For in vitro diagnostic use only.

For Rx use only

ANNUAL REVIEW

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PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Total Protein in human serum on Beckman Coulter AU analyzers. OSR6632 for use on the AU5800, AU2700 and AU5400 systems only.

SUMMARY AND EXPLANATION

Measurements of Total Protein are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic and nutritional disorders.

The total serum protein is the sum of all circulating proteins and is a major component of blood. It is often useful, however, in interpreting the significance of the total protein concentration to have more specific knowledge of individual fractions such as albumins and globulins.¹

METHODOLOGY

This Total Protein procedure is based on the modification of Weichselbaum.² Cupric ions in an alkaline solution react with proteins and polypeptides containing at least two peptide bonds to produce a violet colored complex. The absorbance of the complex at 540/660 nm is directly proportional to the concentration of protein in the sample.

\[
\text{Protein + Cu}^{2+} + \text{OH}^- \rightarrow \text{Blue violet complex}
\]
SPECIMEN

SPECIMEN STORAGE AND STABILITY

Total Protein is stable in serum for one week at room temperature (15 - 25°C) and for one month refrigerated (2 - 8°C). Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum samples, free from hemolysis, are the recommended specimens. Plasma is not recommended since fibrinogen in the sample will add to the protein measured. If plasma must be used, the recommended anticoagulant is heparin.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Total Protein Reagent

Reagent storage location in this laboratory:
WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Sodium hydroxide</td>
<td>200 mmol/L</td>
</tr>
<tr>
<td>Potassium sodium tartrate</td>
<td>32 mmol/L</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>18.8 mmol/L</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>30 mmol/L</td>
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</tbody>
</table>

GHS HAZARD CLASSIFICATION
Total Protein R1

DANGER

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves, protective clothing and eye/face protection.
P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353 IF ON SKIN (or hair): Rinse skin with water.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/physician.

Total Protein R2

DANGER

H314 Causes severe skin burns and eye damage.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
P280 Wear protective gloves, protective clothing and eye/face protection.
P301+P330+P331 IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353 IF ON SKIN (or hair): Rinse skin with water.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/physician.

Sodium Hydroxide 1 - 5%
Copper sulphate 0.5 - 1%

Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Chemistry Calibrator (Cat. No. DR0070)
Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For AU400/400\(^6\)/480, AU640/640\(^0\)/680, AU2700/5400/AU5800 and DxC 700 AU Beckman Coulter Analyzers.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The Total Protein Reagents are ready for use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 - 25°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. Opened reagents are stable for 21 days when stored in the refrigerated compartment of the AU5800 analyzer.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity, precipitate, or any change in color in the Total Protein reagent may indicate degradation and warrant discontinuance of use. R1 should be a clear, colorless solution and R2 should be a clear blue solution.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

Calibration of this total protein procedure is accomplished by the use of the Chemistry Calibrator (Cat # DR0070), which is traceable to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 927a.
Absorption of atmospheric CO₂ by the reagent on board the analyser can impair calibration stability. This effect will vary depending upon the rate of use. Consequently each laboratory should set a calibration frequency in the instrument parameters appropriate to their usage pattern.

Recalibration of this test is required when any of these conditions exist:

1. An observed drift in QC values of > 5%.
2. A change of bottle/Lot number.
3. Major preventative maintenance was performed on the analyzer.
4. A critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU analyzer User Guide/Instructions For Use (IFU). Quality control testing should be performed in accordance with regulatory requirements and each laboratory’s standard procedure.

Location of controls used at this laboratory.

<table>
<thead>
<tr>
<th>CONTROL NAME</th>
<th>SAMPLE TYPE</th>
<th>STORAGE</th>
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TESTING PROCEDURE(S)

A complete list of test parameters and operational procedure can be found in the User Guide/IFU appropriate to the Beckman Coulter AU analyzer.

RESULTS INTERPRETATION

Automatically printed out for each sample in g/dL at 37°C. For SI units (g/L) results must be multiplied by 10.

REPORTING RESULTS

EXPECTED RESULTS
3 years to adult: 6.0 - 8.3 g/dL
Newborns: 4.6 - 7.0 g/dL
Beckman Coulter Determined Reference Range 6.4 - 8.9 g/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Expected reference ranges in this laboratory:

<table>
<thead>
<tr>
<th>INTERVALS</th>
<th>SAMPLE TYPE</th>
<th>UNITS</th>
</tr>
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</table>

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies show that the following substances interfere with this Total Protein procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

- Bilirubin: No significant interference up to 40 mg/dL Bilirubin
- Hemolysis: No significant interference up to 500 mg/dL Hemolysate
- Lipemia: No significant interference up to 1,000 mg/dL Intralipid*

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young for a compilation of reported interferences with this test.

Laboratory specific procedure notes:
PERFORMANCE CHARACTERISTICS

The following data was obtained using the Total Protein Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Total Protein procedure is linear from 3 to 12 g/dL for serum determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

SENSITIVITY

Typical change in absorbance per minute for 1 g/dL of Total Protein is 23.7 mAbsorbance in the AU400/400, 51.3 mAbsorbance in the AU640/640, and 62.5 mAbsorbance in the AU2700/5400/680/480 analyzers.

METHODS COMPARISON

Reference

Patient samples were used to compare this Total Protein Reagent. The table below demonstrates representative performance on AU analyzers.

<table>
<thead>
<tr>
<th>Y Method</th>
<th>DxC 700 AU</th>
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<tbody>
<tr>
<td>X Method</td>
<td>AU5800</td>
</tr>
<tr>
<td>Slope</td>
<td>0.992</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.03</td>
</tr>
<tr>
<td>Correlation Coeff. (r)</td>
<td>0.9988</td>
</tr>
<tr>
<td>No. of Samples (N)</td>
<td>134</td>
</tr>
<tr>
<td>Range (g/dL)</td>
<td>3.5 - 10.5</td>
</tr>
</tbody>
</table>

PRECISION

Reference

Estimates of precision, based on CLSI recommendations, are consistent with typical performance. The within run precision is less than 3% CV and the total precision is less than 4% CV. Assays of control sera were performed and this data reduced following CLSI guidelines above.

<table>
<thead>
<tr>
<th>N = 80</th>
<th>Within-run</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, g/dL</td>
<td>SD</td>
<td>CV%</td>
</tr>
<tr>
<td>3.6</td>
<td>0.02</td>
<td>0.50</td>
</tr>
<tr>
<td>7.3</td>
<td>0.03</td>
<td>0.34</td>
</tr>
<tr>
<td>11.0</td>
<td>0.03</td>
<td>0.26</td>
</tr>
</tbody>
</table>
ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>TP-1U</td>
<td>Total Protein (Serum)</td>
</tr>
</tbody>
</table>

Setting Sheet Footnotes

# User defined

## Lot or Lot + Bottle

† Beckman Coulter System Calibrator Cat No.: DR0070

* Values set for working in g/dL. To work in SI units (g/L) multiply by 10.

‡ Absorption of atmospheric CO2 by the reagent on board the analyser can impair calibration stability. Each laboratory should set a calibration frequency appropriate to their usage pattern.

REVISION HISTORY

Correct error in Spanish Language

Preceding version revision history

Correct error in Spanish Language
REFERENCES


5. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.


8. Data is on file for specific AU analyzers.


Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.