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# Uric Acid (Liquid) Reagent Set

## Intended Use
For the quantitative determination of Uric Acid in serum using the Mindray BS-200 analyzer. For in vitro diagnostic use only.

## Clinical Significance
The determination of uric acid in serum is most commonly performed for the diagnosis of gout. Increased uric acid levels are also found in leukemia, polycythemia, familial idiopathic hyperuricemia, and conditions associated with decreased renal function.

## Test Summary
Uric Acid has been determined by phosphotungstate methods,[1] variations of the phosphotungstate method[2] and iron reduction methods.[3,4] The above methodologies are influenced by many substances in their procedures as well as many contaminating substances on glassware, etc.[5] The enzyme Uricase has been widely used for Uric Acid determinations because of its improved specificity.[6,7] Recently, hydrogen peroxide, a by-product of the Uricase-Uric Acid reaction, has been coupled to other enzymatic reactions to yield a colorimetric end product. The present procedure uses the coupling of 4-aminoantipyrine (4-AAP), 2-Hydroxy-3,5-Dichloro-benzenesulfonate (HDCBS), and hydrogen peroxide in the presence of peroxidase to yield a chromagen measured at 520nm.

## Principle
$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{HDCBS} \xrightarrow{\text{POD}} \text{Chromagen} + 4H_2O$$

Uric Acid is oxidized by Uricase to allantoin and hydrogen peroxide. HDCBS + 4-AAP + hydrogen peroxide, in the presence of peroxidase, produces a red chromagen that is measured at 520nm. The absorbance at 520nm is proportional to the concentration of Uric Acid in the sample.

## Reagent Composition
Uric Acid reagent: 4-AAP >0.2mM, HDCBS 2mM, Uricase (Microbial) >150 U/L, Peroxidase (horseradish) >2,500 U/L, Buffer, pH 8.1 ± 0.1, Non-reactive stabilizers.

## Reagent Preparation
The reagent is ready to use.

## Reagent Storage and Stability
The reagent set is stored at 2-8°C. Under proper storage the reagent will remain stable until the indicated expiration date.

## Precautions
1. This reagent set is for in vitro diagnostic use only.
2. The reagent should not be used if: The reagent is turbid or contains obvious microbial growth. The reagent blank has an absorbance of 0.500 or greater at 520nm. A pink color is normal for this reagent.
3. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)[8]

## Specimen Collection and Storage
1. Unhemolyzed serum is recommended.
2. Uric Acid in serum is stable for three days at 2-8°C and up to six months when frozen.[9]
3. Collect specimens per NCCLS document H4-A3.[10]

## Interferences
1. Elevated ascorbic acid levels can result in falsely depressed uric acid values.
2. Lipemic samples may cause falsely elevated uric acid levels.
3. Hemoglobin to 100 mg/dl has been demonstrated to have a negligible effect (<5%) on uric acid values. Hemoglobin greater than 100 mg/dl may cause falsely elevated uric acid values.
4. Bilirubin to 30 mg/dl has been demonstrated to have a negligible effect (<5%) on uric acid results using this method.
5. See Young, et al[11] for other interfering substances.

## Materials Provided
Uric Acid Reagent

## Materials Required but not Provided
1. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

## Mindray BS-200 Test Parameters

| | | | |
|---|---|---|---|
| Test: | UA | R1: | 180 |
| No.: | 032 | R2: | 0 |
| Full Name: | Uric Acid | Sample Volume: | 4 |
| Standard No.: | | R1 Blank: | |
| Reac. Type: | Endpoint | Mixed Rgt. Blank: | |
| Pri. Wave: | 510nm | Linearity Range: | 0.0 – 20.0 |
| Sec. Wave: | 670nm | Linearity Limit: | |
| Direction: | Increase | Substrate Limit: | |
| Reac. Time: | 0 / 19 | Factor: | |
| | | Compensate: Slope 1.0 | Intercept: 0 |
| Incuba. Time: | 0 | □ Prozone check | |
| Unit: | mg/dl | q1: q2: q3: q4: | |
| Precision: | 0.1 | PC: | Abs: |

## Calibration Parameters

| | | | |
|---|---|---|---|
| Rule: | Two-point linear | Calibrator 1: | Deionized Water |
| Sensitivity: | | Calibrator 2: | Chem Cal |
| Replicates: | 2 | Calibrator 3: | |
| Interval (day): | | Calibrator 4: | |
| Difference Limit: | | Calibrator 5: | |
| SD: | | Calibrator 6: | |
| Blank Response: | | | |
| Error Limit: | | | |
| Coefficient: | 0 | | |

## Limitations
1. If the spectrophotometer being used requires a final volume greater than 1.0ml for accurate reading, use 0.075ml (75ul) of sample to 3.0ml of reagent. Perform the test as described above.
2. The procedure described is linear to 20 mg/dl. Samples with values exceeding 20 mg/dl should be diluted 1:1 with saline, re-assayed, and the results multiplied by two.

3. Lipemic samples will give falsely elevated results and a serum blank must be run. Serum Blank: Add 0.025ml (25ul) of sample to 1.0ml water. Zero spectrophotometer with water. Read and record absorbance and subtract reading from test absorbance. Calculate as usual.

## Calibration
Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

## Calculations (Example)
A = Absorbance

$$\frac{A\ (Unk)}{A\ (Std)} \times \text{Conc. of Std (mg/dl)} = \text{Uric Acid (mg/dl)}$$

Example: A (Unk) =0.126, A (Std) = 0.100, Conc. of Std = 5 mg/dl.

Then: $\frac{0.126}{0.100} \times 5 = 6.3$ mg/dl

## SI Units (mM/L)
To convert to mM/L, multiply the result (mg/dl) by 10 to convert dl to L and divide by 168 (the molecular weight of Uric Acid).

Mg/dl x $\frac{10}{168}$ = mM/L  mg/dl x .0595 = mM/L

Example: 6.3mg/dl x .0595 = 0.374mM/L

## Quality Control
Serum controls with known normal and abnormal uric acid values should be run routinely to monitor the validity of the reaction. These controls should be run at least with every working shift in which uric acid determinations are performed. It is strongly recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

## Expected Values
2.5 - 7.7mg/dl[9]
It is strongly recommended that each laboratory establish its own normal range.

## Performance
1. Assay Range: 0 - 20 mg/dl
2. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer and method, resulting in a correlation coefficient of 0.998 and the regression equation was y=1.015x+0.02.
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[12]

| Within Day (n=20) | | | Day to Day (n=20) | | |
|---|---|---|---|---|---|
| **Mean** | **S.D.** | **C.V.%** | **Mean** | **S.D.** | **C.V.%** |
| 6.63 | 0.31 | 4.6 | 7.11 | 0.14 | 2.0 |
| 9.38 | 0.16 | 1.7 | 10.13 | 0.20 | 2.0 |

4. Sensitivity: The sensitivity of this reagent was investigated by reading the change in absorbance at 520nm for a saline sample, and two serum samples with known concentrations. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Uric Acid (Liquid) reagent showed little or no reagent drift on a zero sample. Also, that an absorbance change of 0.015 was approximately equivalent to 1 mg/dl of Uric Acid.

## References
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