sodium Heparin were found to be compatible with this method.

INTERFERENCE
1. Hemoglobin levels up to 500 mg/dl, Triglyceride levels up to 1000mg/dl and Bilirubin levels up to 30 mg/dl were found to exhibit negligible interference.
2. Lipemic samples >3+ should be ultra-centrifuged and the analysis performed on the infranate.
3. On this method, refer to the work of Young for a review of drug and comprehensive list of substances effect on creatinine level.

EXPECTED VALUE
0.6 -1.3 mg/dL or 53 to 115µmol/L

PRECAUTIONS:
1. For in vitro diagnostic use only.
2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH manual Biosafety in Microbiological and Biomedical Laboratories, and in accordance with National or local regulations related to the safety precautions of such materials.
3. Each laboratory has to perform the quality control test to assure the results being reliable before running the specimen tests.
4. Recommended to test alone.
**PROCEDURES**

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<th>CREA R1:</th>
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<tbody>
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<tr>
<td>Q3: /</td>
<td>Q4: /</td>
<td></td>
</tr>
<tr>
<td>PRECISION:</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>PC: /</td>
<td>ABS.: /</td>
<td></td>
</tr>
</tbody>
</table>

**PERFORMANCE CHARACTERISTICS**

**Analytical Range:** 0.3-20 mg/dL

For Creatinine analyte by Teclo Creatinine Reagent on TC Matrix System, this method has been demonstrated to be linear from 0.3-20 mg/dL.

**Accuracy:** Comparison study was performed on TC Matrix System from 40 samples. Beckman Coulter Creatinine reagent was used to compare with Creatinine Reagent. The results of this study in yield a correlation coefficient of 0.99 with a regression equation of $y=0.99X - 6.4$.

**Precision:** Within Run precision for Creatinine Reagent Set was determined following a modification of NCCLS EP5-A.Two commercial human serum were assayed on TC Matrix System for 25 times.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Mean (mg/dl)</td>
<td>1.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Standard Deviation (mg/dl)</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>10.5</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Run-Day precision for Creatinine Reagent was determined following a modification of NCCLS EP5-A.Two commercial human serum, TECO MULTI Calibrators were assayed on TC Matrix Systems five times per day for five days for the total of 25 values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Mean (mg/dl)</td>
<td>1.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Standard Deviation (mg/dl)</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Coefficient of Variation (%)</td>
<td>10.2</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**REFERENCES:**
1. Jaffe M.1886. 2 tschr physiol whem. 10:391.
CREATININE Enzymatic

End point colorimetric determination of Creatinine in serum, plasma and urine

ORDER INFORMATION

REF Kit size
GA4420 00 1x45 + 1x15 ml
KL4420 00 1x45 + 1x15 ml
BK4420 00 2x(45+15 ml)

INDICATION

Creatinine is a metabolic waste product formed due to non-enzymatic dehydration of creatine derived from creatine phosphoric acid. Determination of serum or urinary creatinine is a useful diagnostic tool for kidney diseases such as acute chronic nephritis and other disorders such as urethropraxis, mercurialism and nephrosis.

METHOD PRINCIPLE

The enzymatic assay for creatinine involves a series of coupled enzymatic reactions including creatininase enzymatic conversion of creatinine into the product creatine which is converted to sarcosine by creatine amidinohydrolase (creatinase), followed by oxidation of sarcosine by sarcosine oxidase (SOD) producing hydrogen peroxide. In the presence of peroxidase (POD) the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye.

Any endogenous creatine present in the sample is removed by creatinase and sarcosine oxidase during preincubation.

COMPOSITION

REAGENT A:
Creatinase 12-60 IU/ml
Sarcosine oxidase 4-17 IU/ml
TOOS 0.07-0.21 mg/ml

REAGENT B:
Creatininase 135-670 IU/ml
Peroxidase 20 kU/l
4-AA 0.3-0.9 mg/ml

STANDARD:
1x2 ml Creatinine 2 mg/ml (177 µmol/l)

Preparation
The reagents are liquids ready to use.

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT
• Automatic pipettes
• Photometer
• Analysis cuvettes (optical path = 1 cm)
• Temperature controlled water bath
• NaCl solution 9 g/l

SAMPLES

Fresh serum, plasma (heparin, EDTA), urine 24h. Dilute urine 1:10 with saline solution.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known creatinine concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>270 µl</td>
<td>270 µl</td>
<td>270 µl</td>
</tr>
<tr>
<td>Distilled H2O</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>8 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>8 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes at 37 °C.
Read the absorbance (A1) of the standard and samples at 550 nm against water. Then add:

| Reagent B | 90 µl | 90 µl |
| Distilled H2O | - | - |

Mix and incubate for 5 minutes at 37 °C.
Read the absorbance (A2) of the standard and samples at 550 nm against water.

CALCULATION OF RESULTS

Calculate ∆A (A2 – A1) for all the samples and for the standard.

Serum:
\[ \text{mg/dl} = \frac{\text{∆A sample}}{\text{∆A standard}} \times 2 \]

Urine:
\[ \text{mg/dl} = \frac{\text{∆A sample}}{\text{∆A standard}} \times 20 \]

Urine: (when 24 hours diuresis is known)
\[ \text{g/24h} = \frac{\text{∆A sample}}{\text{∆A standard}} \times 0.2 \times \text{I/24h} \]

\[ \text{mg/kg/24h} = \frac{\text{urine creatinine, g/24h}}{\text{body mass (kg)}} \times 1000 \]

Clearance: (when 24 hours diuresis is known)
\[ \text{ml/min.} = \frac{\text{urine creatinine, mg/dl x ml/24h}}{\text{serum creatinine, mg/dl x 1440}} \]

Conversion factor
Creatinine [mg/ml] x 88.4 = Creatinine [µmol/l]
Creatinine [µmol/l] x 0.0113 = Creatinine [mg/ml]

Note:
For creatinine values exceeding the linearity limit repeat the determination using sample diluted with saline solution; multiply the result by dilution factor used.

Conversion factor
Creatinine [mg/ml] x 88.4 = Creatinine [µmol/l]
Creatinine [µmol/l] x 0.0113 = Creatinine [mg/ml]
REFERENCE VALUES

<table>
<thead>
<tr>
<th>Sample</th>
<th>Subjects</th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Male</td>
<td>0.6 ÷ 1.3</td>
<td>mg/dl</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.5 ÷ 1.2</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Urine</td>
<td>Adults</td>
<td>1.3 ÷ 1.8</td>
<td>g/24h</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>20 ÷ 26</td>
<td>mg/kg/24h</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14 ÷ 24</td>
<td>mg/kg/24h</td>
</tr>
<tr>
<td>Clearance</td>
<td>Male</td>
<td>107 ÷ 139</td>
<td>ml/minute</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>87 ÷ 107</td>
<td>ml/minute</td>
</tr>
</tbody>
</table>

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Serum

Precision has been evaluated by testing four serum specimens at different creatinine concentration with two runs per day with duplicate over 20 working days. The obtained results are reported in the following tables.

Serum: within-run precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (mg/dl)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum # 1</td>
<td>80</td>
<td>0.74</td>
<td>0.015</td>
<td>2.1</td>
</tr>
<tr>
<td>Serum # 2</td>
<td>80</td>
<td>1.38</td>
<td>0.015</td>
<td>1.1</td>
</tr>
<tr>
<td>Serum # 3</td>
<td>80</td>
<td>4.04</td>
<td>0.029</td>
<td>0.7</td>
</tr>
<tr>
<td>Serum # 4</td>
<td>80</td>
<td>10.28</td>
<td>0.015</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Serum: total precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (mg/dl)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum # 1</td>
<td>80</td>
<td>0.74</td>
<td>0.022</td>
<td>3.0</td>
</tr>
<tr>
<td>Serum # 2</td>
<td>80</td>
<td>1.38</td>
<td>0.026</td>
<td>1.9</td>
</tr>
<tr>
<td>Serum # 3</td>
<td>80</td>
<td>4.04</td>
<td>0.058</td>
<td>1.4</td>
</tr>
<tr>
<td>Serum # 4</td>
<td>80</td>
<td>10.28</td>
<td>0.140</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Urine

Within-run precision has been evaluated by testing 21 replicates of three commercial urine controls at different creatinine concentration in the same run. For total precision, 2 runs of each commercial urine control were performed consecutively for 5 days. The samples were diluted ten-fold with 0.9% saline and tested for creatinine values. The values were multiplied by the dilution factor (i.e. 10) to obtain the final results indicated below.

Urine: within-run precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (mg/dl)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine # 1</td>
<td>21</td>
<td>29.09</td>
<td>0.100</td>
<td>0.36</td>
</tr>
<tr>
<td>Urine # 2</td>
<td>21</td>
<td>87.10</td>
<td>0.270</td>
<td>0.31</td>
</tr>
<tr>
<td>Urine # 3</td>
<td>21</td>
<td>196.70</td>
<td>0.900</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Urine: total precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (mg/dl)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine # 1</td>
<td>20</td>
<td>29.86</td>
<td>0.790</td>
<td>2.64</td>
</tr>
<tr>
<td>Urine # 2</td>
<td>20</td>
<td>87.70</td>
<td>0.670</td>
<td>0.76</td>
</tr>
<tr>
<td>Urine # 3</td>
<td>20</td>
<td>195.00</td>
<td>1.190</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Linearity

The linearity of the procedure is 0.14-13.56 mg/dl in serum and 0.14-141.25 mg/dl in urine.

Sensitivity

Test sensitivity, in terms of limit of detection, is 0.14 mg/dl.

Correlation

Accuracy has been evaluated by assaying serum samples (range: 0.2-13.51 mg/dl) and urine samples (range: 0.14-141 mg/dl) with the present method and a legally marked creatinine assay. The regression curves calculated are the following:

Serum: y = 0.9467x + 0.0643 mg/dl  r = 0.9981
Urine: y = 1.005x - 0.2979 mg/dl  r = 0.9969

Interferences

The following substances produced less than 10% deviation at the listed concentrations:

- Triglycerides 1000 mg/dl
- Hemoglobin 500 mg/dl
- Bilirubin 40 mg/dl
- Bilirubin conjugate 30 mg/dl
- Ascorbic acid 10 mg/dl

Certain drugs can sometimes cause abnormally elevated creatinine values.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

CREATININE
Kinetic colorimetric determination of Creatinine in biological fluids

ORDER INFORMATION
REF Kit size
GA4450 00 5x50 + 5x50 ml
KL4450 00 8x40 + 8x40 ml

INDICATION
Creatinine is a metabolic waste product formed due to non-enzymatic dehydration of Creatine derived from Creatine phosphoric acid. Determination of serum or urinary Creatinine is a useful diagnostic tool for kidney diseases such as acute chronic nephritis and other disorders such as urethrophraxis, mercurialism and nephrosis.

METHOD PRINCIPLE
Serum and urine Creatinine reacts with Picric acid in alkaline solution yielding a yellow-orange coloured compound. The intensity of the colour is directly proportional to the Creatinine concentration present in the sample.

COMPOSITION
REAGENT A:
- Sodium hydroxide 1.25 mmol/l
  Corrosive R34; S(1/2-)26-37/39-45.

REAGENT B:
- Picric acid 20.5 mmol/l

STANDARD:
- 1x5 ml
- Creatinine 2 mg/dl
  Verified against NIST reference material.

PREPARATION OF REAGENTS
Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 1 part of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 10 ml of RA + 10 ml of RB).

Storage and stability
Store at room temperature (15-25 °C). Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

Working reagent is stable for 7 days at 2-8 °C.

ANCILLARY EQUIPMENT
- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution 9 g/l

SAMPLES
Serum, urine 24h. Stable 24 hours at 2-8 °C. Dilute urine 1:25 with distilled water.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL
It is recommended to use commercial Quality Control sera with known Creatinine concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE
Allow the reagents to reach working temperature before using.

Bireagent procedure
Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Sample/Standard</th>
<th>Serum or diluted urine</th>
<th>Standard</th>
<th>Reagent A</th>
<th>Mix and incubate for 5 minutes, then add:</th>
<th>Reagent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>100 µl</td>
<td>-</td>
<td>500 µl</td>
<td>500 µl</td>
<td>500 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>100 µl</td>
<td>100 µl</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix and, after 10 seconds, read absorbance (A1) at 490-510 nm; read again after 1 minute the absorbance (A2). Analysis of samples and standards have to be executed at the same temperature.

Monoreagent procedure
Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Sample/Standard</th>
<th>Serum or diluted urine</th>
<th>Standard</th>
<th>Working reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>100 µl</td>
<td>100 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>100 µl</td>
<td>1000 µl</td>
<td></td>
</tr>
</tbody>
</table>

Mix and, after 10 seconds, read absorbance (A1) at 490-510 nm; read again after 1 minute the absorbance (A2). Analysis of samples and standards have to be executed at the same temperature.

CALCULATION OF RESULTS
Calculate ∆A (A2 – A1) for all the samples and for the standard.

Serum:
mg/dl = \( \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 2 \)

Urine:
mg/dl = \( \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 50 \)

Urine: (when 24 hours diuresis is known)
g/24h = \( \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 0.5 \times \text{l/24h} \)

mg/kg/24h = \( \frac{\text{urine creatinine, g/24h}}{\text{body mass (Kg)}} \times 1000 \)

Clearance: (when 24 hours diuresis is known)
ml/min. = \( \frac{\text{urine creatinine, mg/dl x ml/24h}}{\text{serum creatinine, mg/dl x 1440}} \)

Note:
1. For creatinine values higher than 6 mg/dl, repeat the determination using sample diluted 1:5 in saline solution; multiply the result by 5.
2. For the children the body surface (m²) must be taken into account in the calculation, multiplying the previous result by 1.73/m².
3. This method can be applied, by proportionally varying the working volumes, to all automatic instruments where the serum works as starter.
REFERENCE VALUES

<table>
<thead>
<tr>
<th>Sample</th>
<th>Subjects</th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Male</td>
<td>0.6 ÷ 1.3</td>
<td>mg/dl</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.5 ÷ 1.2</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Urine</td>
<td>Adults</td>
<td>1.3 ÷ 1.8</td>
<td>g/24h</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>20 ÷ 26</td>
<td>mg/Kg/24h</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14 ÷ 24</td>
<td>mg/Kg/24h</td>
</tr>
<tr>
<td></td>
<td>Clearance</td>
<td>107 ÷ 139</td>
<td>ml/minute</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>87 ÷ 107</td>
<td>ml/minute</td>
</tr>
</tbody>
</table>

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different Creatinine concentrations. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (mg/dl)</th>
<th>Within Run</th>
<th>Between Run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>%CV</td>
<td>SD</td>
</tr>
<tr>
<td>Serum 1</td>
<td>1.72</td>
<td>0.02</td>
<td>1.2</td>
</tr>
<tr>
<td>Serum 2</td>
<td>3.05</td>
<td>0.03</td>
<td>1.0</td>
</tr>
<tr>
<td>Serum 3</td>
<td>4.52</td>
<td>0.04</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear up to 6 mg/dl.

Sensitivity
Test sensitivity, in terms of limit of detection, is 0.1 mg/dl.

Correlation
A correlation study comparing the present method and a commercial one gave the following results:

\[ y = 1.0534x + 0.6955 \text{ mg/dl} \quad r = 0.9541 \]

Interferences
- Hemoglobin > 200 mg/dl
- Bilirubin > 20 mg/dl
- Triglycerides > 1000 mg/dl

PRECAUTIONS IN USE

Reagent A is harmful (Corrosive).
Refer to Safety Data Sheet.
Reagent B and Standard are not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY
1. HENRY J.B., Clinical Diagnosis and Management; 17th edition, Saunder Publisher (1984)
UREA U.V.
Enzymatic method for the quantitative determination of Urea in serum, plasma and urine

ORDER INFORMATION
REF Kit size
GA4960 00 10x40 + 5x20 ml
KL4960 00 10x40 + 10x10 ml
BK4960 00 5x(60+15 ml)

INDICATION
Urea concentration is an indicator of kidney function. Conditions associated to high urea values are related to ioperuemia and azotemia.

METHOD PRINCIPLE
Urease hydrolyzes urea into ammonia and carbon dioxide. Glutamate dehydrogenase catalyzes the reaction of ammonia with 2-ketoglutarate and oxidizes NADH into NAD⁺.

\[
\text{Urea} + 2 \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2 \text{NH}_4^+ + 2 \text{HCO}_3^-
\]

2-Ketoglutarate + NH₄⁺ + NADH \xrightarrow{\text{GLDH}} L-Glutamate + NAD⁺ + H₂O

The decrease of absorbance of NADH, measured at 340 nm, is proportional to the urea present in the sample.

COMPOSITION
REAGENT A:
- TRIS pH 7.8 150 mmol/l
- 2-Ketoglutarate 8.75 mmol/l
- ADP 0.75 mmol/l
- Urease ≥ 7.5 kU/l
- GLDH (Glutamate-dehydrogenase) ≥ 1.25 kU/l
- Sodium azide ≤ 0.95 g/l

REAGENT B:
- NADH 1.32 mmol/l
- Sodium azide ≤ 0.95 g/l

STANDARD:
- 1x5 ml Urea 50 mg/dl
Verified against NIST reference material.

PREPARATION OF REAGENTS
Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g. 20 ml of RA + 5 ml of RB). Let stand working reagent at least 30 minutes at room temperature before use.

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 28 days at 2-8 °C or 5 days at 15-25 °C, protected from light.

ANCILLARY EQUIPMENT
- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES
Serum, plasma, 24h urine.
Do not use anticoagulants containing fluoride or ammonium ions.
Dilute urine 1:20 with distilled water.

Stability:
<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/plasma</td>
<td>20-25 °C</td>
<td>7 days</td>
</tr>
<tr>
<td>Serum/plasma</td>
<td>4-8 °C</td>
<td>7 days</td>
</tr>
<tr>
<td>Serum/plasma</td>
<td>-20 °C</td>
<td>1 year</td>
</tr>
<tr>
<td>Urine</td>
<td>2 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Urine</td>
<td>1 month</td>
<td></td>
</tr>
</tbody>
</table>

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL
It is recommended to use commercial Quality Control sera with known urea concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE
Working temperature 37 °C
Wavelength 340 nm (334 nm, 365 nm)
Optical path 1 cm
Reaction fixed time (decrease)

Allow the reagents to reach working temperature before using.

Bireagent procedure
Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A: 800 µl</td>
<td>800 µl</td>
<td>800 µl</td>
</tr>
<tr>
<td>Standard: -</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample: -</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes at 37 °C. Then add:
Reagent B: 200 µl
200 µl
200 µl

Mix, incubate for 30 seconds at 37 °C, than read A₁ of sample, standard and Blank. After precisely 60 seconds read absorbance A₂.

Determine:
\[ \Delta A = [(A₁-A₂) \text{ sample or standard}] - [(A₁-A₂) \text{ Blank}] \]

Monoreagent procedure
Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent: 1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard: -</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample: -</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Mix, incubate for 30 seconds at 37 °C, than read A₁ of sample, standard and Blank. After precisely 60 seconds read absorbance A₂.

Determine:
\[ \Delta A = [(A₁-A₂) \text{ sample or standard}] - [(A₁-A₂) \text{ Blank}] \]

Note
- Reaction volumes can be proportionally changed.
- The method is optimized for "two points" determinations. It is absolutely necessary to incubate reagent blank, standard and samples exactly for the same time. The same preincubation time for reagent blank, standard and sample is also necessary.
CALCULATION OF RESULTS

Serum-plasma:

\[
\text{Urea, mg/dl} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 50
\]

Urine (when 24h diuresis is known):

\[
\text{Urea, g/24h} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 10 \times l/24h
\]

Conversion factor

Urea [mg/dl] \times 0.1665 = Urea [mmol/l]

Urea [mg/dl] \times 0.467 = BUN* [mg/dl]

* Blood Urea Nitrogen

REFERENCE VALUES

Serum-plasma: 18÷53 mg/dl (adults)

Urine 24h: 6÷17 mg/24h

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of two sera with different urea concentration. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (mg/dl)</th>
<th>SD</th>
<th>%CV</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siero 1</td>
<td>42.8</td>
<td>1.54</td>
<td>3.6</td>
<td>1.50</td>
<td>3.5</td>
</tr>
<tr>
<td>Siero 2</td>
<td>161.7</td>
<td>3.60</td>
<td>2.2</td>
<td>7.51</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Linearity

The assay is linear up to 300 mg/dl.

Sensitivity

Test sensitivity, in terms of limit of detection, is 2 mg/dl.

Correlation

A correlation study comparing the present method and a commercial one gave the following results:

\[ y = 1.0436x - 1.1064 \text{ mg/dl} \quad r = 0.9924 \]

Interferences

- Hemoglobin > 500 mg/dl
- Bilirubin > 40 mg/dl
- Triglycerides > 2000 mg/dl
- Ascorbic acid > 30 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

**ORDER INFORMATION**

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA4865 00</td>
<td>12x50 ml</td>
</tr>
<tr>
<td>KL4865 00</td>
<td>10x60 ml</td>
</tr>
<tr>
<td>BK4865 00</td>
<td>4x60 ml</td>
</tr>
</tbody>
</table>

**INDICATION**

Uric acid determination is used for the diagnosis of gout, nitrogen retention, for the monitoring of nephropathies and it is used in all cytolytic therapies.

**METHOD PRINCIPLE**

Uric acid is oxidized by uricase into allantoin with production of hydrogen peroxide which, under the catalytic influence of peroxidase, reacts with 4-aminofenazone and N-ethyl-N-(hydroxi-3-sulphopropil)-p-toluidine (ESPT) to form a blue-violet colour:

\[
\text{Uricase} \quad \text{Uric acid} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Allantoine} + \text{CO}_2 + \text{H}_2\text{O}_2
\]

\[
\text{POD} \quad \text{ESPT} + 4\text{-Aminophenazone} + 2\text{ H}_2\text{O}_2 \rightarrow \text{Indamine} + 3\text{ H}_2\text{O}
\]

The colour intensity, measured at 550 nm, is proportional to the uric acid present in the sample. The presence of ascorbate oxidase avoids interferences by ascorbic acid and other reducing agents.

**COMPOSITION**

**REAGENT A:**
- Borate Buffer pH 7.0 180 mmol/l
- Uricase > 50 U/l
- Cholesterol esterase (CHE) > 300 U/l
- 4-aminophenazone 0.25 mmol/l
- ESPT 1 mmol/l
- Peroxidase (POD) > 100 U/l
- NaN₃ < 0.095 g/l

**STANDARD:**
1x5 ml Uric Acid 6 mg/dl

Verified against NIST reference material.

**PREPARATION**

Reagents are liquids ready to use.

**STORAGE AND STABILITY**

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

**ANCILLARY EQUIPMENT**

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

**SAMPLES**

Serum, heparin or EDTA plasma, urine 24h diluted 1:10 with distilled water.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Serum/plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25 °C</td>
<td>3 days</td>
<td>4 days</td>
</tr>
<tr>
<td>4-8 °C</td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td>&lt; 20 °C</td>
<td>6 months</td>
<td></td>
</tr>
</tbody>
</table>

**Specimen collection / Preanalytical factors**

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

**INTERNAL QUALITY CONTROL**

It is recommended to use controls with known uric acid concentration. Check that the values obtained are within the reference range provided.

**ANALYTICAL PROCEDURE**

Allow the reagents to reach working temperature before using.

**CALCULATION OF RESULTS**

Serum, plasma:

\[
\text{Uric Acid, mg/dl} = \frac{A \text{ sample}}{A \text{ standard}} \times 6
\]

Urine:

\[
\text{Uric Acid, mg/24h} = \frac{A \text{ sample}}{A \text{ standard}} \times 600 \times l/24h
\]

**Conversion factor**

Uric Acid [mg/dl] x 59.48 = Uric Acid [µmol/l]

Uric Acid [mg/dl] x 0.05948 = Uric Acid [mmol/l]

**REFERENCE VALUES**

<table>
<thead>
<tr>
<th></th>
<th>Female (µmol/l)</th>
<th>Male (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5 days</td>
<td>1.5-7.9 (113-470)</td>
<td>1.9-7.9 (113-470)</td>
</tr>
<tr>
<td>1-4 years</td>
<td>1.7-5.1 (101-303)</td>
<td>2.2-5.7 (131-470)</td>
</tr>
<tr>
<td>5-11 years</td>
<td>3.0-6.4 (178-381)</td>
<td>3.0-6.4 (178-381)</td>
</tr>
<tr>
<td>12-14 years</td>
<td>3.2-6.1 (190-381)</td>
<td>3.2-7.4 (190-440)</td>
</tr>
<tr>
<td>15-17 years</td>
<td>3.2-6.4 (190-381)</td>
<td>4.5-8.1 (190-440)</td>
</tr>
</tbody>
</table>

Urine

\[
\begin{align*}
\text{Uric Acid, mg/24h} \leq 800 & \text{ balanced diet} \\
\text{Uric Acid, mg/24h} \leq 600 & \text{ low purine diet}
\end{align*}
\]

Each laboratory should establish reference ranges for its own patients population.
ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of three controls at different uric acid concentration. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (mg/dl)</th>
<th>SD</th>
<th>%CV</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>4.6</td>
<td>0.08</td>
<td>1.8</td>
<td>0.17</td>
<td>3.8</td>
</tr>
<tr>
<td>Serum 2</td>
<td>12.1</td>
<td>0.22</td>
<td>1.8</td>
<td>0.53</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear up to 20 mg/dl (1190 µmol/l).

Sensitivity
Test sensitivity, in terms of limit of detection, is 0.3 mg/dl (17.84 µmol/l).

Correlation
A correlation study comparing the present method with a commercial one gave the following results:

\[ y = 1.1974x - 0.4471 \text{ mg/dl} \quad r = 0.9947 \]

Interferences
- Bilirubin > 20 mg/dl
- Hemoglobin > 50 mg/dl
- Triglycerides > 2000 mg/dl
- Ascorbic acid > 30 mg/dl

PRECAUTIONS IN USE
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY
INTENDED USE
For the quantitative determination of uric acid in serum.

INTRODUCTION
Uric acid is the end product of purine metabolism. Nearly half of the total uric acid is eliminated and replaced each day by way of urinary excretion and through microbial degradation in the intestinal tract. Increased uric acid levels are commonly associated with both nitrogen retention and urea, creatine, and other non-protein constituents. The quantitation of uric acid is an aid in the diagnosis of gout, decreased renal function, myeloproliferative disorders, and other conditions in which the cause for the hyper-uricemia is not well known.1

Uric acid is most commonly determined by a phosphotungstate method2 and iron reduction method.3 Due to serum interferences, the enzyme uricase has been widely used instead. Uricase is more specific for uric acid since uricase acts only on uric acid.4,5

PRINCIPLE
The enzymatic reaction sequence employed in the assay of uric acid is as follows:

\[
\text{Uricase} \quad \text{Uric acid} + \text{O}_2 + 2 \text{H}_2\text{O} \xrightarrow{\text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2} \\
\text{Peroxidase} \quad 2 \text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{chromogen} + 4 \text{H}_2\text{O}}
\]

Uric Acid is converted by uricase into allantoin and hydrogen peroxides. The hydrogen peroxide initiates the coupling of 4-aminoantipyrine to 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) to form the chromogen which is measured at 520nm and which is proportional to the amount of hydrogen peroxide generated from uric acid.

REAGENTS
1. Uric acid reagent: 4-Aminoantipyrine 4mM, 3,5 Dichloro-2-hydroxybenzenesulfonic acid 2mM, Stabilizer and Surfactant, buffer pH 7.5.
2. Uric acid standard (5 mg/dl).

WARNINGS AND PRECAUTIONS
1. For in vitro diagnostic use.
   CAUTION: The reagents may be hazardous. Handle in accordance with good laboratory procedures, which dictate avoiding ingestion, and eye or skin contact.
2. Serum specimens should be considered infectious and handled appropriately.

STORAGE AND STABILITY
The reagent set is stored refrigerated (2 - 8°C). DO NOT FREEZE. Bring reagent to room temperature before use.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Turbidity has occurred; turbidity may be a sign of contamination.
2. There is evidence of discoloration. A slight pink color is normal.

SPECIMEN COLLECTION
1. Test specimen should be serum and free from hemolysis.
2. Bacterial contamination should be avoided to preserve the loss of uric acid.
3. Uric acid in serum is stable for three (3) days at 2 - 8°C and up to six (6) months when frozen.6

INTERFERING SUBSTANCES
1. Bilirubin and ascorbic acid can result in falsely depressed uric acid levels.
2. Lipemic samples may cause falsely elevated uric acid levels.
3. Collection tubes containing formaldehyde as a preservative must be avoided.
4. For a comprehensive review of drug interferences refer to Young et al.7

MATERIALS REQUIRED BUT NOT PROVIDED
1. Pipette devices
2. Test tubes/rack
3. Timing device
4. Heating block (37°C)
5. Spectrophotometer capable of reading at 520 nm

GENERAL INSTRUCTIONS
The reagent for uric acid is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

AUTOMATED PROCEDURE
Refer to appropriate application manual available.

MANUAL PROCEDURE
1. Label test tubes, "reagent blank", "standard", "control", "unknown", etc.
2. Pipette 1.0 ml of working reagent into all tubes.
3. Pre-warm all tubes at 37°C for three (3) minutes.
4. Add 0.025 ml (25 µl) of sample to respective tubes and mix.
5. Incubate all tubes at 37°C for ten (10) minutes.
6. After incubation, zero the spectrophotometer with the reagent blank at 520 nm and read/record the absorbance of all tubes. (Wavelength range: 500 – 550 nm).
7. Repeat procedure for each sample.

* TC MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD

ALTERNATE VOLUMES
If the spectrophotometer being used requires a final volume greater than 1.0 ml for accurate reading, use 0.05 ml (50 µl) of sample to 3.0 ml of Reagent. Perform the test as described above.

PROCEDURAL LIMITATIONS
The reagent is linear to 25 mg/dl uric acid. Samples with values exceeding 25 mg/dl should be diluted 1:1 with saline, reassayed and the results multiplied by two (2). Lipemic samples will give falsely elevated results and a serum blank must be run.

Serum blank: Add 0.025 ml (25 µl) of sample to 1.0 ml water. Zero the spectrophotometer with water. Read and record absorbance and subtract reading from test absorbance.

CALCULATIONS (RATIO METRIC)
A = Absorbance
A unknown x concentration = value for unknown (mg/dl)
A standard of standard

Example: If the unknown A = 0.170, standard A = 0.180, concentration standard = 5 mg/dl, then:

\[ \frac{0.170 \times 5}{0.180} = 4.7 \text{ mg/dl} \]

SI UNITS (mmol/L): Multiply the result (mg/dl) by 10 to convert dl to liter and divide by 168 (the molecular weight of uric acid).

\[ \frac{\text{mg/dl} \times 10}{168} = \text{mmol/L} \]

\[ \text{mg/dl} \times 0.0595 = \text{mmol/L} \]

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with established uric acid values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES
1.5 - 7.0 mg/dl
It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS
1. Linearity: 25 mg/dl
2. Sensitivity: Based on an instrument resolution of 0.001 absorbance, the present procedure has a sensitivity of 0.03 mg/dl.

3. Comparison: A comparison with another commercial enzymatic uric acid procedure yielded a correlation coefficient of 1.00 with a regression equation of \( y = 1.02 x - 0.22 \).

4. Precision studies:

<table>
<thead>
<tr>
<th></th>
<th>Mean (mg/dl)</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within Run</td>
<td>3.9</td>
<td>0.06</td>
<td>2.0%</td>
</tr>
<tr>
<td>Run-to-Run</td>
<td>7.9</td>
<td>0.04</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

5. REFERENCES

U582: 10/01

Manufactured by:

TECO DIAGNOSTICS
1268 N. LAKEVIEW AVE.
ANAHEIM, CA 92807
U.S.A.

Authorized Representative:
Emergo Europe
P.O. Box 149
4300 AC Zierikzee
The Netherlands
CK NAC
Kinetic method UV optimized IFCC-DGKC for measurement of Creatine Kinase (CK) activity in serum

ODER INFORMATION
REF Kit size
GD5432 00 4x16 + 1x16 ml
KL5432 00 5x16 + 5x4 ml
BK5432 00 2x(40+10 ml)

INDICATION
The enzyme Creatine Kinase (CK) is found mainly in skeletal and heart muscle. It is a dimer formed by the association of two subunits conventionally named M (from muscle) and B (from brain). The different association of the two subunits is at the base of the differentiation of the three known isoenzymes: MM, MB and BB. CK activity values are high in patients with myocardial infarction, progressive muscular dystrophy, alcoholic myopathy, and delirium tremens, but normal in patients with hepatitis and other forms of liver disease. The high values in patients with hypothyroidism reflect the muscle changes in this condition. Although CK is an almost specific index of injury of myocardium and muscle, more recent reports indicate that inexplicably high serum CK values can occur in patients with pulmonary infarction and pulmonary edema.

At present, it should be regarded as a useful but not completely specific adjunct in the diagnosis of myocardial and muscle disease. Specificity of CK assay is enhanced by measurement of its isoenzymes.

PRINCIPLE OF THE METHOD
The CK activity is measured by the increasing rate of absorbance resulting from the following reactions:

\[
\text{CK (AMP, NAC)} \\
\begin{align*}
\text{Creatine phosphate + ADP} & \quad \rightarrow \quad \text{Creatine} + \text{ATP} \\
\text{ATP + Glucose} & \quad \rightarrow \quad \text{ADP} + \text{G6P} \\
\text{G6P + NADP} & \quad \rightarrow \quad \text{Gluconate-6P} + \text{NADPH} + \text{H}^+ \\
\end{align*}
\]

COMPOSITION
REAGENT A:
Imidazol buffer, pH 6.7 100 mmol/l
N-acetyl cysteine (NAC) 20 mmol/l
Magnesium acetate 10 mmol/l
Glucose 20 mmol/l
HK ≥ 4 KU/l

REAGENT B:
Creatine phosphate 30 mmol/l
AMP 5 mmol/l
ADP 2 mmol/l
Di(adenosine-5')pentaphosphate 10 µmol/l
G6P-DH ≥ 1.5 KU/l

PREPARATION OF THE REAGENTS
Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g.: 20 ml of RA + 5 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 30 days at 2-8 °C or 10 days at 20-25 °C, protected from light.
Do not utilize the reagent if the absorbance at 340 nm against water is > 0.300, or if the controls values are not inside the declared ranges.

ANCILLARY EQUIPMENT
• Automatic pipettes
• Photometer
• Analysis cuvettes (optical path = 1 cm)
• Temperature controlled water bath
• NaCl solution 9 g/l

SAMPLE
Serum. Stable 8 days at 2-8 °C or 30 days at –20 °C. Chill the samples as rapidly as possible after collection. Avoid using hemolyzed samples.

Specimen collection / preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL
It is recommended to use commercial control sera with known enzymatic activity. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE
Working Temperature 37 °C
Wavelength 340 (334-365) nm
Lightpath 1 cm
Type of reaction Kinetic (increase)

Allow the reagents to reach working temperature before using.

Bireagent procedure
Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B</td>
<td>250 µl</td>
<td>250 µl</td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>50 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Mix and incubate 2 minutes at 37 °C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>1000 µl</th>
<th>1000 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix and incubate 5 minutes at 37 °C. Read initial absorbance. Read absorbance again 1, 2, 3 minutes thereafter against Blank. Calculate ΔA/min.
Monoreagent procedure

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>40 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>40 µl</td>
</tr>
</tbody>
</table>

Mix and incubate 5 minutes at 37 °C. Read initial absorbance. Read absorbance again 1, 2, 3 minutes thereafter against Blank. Calculate ∆A/min.

Note:
Samples with activities higher than 900 U/l should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

CALCULATION OF RESULTS

CK activity (U/l) = ∆A/min x 4127 (37 °C)

REFERENCE VALUES

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Test temperature, 37 °C</td>
<td></td>
</tr>
<tr>
<td>CK Male</td>
<td>≤ 174 U/l</td>
</tr>
<tr>
<td>CK Female</td>
<td>≤ 140 U/l</td>
</tr>
<tr>
<td>Childrens</td>
<td>≤ 225 U/l</td>
</tr>
</tbody>
</table>

Each laboratory is advised to establish the reference interval in relation to its own geographic area.

ANALYTICAL PERFORMANCES

Precision

<table>
<thead>
<tr>
<th></th>
<th>Within Run (Replicates: 10 for each level)</th>
<th>Between Run (Replicates: 5 for each level, for 5 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=10 mean (U/l)</td>
<td>SD (U/l)</td>
</tr>
<tr>
<td>Sample 1</td>
<td>153</td>
<td>2.4</td>
</tr>
<tr>
<td>Sample 2</td>
<td>546</td>
<td>4.6</td>
</tr>
<tr>
<td>Sample 3</td>
<td>789</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear up to 900 U/l.

Sensitivity
Test sensitivity, in terms of limit of detection, is 4 U/l.

Correlation
A correlation study comparing the present method and a commercial one gave the following results:

\[ y = 1.098x + 6.8 \text{ U/l} \]

\[ r = 0.999 \]

Interferences
A number of drugs has been listed that will affect the CK determination.\(^{(3)}\)

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY

ODER INFORMATION

REF          Kit size
GD5430 00   1x20 + 1x5 ml
KL5430 00   5x16 + 5x4 ml
BK5430 00   3x(16+4 ml)

INDICATION
The enzyme Creatine Kinase (CK) is found mainly in skeletal and heart muscle. It is a dimer formed by the association of two subunits conventionally named M (from muscle) and B (from brain). The different association of the two subunits is at the base of the differentiation of the three known isoenzymes: MM, MB and BB. An increase of the CK activity may be associated to myocardial infarction, acute cerebrovascular diseases, traumas or diseases involving muscles. After myocardial infarction, CK level begins to increase between the fourth and the sixth hour following the event, reaching a peak between the eighteenth and the thirtieth hour, returning to the normal range during the thirth day. CK-MB determination within the proper time frame after infarction is most critical, the useful interval being from about 10 to 24 hour after the infarction. Its detection is of importance in determining the degree of the injury and the efficacy of the treatment.

PRINCIPLE OF THE METHOD
The immunoinhibition from a specific antibody of both, MM subunits and the single M subunit of CK-MB, allows the determination of the B subunit. The CK-B activity, corresponding to half of CK-MB, is measured by the increasing rate of absorbance resulting from the following reactions:

\[
\text{CK (AMP, NAC)} \rightarrow \text{Creatine phosphate + ADP} \rightarrow \text{Creatine + ATP} \\
\text{ATP + Glucose} \rightarrow \text{ADP + G6P} \\
\text{G6P + NADP}^+ + \text{H}_2\text{O} \rightarrow \text{Gluconate-6P + NADPH + H}^+
\]

COMPOSITION

REAGENT A:
- Imidazol buffer, pH 6.7 100 mmol/l
- N-acetyl cysteine (NAC) 20 mmol/l
- Magnesium acetate 10 mmol/l
- Glucose 20 mmol/l
- NADP 2.5 mmol/l
- HK \( \geq \) 4 KU/l

REAGENT B:
- Creatine phosphate 30 mmol/l
- AMP 5 mmol/l
- ADP 2 mmol/l
- Di(adenosine-5')pentaphosphate 10 \( \mu \)mol/l
- G6P-DH \( \geq \) 1.5 KU/l
- Sufficient CK-M human antibody to inhibit \( \geq \) 3000 U/l of CK-MM at 37 °C.

PREPARATION OF THE REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (e.g.: 20 ml of RA + 5 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 30 days at 2-8 °C or 5 days at 20-25 °C, protected from light. Do not utilize the reagent if the absorbance at 340 nm against water is > 0.800, or if the controls values are not inside the declared ranges.

ANCILLARY EQUIPMENT
- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLE
Serum. Stable 8 days at 2-8 °C or 30 days at –20 °C. Chill the samples as rapidly as possible after collection. Avoid using hemolyzed samples.

Specimen collection / preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL
It is recommended to use commercial control sera with known enzymatic activity. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE
Working Temperature 37 °C
Wavelength 340 (334-365) nm
Lightpath 1 cm
Type of reaction Kinetic (increase)

Allow the reagents to reach working temperature before using.

Bireagent procedure
Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B</td>
<td>250 ( \mu )l</td>
<td>250 ( \mu )l</td>
</tr>
<tr>
<td>Distilled ( \text{H}_2\text{O} )</td>
<td>50 ( \mu )l</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>50 ( \mu )l</td>
</tr>
<tr>
<td>Mix and incubate 2 minutes at 37 °C. Then add:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent A</td>
<td>1000 ( \mu )l</td>
<td>1000 ( \mu )l</td>
</tr>
<tr>
<td>Mix and incubate 5 minutes at 37 °C. Read initial absorbance. Read absorbance again 1, 2, 3 minutes thereafter against Blank. Calculate ( \Delta \text{A/min} ).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Monoreagent procedure

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>40 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>40 µl</td>
</tr>
</tbody>
</table>

Mix and incubate 5 minutes at 37 °C.
Read initial absorbance. Read absorbance again 1, 2, 3 minutes thereafter against Blank. Calculate ΔA/min.

CALCULATION OF RESULTS

CK-B activity (U/l) = ΔA/min x 4127 (37 °C)
CK-MB activity (U/l) = CK-B activity x 2

REFERENCE VALUES

CK-MB, adults: 2.0 U/l ÷ 19.5 U/l (at 37 °C)

Newborns, infants, and children have higher serum CK-MB values than adults.

A ratio between CK-MB and total CK activities above 4% should be considered suspicious, and above 10% consistent with acute myocardial infarction.(5)

Each laboratory is advised to establish the reference interval in relation to its own geographic area.

ANALYTICAL PERFORMANCES

Precision

<table>
<thead>
<tr>
<th></th>
<th>Within Run (Replicates: 10 for each level)</th>
<th>Between Run (Replicates: 10 for each level, for 6 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=10</td>
<td>mean (U/l)</td>
<td>SD (U/l)</td>
</tr>
<tr>
<td>Sample 1</td>
<td>16.3</td>
<td>0.32</td>
</tr>
<tr>
<td>Sample 2</td>
<td>56.3</td>
<td>0.64</td>
</tr>
<tr>
<td>Sample 3</td>
<td>96.7</td>
<td>1.07</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear up to 330 U/l.

Sensitivity
Test sensitivity, in terms of limit of detection, is 4 U/l.

Correlation
A correlation study comparing the present method and a commercial one gave the following results:

\[ y = 0.958x + 0.55 \text{ U/l} \quad r = 0.997 \]

Interferences
A number of drugs has been listed that will affect the CK determination.(5). The present method also measures any CK-BB present in serum, this activity is usually negligible.

PRECAUTIONS IN USE
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY
**LDH FS**

**IFCC**

Diagnostic reagent for quantitative in vitro determination of lactate dehydrogenase (LDH) in serum or plasma on photometric systems

### Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>14211 99 10 021</td>
<td>R1 5 x 20 ml + R2 1 x 25 ml</td>
<td></td>
</tr>
<tr>
<td>14211 99 10 704</td>
<td>R1 8 x 50 ml + R2 8 x 12,5 ml</td>
<td></td>
</tr>
<tr>
<td>14211 99 10 917</td>
<td>R1 8 x 60 ml + R2 8 x 15 ml</td>
<td></td>
</tr>
<tr>
<td>14211 99 10 191</td>
<td>R1 4 x 36 ml + R2 4 x 9 ml</td>
<td></td>
</tr>
</tbody>
</table>

### Summary [1,2]

Lactate dehydrogenase (LDH) is an enzyme, consisting of five different isoenzymes that catalyze the interconversion of L-lactate and pyruvate. LDH is present in the cytoplasm of all human tissues with higher concentrations in liver, heart and skeletal muscle, and lower in erythrocytes, pancreas, kidney and stomach. Increased LDH activities are found in a variety of pathological conditions such as myocardial infarction, liver diseases, blood diseases, cancer or muscle diseases. However, because of the lack of organ specificity, determination of its isoenzymes or other enzymes such as alkaline phosphatase or ALAT / ASAT is necessary for differential diagnosis.

### Method

Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) and DGKC (German Society of Clinical Chemistry)

### Principle

\[
\text{L-Lactate} + \text{NAD}^+ \xrightarrow{<LDH>} \text{Pyruvate} + \text{NADH} + \text{H}^+ 
\]

### Reagents

**Components and Concentrations**

\[N.B:\text{ Concentrations are those in the final test mixture.}\]

<table>
<thead>
<tr>
<th>R1: N-Methyl-D-Glucamine</th>
<th>pH 9.40</th>
<th>325 mmol/l</th>
<th>L-Lactate</th>
<th>50 mmol/l</th>
<th>NAD(^+)</th>
<th>10 mmol/l</th>
</tr>
</thead>
</table>

**Storage Instructions and Reagent Stability**

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C and contamination is avoided. Do not freeze the reagents! Reagent 2 must be protected from light.

### Warnings and Precautions

Take the necessary precautions for the use of laboratory reagents.

### Waste Management

Please refer to local legal requirements.

### Assay Procedure

**Application sheets for automated systems are available on request.**

| Wavelength | 340 nm, Hg 365 nm, Hg 334 nm |
| Optical path | 1 cm |
| Temperature | 37 °C |
| Measurement | Against reagent blank |

#### Substrate start

\[
\Delta A \text{/min Sample} = [\Delta A \text{/min} \text{Sample}] - [\Delta A \text{/min blank}]
\]

#### Sample start

\[
\Delta A \text{/min} = [\Delta A \text{/min Sample}] - [\Delta A \text{/min blank}]
\]
**Calculation**

From absorbance readings calculate \( \Delta A/\text{min} \) and multiply by the corresponding factor from table below:

\[
\Delta A/\text{min} \times \text{factor} = \text{LDH activity [U/l]}
\]

<table>
<thead>
<tr>
<th>Substrate Start</th>
<th>( \text{Mean} ) [U/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 nm</td>
<td>10080</td>
</tr>
<tr>
<td>334 nm</td>
<td>10275</td>
</tr>
<tr>
<td>365 nm</td>
<td>18675</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Start</th>
<th>( \text{Mean} ) [U/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>340 nm</td>
<td>8095</td>
</tr>
<tr>
<td>334 nm</td>
<td>8250</td>
</tr>
<tr>
<td>365 nm</td>
<td>15000</td>
</tr>
</tbody>
</table>

**Controls**

For internal quality control DiaSys TruLab N and P controls should be assayed with each batch of samples.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 062 x 20 x 5 ml</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 062 x 20 x 5 ml</td>
</tr>
</tbody>
</table>

**Performance Characteristics**

**Measuring range**

The test has been developed to determine LDH activities which correspond to a maximal \( \Delta A/\text{min} \) of 0.15 at 340 and 334 nm or 0.08 at 365 nm.

If these values are exceeded the sample should be diluted 1 + 10 with NaCl solution (9 g/l) and results multiplied by 11.

**Specificity / Interferences**

No interference was observed by ascorbic acid up to 30 mg/dl, bilirubin up to 40 mg/dl and lipemia up to 2,000 mg/dl triglycerides. Hemoglobin interferes starting with a concentration of 50 mg/dl.

**Sensitivity / Limit of Detection**

The lower limit of detection is 5 U/l.

**Precision**

<table>
<thead>
<tr>
<th>Intra-assay precision</th>
<th>( \text{Mean} ) [U/l]</th>
<th>( \text{SD} ) [U/l]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>178</td>
<td>2.00</td>
<td>1.12</td>
</tr>
<tr>
<td>Sample 2</td>
<td>187</td>
<td>2.12</td>
<td>1.14</td>
</tr>
<tr>
<td>Sample 3</td>
<td>566</td>
<td>2.27</td>
<td>0.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inter-assay precision</th>
<th>( \text{Mean} ) [U/l]</th>
<th>( \text{SD} ) [U/l]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>170</td>
<td>1.62</td>
<td>0.95</td>
</tr>
<tr>
<td>Sample 2</td>
<td>176</td>
<td>2.48</td>
<td>1.41</td>
</tr>
<tr>
<td>Sample 3</td>
<td>566</td>
<td>3.61</td>
<td>0.64</td>
</tr>
</tbody>
</table>

**Method Comparison**

A comparison between DiaSys LDH FS IFCC (y) and the IFCC reference reagent (x) using 51 samples gave following results: \( y = 0.949 x + 8.451 \text{ U/l}; r = 0.990 \).

A comparison with a commercially available test with 51 samples gave following results:

\( y = 0.992 x + 10.72 \text{ U/l}; r = 0.997 \).

**Reference Range**

<table>
<thead>
<tr>
<th>Age</th>
<th>Female [U/l]</th>
<th>Male [U/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults [3]</td>
<td>&lt; 247 U/l</td>
<td>&lt; 248 U/l</td>
</tr>
<tr>
<td>Children [5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 30 days</td>
<td>145 – 765 U/l</td>
<td>125 – 735 U/l</td>
</tr>
<tr>
<td>31 days – 1 year</td>
<td>190 – 420 U/l</td>
<td>170 – 450 U/l</td>
</tr>
<tr>
<td>1 – 3 year(s)</td>
<td>165 – 395 U/l</td>
<td>155 – 345 U/l</td>
</tr>
<tr>
<td>4 – 6 years</td>
<td>135 – 345 U/l</td>
<td>135 – 345 U/l</td>
</tr>
<tr>
<td>7 – 9 years</td>
<td>140 – 280 U/l</td>
<td>145 – 300 U/l</td>
</tr>
<tr>
<td>10 – 12 years</td>
<td>120 – 260 U/l</td>
<td>120 – 325 U/l</td>
</tr>
<tr>
<td>13 – 15 years</td>
<td>100 – 275 U/l</td>
<td>120 – 290 U/l</td>
</tr>
<tr>
<td>16 – 18 years</td>
<td>105 – 230 U/l</td>
<td>105 – 235 U/l</td>
</tr>
</tbody>
</table>

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

**Literature**


**Manufacturer**

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany
**ORDER INFORMATION**

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA4910 00</td>
<td>5x40 + 1x20 ml</td>
</tr>
<tr>
<td>GA4911 00</td>
<td>10x40 + 2x20 ml</td>
</tr>
<tr>
<td>KL4910 00</td>
<td>8x50 + 8x5 ml</td>
</tr>
<tr>
<td>BK4910 00</td>
<td>2x(80+8 ml)</td>
</tr>
</tbody>
</table>

**ANCILLARY EQUIPMENT**

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

**SAMPLES**

Serum, (heparin or EDTA) plasma. Do not use haemolysed samples because haemolysis can cause falsely positive results. Do not use anticoagulants containing ammonium salts (ex. ammonium heparin).

Loss of activity within 3 days: at 2-8 °C < 8% at 15-25 °C < 10%

Stability at - 20 °C at least 3 months.

**Specimen collection / Preanalytical factors**

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

**INTERNAL QUALITY CONTROL**

It is recommended to use commercial Quality Control sera with known GOT/AST activity. Check that the values obtained are within the reference range provided.

**ANALYTICAL PROCEDURE**

- **Working temperature**: 37 °C
- **Wavelegnth**: 340 nm (334 nm, 365 nm)
- **Optical path**: 1 cm
- **Reaction**: Kinetic (decrease)

Allow the reagents to reach working temperature before using.

**Bireagent procedure**

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Sample</th>
<th>µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1000</td>
</tr>
<tr>
<td>Sample</td>
<td>100</td>
</tr>
</tbody>
</table>

Mix and incubate at 37 °C for 5 minutes, then add:

<table>
<thead>
<tr>
<th>Sample</th>
<th>µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B</td>
<td>100</td>
</tr>
</tbody>
</table>

Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ∆A/min.

**Monoreagent procedure**

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Sample</th>
<th>µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1000</td>
</tr>
<tr>
<td><strong>Incubate at 37 °C for 5 minutes, then add</strong>:</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>100</td>
</tr>
</tbody>
</table>

Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ∆A/min.

**Note**

- Reaction volumes can be proportionally changed.
- For values upper than 440 U/l dilute samples 1+9 with saline solution and multiply result by 10.
CALCULATION OF RESULTS

Activity (U/l) = ∆A/min x factor (f) indicated in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Bireagent procedure</th>
<th>Monoreagent procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>340 nm f = 1905</td>
<td>340 nm f = 1746</td>
</tr>
<tr>
<td></td>
<td>334 nm f = 1945</td>
<td>334 nm f = 1780</td>
</tr>
<tr>
<td></td>
<td>365 nm f = 3529</td>
<td>365 nm f = 3235</td>
</tr>
</tbody>
</table>

Note:
As the factor "f" used to calculate results depends on several variables (wavelength, temperature, sample volume, reaction volume...), it is recommended to use commercial calibration sera to asset the instruments.

REFERENCE VALUES

Male: 10÷50 U/l
Female: 10÷35 U/l

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of two samples at different enzymatic activities. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (U/l)</th>
<th>Within Run</th>
<th>Between Run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SD %CV</td>
<td>SD %CV</td>
</tr>
<tr>
<td>Serum 1</td>
<td>44.7</td>
<td>0.69 1.5</td>
<td>2.23 5.0</td>
</tr>
<tr>
<td>Serum 2</td>
<td>132.4</td>
<td>2.00 1.5</td>
<td>6.74 5.1</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear up to 440 U/l.

Sensitivity
Test sensitivity, in terms of limit of detection, is 1 U/l.

Correlation
A correlation study comparing the present method an a commercial one gave the following results:

y = 1.0457x - 0.8281 U/l  r = 0.9853

Interferences
- Bilirubin > 40 mg/dl
- Triglycerides > 2000 mg/dl
- Ascorbic acid > 30 mg/dl
- Hemoglobin The presence of hemoglobin in serum indicates destruction of erythrocytes with release of AST, producing high interference.

PRECAUTIONS IN USE

Reagent A is harmful (Nocive).
Refer to Safety Data Sheet.
Reagent B is not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

BIBLIOGRAPHY

SCE recommended method for quantitative determination of alanine aminotransferase (ALT) activity in serum and plasma

ORDER INFORMATION

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA4920 00</td>
<td>5x40 + 1x20 ml</td>
</tr>
<tr>
<td>GA4921 00</td>
<td>10x40 + 2x20 ml</td>
</tr>
<tr>
<td>KL4920 00</td>
<td>8x50 + 8x5 ml</td>
</tr>
<tr>
<td>BK4920 00</td>
<td>2x(80+8 ml)</td>
</tr>
</tbody>
</table>

INDICATION

Measurement of the activity of serum aminotransferases (formerly called transaminases) is indicated in the diagnosis of acute hepatic disorders and in monitoring their evolution. As a liver specific enzyme alanine aminotransferase (ALT) is only significantly elevated in hepatobiliary diseases.

METHOD PRINCIPLE

Optimized UV test according to SCE (Scandinavian Committee on Enzymes) recommendations. The principle of the method is based on the following enzymatic reactions:

\[
\text{ALT} \quad \text{L-Alanine + 2-Oxoglutarate} \leftrightarrow \text{L-Glutamate + Pyruvate} \\
\text{LDH} \quad \text{Pyruvate + NADH + H^+} \leftrightarrow \text{L-Lactate + NAD}^+
\]

Decrease in absorbance value at 340 nm, due to the oxidation of NADH to NAD\(^+\), is directly proportional to the AST activity in the sample.

COMPOSITION

**REAGENT A:**
- TRIS 28 mmol/l
- EDTA-Na\(_2\) 5.68 mmol/l
- L-Alanine 284 mmol/l
- LDH \(\geq 1200 \text{ U/l}\)
- Sodium azide 2 g/l
- Nocive \((x_n)\) R28-32; \(S(\frac{1}{2})\) 28-45-60-61

**REAGENT B:**
- 2-Oxoglutarate 68 mmol/l
- NADH 1.12 mmol/l
- Sodium azide 0.095 g/l

PREPARATION OF REAGENTS

**Bireagent procedure:**
The reagents are liquids ready to use.

**Monoreagent procedure:**
Mix 10 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 2 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 5 days at 15-25 °C or 28 days at 2-8 °C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, (heparin or EDTA) plasma. Do not use haemolysed samples because haemolysis can cause falsely positive results. Do not use anticoagulants containing ammonium salts (ex. ammonium heparin).

Loss of activity within 3 days:
- at 2-8 °C < 10%
- at 15-25 °C < 17%

Stability at - 20 °C at least 3 months.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known GPT/ALT activity. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature 37 °C
Wavelength 340 nm (334 nm, 365 nm)
Optical path 1 cm
Reaction Kinetic (decrease)

Allow the reagents to reach working temperature before using.

**Bireagent procedure**

Pipette into disposable or well clean cuvettes:
- Reagent A 1000 µl
- Sample 100 µl
- Mix and incubate at 37 °C for 5 minutes, then add:
  - Reagent B 100 µl
  - Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm.
  - Read absorbance again 1, 2, 3 minutes thereafter.
  - Calculate \(\Delta A/\text{min}\).

**Monoreagent procedure**

Pipette into disposable or well clean cuvettes:
- Working reagent 1000 µl
- Incubate at 37 °C for 5 minutes, then add:
  - Sample 100 µl
  - Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 340 nm.
  - Read absorbance again 1, 2, 3 minutes thereafter.
  - Calculate \(\Delta A/\text{min}\).

Note
- Reaction volumes can be proportionally changed.
- For values upper than 400 U/l dilute samples 1+9 with saline solution and multiply result by 10.
**CALCULATION OF RESULTS**

Activity (U/l) = \(\Delta A/\text{min} \times \text{factor (f)}\) indicated in the following table:

**Bireaegent procedure**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Factor (f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>1905</td>
</tr>
<tr>
<td>334</td>
<td>1945</td>
</tr>
<tr>
<td>365</td>
<td>3529</td>
</tr>
</tbody>
</table>

**Monoreagent procedure**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Factor (f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>1746</td>
</tr>
<tr>
<td>334</td>
<td>1780</td>
</tr>
<tr>
<td>365</td>
<td>3235</td>
</tr>
</tbody>
</table>

**Note:**

As the factor "f" used to calculate results depends on several variables (wavelength, temperature, sample volume, reaction volume...), it is recommended to use commercial calibration sera to asset the instruments.

**REFERENCE VALUES**

- **Male:** 10÷60 U/l
- **Female:** 8÷40 U/l

Each laboratory should establish reference ranges for its own patients population.

**ANALYTICAL PERFORMANCES**

**Precision**

Within-run and between-run coefficients of variation have been calculated on replicates of two samples at different enzymatic activities. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (U/l)</th>
<th>SD</th>
<th>%CV</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>42.7</td>
<td>0.46</td>
<td>1.1</td>
<td>1.55</td>
<td>3.6</td>
</tr>
<tr>
<td>Serum 2</td>
<td>117.2</td>
<td>3.86</td>
<td>3.3</td>
<td>4.16</td>
<td>3.6</td>
</tr>
</tbody>
</table>

**Linearity**

The assay is linear up to 400 U/l.

**Sensitivity**

Test sensitivity, in terms of limit of detection, is 3 U/l.

**Correlation**

A correlation study comparing the present method an a commercial one gave the following results:

\[ y = 0.9061x + 1.9277 \text{ U/l} \quad r = 0.9809 \]

**Interferences**

- Bilirubin > 40 mg/dl
- Triglycerides > 2000 mg/dl
- Ascorbic acid > 30 mg/dl
- Hemoglobin > 150 mg/dl

**PRECAUTIONS IN USE**

**Reagent A is harmful (Nocive).**

Refer to Safety Data Sheet.

Reagent B is not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

**Waste Management**

Please refer to local legal requirements.

**BIBLIOGRAPHY**

**ORDER INFORMATION**

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA4933 00</td>
<td>5x40 + 1x50 ml</td>
</tr>
<tr>
<td>KL4933 00</td>
<td>8x16 + 8x4 ml</td>
</tr>
<tr>
<td>BK4933 00</td>
<td>2x(40+10 ml)</td>
</tr>
</tbody>
</table>

**PREPARATION OF REAGENTS**

**Bireagent procedure:**

The reagents are liquids ready to use.

**Monoreagent procedure:**

Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 5 ml of RB).

**Storage and stability**

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 5 days at 20-25 °C or 30 days at 2-8 °C.

**ANCILLARY EQUIPMENT**

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

**SAMPLES**

Serum or heparinized samples. Avoid use of anticoagulants like oxalate, EDTA and citrate as they inhibit enzyme activity. Do not utilize hemolyzed sample. Carry out the sample after at least 8 hours fasting. Stable 7 days at 2-8 °C.

**Specimen collection / Preanalytical factors**

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

**INTERNAL QUALITY CONTROL**

It is recommended to use commercial Quality Control sera with known ALP activity. Check that the values obtained are within the reference range provided.

**ANALYTICAL PROCEDURE**

**Working temperature** 37 °C

**Wavelength** 405 nm (400-410 nm)

**Optical path** 1 cm

**Reaction** kinetic (increase)

Allow the reagents to reach working temperature before using.

**Bireagent procedure**

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>800 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

Mix and incubate at 37 °C for 5 minutes, then add:

<table>
<thead>
<tr>
<th></th>
<th>Reagent B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 µl</td>
</tr>
</tbody>
</table>

Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 405 (400-410) nm against water. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ∆A/min.
Monoreagent procedure

Pipette into disposable or well clean cuvettes:

<table>
<thead>
<tr>
<th>Sample</th>
<th>µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1000</td>
</tr>
<tr>
<td>Incubate at 37 °C for 5 minutes, then add:</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>20</td>
</tr>
</tbody>
</table>

Mix and incubate at 37 °C. After 1 minute read the absorbance (A) at 405 (400-410) nm against water. Read absorbance again 1, 2, 3 minutes thereafter. Calculate ∆A/min.

CALCULATION OF RESULTS

Activity (U/l) = ∆A/min x 2764

REFERENCE VALUES

Children: 180÷1200 U/l
Adults: 100÷290 U/l

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of two samples at different enzymatic activities. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Within Run</th>
<th>Between Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean (U/l)</td>
</tr>
<tr>
<td>Serum 1</td>
<td>171.4</td>
</tr>
<tr>
<td>Serum 2</td>
<td>449.8</td>
</tr>
</tbody>
</table>

Linearity

The assay is linear up to 2000 U/l.

Sensitivity

Test sensitivity, in terms of limit of detection, is 6 U/l.

Correlation

A correlation study comparing the present method an a commercial one gave the following results:

\[ y = 1.0392x + 3.2788 \quad U/l \quad r = 0.9961 \]

Interferences

- Bilirubin: > 20 mg/dl
- Triglycerides: > 1000 mg/dl

A list of drugs and substances which cause changes in ALP levels or interfere with its measurement can be found in literature².

PRECAUTIONS IN USE

Reagent A is harmful (Nocive).

Refer to Safety Data Sheet.

Reagent B is not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

Triglycerides - L
Enzymatic colorimetric method for the quantitative determination of Triglycerides in plasma and serum
REF GD0815 00 - REF GA4815 00

INDICATION
Triglycerides determination is used for the diagnosis and monitoring of lipidic dysfunction for the evaluation risk of the atherosclerotic disease. Recent studies have demonstrated that high levels of triglycerides, accompanied to an increase of low density lipoproteins (LDL), constitute a particularly elevated risk for "coronary heart disease" (CHD). High triglycerides concentrations are present in several kidney, liver and pancreas diseases. [1, 2]

METHOD PRINCIPLE
Glycerol, released from triglycerides after hydrolysis with lipoproteinlipase, is transformed by glycerolkinase into glycerol-3-phosphate which is oxidized by glycerolphosphate oxidase into dihydroxyaceto ne phosphate and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide oxidizes the chromogen 4-aminophenazone/N-ethyl-methylanilin-propan-sulphonate sodic (ESPT) to form purple quinoneimine whose intensity is proportional to the concentration of triglycerides in the sample.

COMPOSITION
REAGENT A: REF GD0815 00 4 x 100 ml
REF GA4815 00 12 x 50 ml
Good Buffer pH 7.2 50 mmol/L
ESPT 4 mmol/L
ATP 2 mmol/L
Mg++ 2 mmol/L
Lipoproteinlipase (LPL) ≥ 4 kU/L
Glycerol kinase (GK) ≥ 0. 4 kU/L
Glycerolphosphate oxidase (GPO) ≥ 1.5 kU/L
4-Amminoantipirine 0.5 mmol/L
Peroxidase (POD) ≥ 2 kU/L
NaN3 ≤ 0.095 g/L

STANDARD 200 mg/dl: 1 x 5 ml
Glycerol 200 mg/dl
NaN3 ≤ 0.095 g/L
Verified against NIST reference material.

PREPARATION OF THE REAGENTS
REAGENT A and STANDARD are supplied as liquids ready to use.

Storage and stability
Store at 2-8 °C. Do not freeze the reagents!
If absorbance value of REAGENT A is > 0.300 at 546 nm, it is recommended to verify the results before their use.

After opening
- REAGENT A and STANDARD are stable up to the date stated on the label, if contamination and evaporation are avoided.
- The above conditions are valid if the vials are opened just for the time to take the reagent, closed immediately with their cup and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT
- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl (9 g/L) solution

SPECIMENS
Specimens type
Serum, plasma with eparine or EDTA.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS H11-A3.[5] Fasting for at least 10-12 hours is recommended.

Storage and stability

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Samples</th>
<th>Serum/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25 °C</td>
<td>2 days</td>
<td>7 days</td>
</tr>
<tr>
<td>4-8 °C</td>
<td>7 days</td>
<td>at least 1 year</td>
</tr>
</tbody>
</table>

INTERNAL QUALITY CONTROL
It is recommended, in the assay, to use control sera with known Triglycerides values, to check the correspondence of the obtained results with those expected and validate the data.

PROCEDURE
Application sheets for automated systems are available upon request

Working temperature  20-25, 37 °C
Wavelength 550 nm; range 540 - 560 nm
Optical path 1 cm
Reaction End point

Allow reagents to reach working temperature before using.

<table>
<thead>
<tr>
<th>Pipette into cuvettes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Blank</td>
</tr>
<tr>
<td>REAGENT A</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
<tr>
<td>Standard</td>
</tr>
<tr>
<td>Sample</td>
</tr>
</tbody>
</table>

Mix and incubate for 10 min., read standard and sample absorbances against blank. Colour is stable for 1 hour at least.

Reaction volumes can be proportionally changed.

CALCULATION OF RESULT
Utilize the following formula:

\[
\text{Triglycerides} = \frac{\Delta A_{\text{Sample}}}{\Delta A_{\text{Standard/Cal}}} \times \text{Standard/Cal. Conc.} \ [\text{mg/dL}]
\]

For the free glycerol corrected values, subtract 10 mg/dL (0.11 mmol/L) from the value calculated as described above.

Conversion factor

\[
\text{Triglyceries, mg/dL} \times 0.1126 = \text{Triglycerides, mmol/L}
\]
**REFERENCE VALUES** [2]

(On fasting)

**Recommended values:**  
< 200 mg/dL (2.3 mmol/L)

**Upper limit:**  
200-400 mg/dL (2.3-4.5 mmol/L)

**High values:**  
> 400 mg/dL (4.5 mmol/L)

Epidemiological studies have revealed that a combination of triglycerides > 180 mg/dL (> 2.0 mmol/L) in plasma and cholesterol HDL < 40 mg/dL (< 1.0 mmol/L) cause high risk of CHD. Limit levels (> 200 mg/dL) should be evaluated together with others risk factors for CHD. [3]

**Note**  
Each laboratory should establish reference ranges for its own patient population.

**ANALYTICAL PERFORMANCES**

**Imprecision**

<table>
<thead>
<tr>
<th></th>
<th>WHITIN RUN</th>
<th>BETWEEN RUN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SERUM</strong></td>
<td>Level 1</td>
<td>Level 2</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>Mean [mg/dL]</strong></td>
<td>80.4</td>
<td>106</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>1.23</td>
<td>1.94</td>
</tr>
<tr>
<td><strong>CV %</strong></td>
<td>1.53</td>
<td>1.82</td>
</tr>
</tbody>
</table>

**Sensitivity**

Test sensitivity, in terms of detection limit is 1 mg/dL (0.01 mmol/L).

**Measuring range**

The test has been developed to determine Triglycerides concentration from 1 up to 1000 mg/dL (0.01–11 mmol/L). If such range is exceeded, the sample should be diluted 1+4 with NaCl solution (0.9 g/L) and result multiplied by 5.

**Correlation**

A study based comparing this method with GPO-POD method gave the following results:

N = 77  
\[ y = 0.98x + 1.28 \]  
\[ r = 0.993 \]

**Interferences**

Bilirubin does not interfere up to 40 mg/dL, hemoglobin up to 250 mg/dL and ascorbic acid up to 6 mg/dL.

**PRECAUTIONS IN USE**

All reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of the laboratory reagents according to good laboratory practice is recommended. [3]

**Waste Management**

Please refer to local legal requirements.

**REFERENCES**


Edition: 2005/11/22

**Manufacturer:**

**Globe Diagnostics S.r.l.**
Via Galileo Galilei 38
Seggiano di Pioltello (Milan) ITALY
Tel: ++39 02 929189 1 - Fax: ++39 02 929189 39

Legend of the symbols used on the labels:

- CE Mark (requirement of 98/79/CE regulation)
- For in vitro diagnostic use
- Batch code
- Catalogue number
- Storage temperature
- Expiry date (year-month)
- Consult accompanying documents
- Consult operating instructions
- Biohazard
- Manufacturer
- Size
Cholesterol Total - L

Enzymatic colorimetric method for the quantitative determination of total Cholesterol in plasma and serum

REF GD0340 00 - GA4340 00

INDICATION
Cholesterol determination is used for the diagnosis and monitoring of lipid metabolism diseases.

METHOD PRINCIPLE
The measurement is based on the following enzymatic reactions:

\[
\text{CHE} \quad \text{cholesterol esters} + \text{H}_2\text{O} \rightarrow \text{Cholesterol} + \text{fatty acids}
\]

\[
\text{CHOD} \quad \text{Cholesterol} + \text{O}_2 \rightarrow \text{Cholest-4-en-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{hydroxybenzoate} + 4\text{-Amminoantipyridine} \rightarrow \text{red complex} + 4\text{ H}_2\text{O}
\]

The intensity of the red complex is proportional to the total cholesterol present in the sample.

COMPOSITION
REAGENT A: REF GD 0340 00    4 x 100 ml
REF GA 4340 00         12 x 50 ml

- Good Buffer pH 6.7 100 mmol/L
- Cholesterol oxidase (CHOD) > 100 U/L
- Cholesterol esterase (CHE) > 300 U/L
- Hydroxybenzoic acid 20 mmol/L
- 4-Amminophenazone 0.5 mmol/L
- Peroxidase (POD) > 200 kU/L
- NaN₃ ≤ 0.095 g/L

STANDARD 200 mg/dl: 1 x 5 ml
Standard Cholesterol 200 mg/dl verified against NIST reference material.

Warning: the standard is irritant (Xi) R41; S7-16-24-26-39.

PREPARATION OF THE REAGENTS
REAGENT A and the STANDARD are supplied as liquids ready to use.

Storage and stability
Store at 2-8 °C. Do not freeze the reagents!
A slight pink colouring of Reagent A does not interfere with the results. Reagent A has to be limpid, eliminate turbidity, if present.

After opening
- Reagent A and Standard are stable up to the date stated on the label, if contamination and evaporation are avoided.
- The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cup and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT
- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl (9 g/L) solution

SPECIMENS
Specimens type
Serum, plasma (heparin, EDTA).

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS H11-A3. Separate serum from cells as soon as possible. Do not use fluorure, citrate and oxalate as anticoagulant.

Storage and stability
Cholesterol is stable in the samples 6 days at 2-8 °C.

INTERNAL QUALITY CONTROL
It is recommended, in the assay, to use control sera with known Cholesterol values, to check the correspondence of the obtained results with those expected and validate the data.

PROCEDURE
Application sheets for automated systems are available upon request

Working temperature 37 °C
Wavelength 510 nm; Intervallo 500-546 nm
Optical path 1 cm
Reaction End point

Allow reagents to reach working temperature before using.

<table>
<thead>
<tr>
<th>Pipette into cuvettes:</th>
<th>Reagent Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>REAGENT A</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.01 mL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.01 mL</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>0.01 mL</td>
</tr>
</tbody>
</table>

Mix and incubate for 10 min., read standard and sample absorbances against blank. Colour is stable for 1 hour at least, protected from light and at 15-25 °C.

Reaction volumes can be proportionally changed.

CALCULATION OF RESULT
Utilize the following formula:

\[
\text{Cholesterol} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard/Cal}} \times \frac{\text{Standard/Cal. Conc.}}{\text{mg/dL}}
\]

Conversion factor
Cholesterol [mg/dL] x 0.02586 = Cholesterol [mmol/L]
REFERENCE VALUES [3]

Cholesterol values according to a study on a population of adults in absence of coronary disease:

recommended values: < 200 mg/dL (< 5.17 mmol/L)
upper limit: 200-239 mg/dL (5.2-6.2 mmol/L)
high values: ≥ 240 mg/dL (≥ 6.21 mmol/L)

Note
Each laboratory should establish reference ranges for its own patient population.

ANALYTICAL PERFORMANCES

Imprecision

<table>
<thead>
<tr>
<th>WHITIN RUN</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean [mg/dL]</td>
<td>131.82</td>
<td>171.85</td>
<td>325.54</td>
</tr>
<tr>
<td>SD</td>
<td>3.64</td>
<td>2.96</td>
<td>3.43</td>
</tr>
<tr>
<td>CV %</td>
<td>2.76</td>
<td>1.72</td>
<td>1.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BETWEEN RUN</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean [mg/dL]</td>
<td>131.82</td>
<td>171.85</td>
<td>325.54</td>
</tr>
<tr>
<td>SD</td>
<td>1.21</td>
<td>1.03</td>
<td>2.21</td>
</tr>
<tr>
<td>CV %</td>
<td>0.92</td>
<td>0.60</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Sensitivity
Test sensitivity, in terms of detection limit is 4 mg/dL (0.103 mmol/L).

Measuring range
The test has been developed to determine Cholesterol concentration from 4 up to 700 mg/dL (0.103 – 18.1 mmol/L).
If such range is exceeded, the sample should be diluted 1+9 with NaCl solution (0.9 g/L) and result multiplied by 10.

Correlation
A study based comparing this method with an analogous one gave the following results:

N = 60 \ y = 0.95411x + 5.144 \ r = 0.9994

Interferences
Bilirubin does not interfere up to 15 mg/dL, hemoglobin up to 500 mg/dL. No interferences by lipids have been found with triglycerides up to 1000 mg/dL.

PRECAUTIONS IN USE

All reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of the laboratory reagents according to good laboratory practice is recommended.[7]

Waste Management
Please refer to local legal requirements.

REFERENCES
**HDL PRECIPITANT**

**REF GD0370 00**

Liquid HDL precipitant to measure HDL-cholesterol.
Auxiliary reagent of REF GD0370 00

**PRINCIPLE OF THE METHOD**

HDL lipoproteins are assayed, after precipitation of LDL and VLDL lipoproteins with PEG 6000, measuring their content of cholesterol or phospholipids. This measurement seems to be more reliable than high density proteins one.

**COMPOSITION**

Solution of PEG 6000 (4 x 50 ml) at 14.5%; preservatives and surfactant.

Store at 2 - 8°C.

**PROCEDURE**

Pipet into conic test tubes:
0.5 ml serum
0.5 ml precipitating reagent

Mix gently by inversion, wait 5 minutes and centrifuge at 5000 g (3000 RPM) for 20 minutes.

Proceed on supernatant as indicated in the instruction sheets of HDL-Cholesterol-E (REF GD0370 00).

**REFERENCE INTERVALS**

<table>
<thead>
<tr>
<th></th>
<th>low values (high risks)</th>
<th>medium values</th>
<th>high values (low risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-Cholesterol mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>men</td>
<td>&lt; 35</td>
<td>35 - 55</td>
<td>&gt; 65</td>
</tr>
<tr>
<td>women</td>
<td>&lt; 45</td>
<td>45 - 65</td>
<td>&gt; 65</td>
</tr>
</tbody>
</table>

**NOTES**

1. The supernatant should be clear. Carefully remove the supernatant within 15 minutes after precipitation, without disturbing the precipitate, and transfer to clean, dry tube.

**BIBLIOGRAPHY REFERENCES**


**MANUFACTURER:**

Globe Diagnostics S.r.l.
Via Galileo Galilei, 38
20096 Seggiano di Piolello (Milan) ITALY
**ORDER INFORMATION**

**REF** Kit size
GA4325 00 5x50 + 5x50 ml
KL4325 00 4x20 + 4x20 ml

**INDICATION**
Conditions such as parathyroid disorders, neoplasms with or without bone metastasis, myelomas or other bone diseases can cause alterations in calcium levels.

**METHOD PRINCIPLE**
Calcium ions react, in alkaline medium, with O-Cresolphthalein forming a red-violet colour whose intensity is directly proportional to the calcium concentration in the sample.

\[ \text{o-Cresolphthalein} + \text{Ca}^{++} \rightarrow \text{Ca}^{++}\text{OCP complex} \]

Interference by magnesium is eliminated by addition of 8-hydroxyquinoline.

**COMPOSITION**

**REAGENT A:**
Ethanolamine buffer pH 10.7 1 mol/l

**REAGENT B:**
O-Cresolphthalein 0.3 mmol/l
8-hydroxyquinoline 34.5 mmol/l

**STANDARD:**
1x5 ml
\( \text{Ca}^{++} 10 \text{ mg/dl} \)
\( \text{NaN}_3 0.95 \text{ g/l} \)
Verified against NIST reference material.

**PREPARATION OF REAGENTS**

**Bireagent procedure:**
The reagents are liquids ready to use.

**Monoreagent procedure:**
Mix 1 part of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 10 ml of RA + 10 ml of RB).

**Storage and stability**
Store at room temperature (15-25 °C). Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

Working reagent is stable for 1 day if stored at 20-25 °C and 3 days at 2-8 °C.

**ANCILLARY EQUIPMENT**
- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

**SAMPLES**
Serum, plasma (with heparin), 24h urine. Do not use anticoagulants such as EDTA, fluoride or oxalate.
After collection, separate the serum and plasma from the red blood cells as soon as possible to avoid the uptake of calcium by the erythrocytes.
Add 10 ml of concentrated HCl to 24h urine and heat the specimen to dissolve calcium oxalate, dilute 1:3 with distilled water.

**Stability:**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Serum, plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25 °C</td>
<td>7 days</td>
<td>2 days</td>
</tr>
<tr>
<td>2-8 °C</td>
<td>3 days</td>
<td>4 days</td>
</tr>
<tr>
<td>-20 °C</td>
<td>8 days</td>
<td>3 days</td>
</tr>
</tbody>
</table>

**Specimen collection / Preanalytical factors**
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

**INTERNAL QUALITY CONTROL**
It is recommended to use commercial Quality Control sera with known calcium concentration. Check that the values obtained are within the reference range provided.

**ANALYTICAL PROCEDURE**

**Working temperature** 25, 30, 37 °C
**Wavelength** 575 nm (560-590 nm)
**Optical path** 1 cm
**Reaction** End point (increase)

Allow the reagents to reach working temperature before using.

**Bireagent procedure**

<table>
<thead>
<tr>
<th>Pipette into disposable or well clean cuvettes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
</tr>
<tr>
<td>Reagent A</td>
</tr>
<tr>
<td>Distilled H₂O</td>
</tr>
<tr>
<td>Standard</td>
</tr>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>Mix, incubate <strong>1 minute</strong>. Then add:</td>
</tr>
<tr>
<td>Reagent B</td>
</tr>
</tbody>
</table>

Mix and incubate for **5 minutes** at the desired temperature. Read absorbance value (A) of standard and samples against Blank.

**Monoreagent procedure**

<table>
<thead>
<tr>
<th>Pipette into disposable or well clean cuvettes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
</tr>
<tr>
<td>Working reagent</td>
</tr>
<tr>
<td>Distilled H₂O</td>
</tr>
<tr>
<td>Standard</td>
</tr>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>Mix and incubate for <strong>5 minutes</strong> at the desired temperature. Read absorbance value (A) of standard and samples against Blank.</td>
</tr>
</tbody>
</table>

**Note:**
- Reaction volumes may be proportionally changed.
- When plasma or serum concentration exceeds 20 mg/dl sample should be diluted 1:2 with NaCl solution (9 g/l) and the result multiplied by 2.
CALCULATION OF RESULTS

Siero, plasma:
\[
\text{Calcium, mg/dl} = \frac{\text{A sample}}{\text{A standard}} \times 10
\]

Urine (when 24h urine volume in known):
\[
\text{Calcium, mg/24h} = \frac{\text{A sample}}{\text{A standard}} \times 300 \times \frac{1}{24h}
\]

Conversion factor
\[
\text{Calcium [mg/dl]} \times 0.2495 = \text{Calcium [mmol/l]}
\]
\[
\text{Calcium [mg/dl]} \times 0.4990 = \text{Calcium [mEq/l]}
\]

REFERENCE VALUES

Serum-plasma: 8÷10 mg/dl (adults)
Urine 24h: 100÷300 mg/24h (varying according to diet)

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision
Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different calcium concentrations. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (mg/dl)</th>
<th>SD</th>
<th>%CV</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>9.3</td>
<td>0.22</td>
<td>2.4</td>
<td>0.21</td>
<td>2.2</td>
</tr>
<tr>
<td>Serum 2</td>
<td>11.5</td>
<td>0.23</td>
<td>2.0</td>
<td>0.31</td>
<td>2.7</td>
</tr>
<tr>
<td>Serum 3</td>
<td>12.7</td>
<td>0.31</td>
<td>2.4</td>
<td>0.40</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Linearity
The assay is linear up to 20 mg/dl.

Sensitivity
Test sensitivity, in terms of detection limit, is 0.2 mg/dl.

Correlation
A study based comparing this method with a commercial one gave the following results:
\[
y = 0.985x + 0.15 \text{ mg/dl} \quad r = 0.99
\]

Interferences

<table>
<thead>
<tr>
<th>Interference</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>&gt; 40 mg/dl</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>&gt; 30 mg/dl</td>
</tr>
<tr>
<td>Magnesium</td>
<td>&gt; 15 mg/dl</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&gt; 500 mg/dl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&gt; 2000 mg/dl</td>
</tr>
</tbody>
</table>

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of the laboratory reagents according to good laboratory practice is recommended.

As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination.

Only use disposable materials.

BIBLIOGRAPHY

CHOLINESTERASE - L

DGKC kinetic colorimetric method for the determination of Cholinesterase activity in serum and plasma

ORDER INFORMATION

<table>
<thead>
<tr>
<th>REF</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA4975 00</td>
<td>2x40 + 1x20 ml</td>
</tr>
<tr>
<td>KL4975 00</td>
<td>1x48 + 1x12 ml</td>
</tr>
<tr>
<td>BK4975 00</td>
<td>2x(50+13 ml)</td>
</tr>
</tbody>
</table>

INDICATION

The term Cholinesterase indicates a group of enzymes having in common the capacity of hydrolyze choline esters. Cholinesterase is synthesized by the liver, for this reason the determination of its activity is indicated for diagnosis and control of hepatic diseases. Cholinesterase activity results slightly decreased in chronic and acute hepatitis; the decrease results more accentuate in case of hepatic cirrhosis. Decrease in enzyme activity is also observed in case of hepatic cells carcinoma, biliary ducts diseases, chronic infectious, acute paramyeloblastic leukemia and in case of phosphoric ester poisonind (e.g. pesticides).

Elevated values of cholinesterase activity are revealed in case of hepatic steatosis caused by alcoholism without inflammatory activity, and in case of diseases with increased synthesis of albumin (e.g. nephrosis, exudative enterophaty and thyrotoxicosis).

Cholinesterase activity determination must be executed before treating patients with the muscle relaxant succinylthiocholine. If enzyme activity is deceased, both for hepatic damages and genetic causes, this substance can cause prolonged apnea.

METHOD PRINCIPLE

In the present method (DGKC), Cholinesterase catalyzes the hydrolysis of butyrilthiocholine into butyrate and thiocholine. Thiocholine reduces ferricyanide ion into ferrocyanide. Absorbance value at 405 nm decreases proportionally to the enzyme activity in the sample.

COMPOSITION

**REAGENT A:**

- Sodium pyrophosphate 75 mmol/l
- Potassium esacyanoferrate III 2 mmol/l
- Sodium azide 0.095%

**REAGENT B:**

- Good buffer, pH 4.5 25.3 mmol/l
- Butyrilthiocholine 400 mmol/l

PREPARATION OF REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.

Monoreagent procedure:
Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 5 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 3 days if stored at 2-8 °C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, plasma not hemolyzed serum. Do not utilize sodium fluoride as anticoagulant as it inhibits enzyme activity. Immediately separate serum or plasma from erythrocytes as they contain cholinesterase. Cholinesterase activity increases of about 25-30% a day if serum or plasma are in contact with red blood cells.

Stable 1 month at 2-8°C.

Specimen collection / Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known Cholinesterase activities. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

- Working temperature 37 °C
- Wavelength 405 nm (400-410 nm)
- Optical path 1 cm
- Reaction kinetic (decrease)

Allow the reagents to reach working temperature before using.

Bireagent procedure

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>800 µl</td>
<td></td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>1000 µl</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>15 µl</td>
<td></td>
</tr>
<tr>
<td>Mix and incubate 5 minutes at 37 °C, then add:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent B</td>
<td>200 µl</td>
<td></td>
</tr>
<tr>
<td>Mix and incubate 1 minute at 37 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Read initial absorbance and repeat absorbance reading after 1, 2, 3 minutes against blank. Calculate ∆A/minute.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Monoreagent procedure

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td></td>
<td>1000 µl</td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>1000 µl</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>15 µl</td>
<td></td>
</tr>
<tr>
<td>Mix and incubate 1 minute at 37 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Read initial absorbance and repeat absorbance reading after 1, 2, 3 minutes against blank. Calculate ∆A/minute.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CALCULATION OF RESULTS

Cholinesterase U/l = ∆A/min. x 62000
REFERENCE VALUES

Male  5600÷11200 U/l
Female  4200÷10800 U/l

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different enzymatic activity. The obtained results are reported in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (U/l)</td>
<td>SD</td>
</tr>
<tr>
<td>Serum 1</td>
<td>3989</td>
<td>110.3</td>
</tr>
<tr>
<td>Serum 2</td>
<td>2245</td>
<td>40.8</td>
</tr>
<tr>
<td>Serum 3</td>
<td>1848</td>
<td>27.1</td>
</tr>
</tbody>
</table>

Linearity

The assay is linear up to 12000 U/l.

Sensitivity

Test sensitivity, in terms of detection limit, is 120 U/l.

Correlation

A study based comparing this method with a commercial one gave the following results:

\[ y = 1.0448x - 79.36 \text{ U/l} \]
\[ r = 0.9388 \]

Interferences

Hemoglobin  > 200 mg/dl
Bilirubin  > 20 mg/dl
Triglycerides > 1000 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of the laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

1. PASQUINELLI F., Diagnostica e Tecniche di Laboratorio, Rosini Ed. (1979)