INTRODUCTION AND SUMMARY

Immunoglobulins are an important part of the body’s immune response. The immunoglobulin G (IgG) molecule is composed of two light chains (kappa and lambda) and two gamma heavy chains. Approximately 80% of the serum immunoglobulin is IgG. The IgG’s main function is to resist infectious agents. For IN VITRO DIAGNOSTIC USE.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent, pH 7.6 4 x 20 mL
Tris(hydroxymethyl)aminomethane (100mM)

R2: Antiserum Reagent, pH 7.6 4 x 20 mL
Anti-human IgG goat antiserum (20%)

WARNINGS AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to “Decontamination of Laboratory Sink Drains to Remove Azide Salts,” in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

REAGENT PREPARATION

The reagents are ready to use. They do not need to be reconstituted.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the packaging and bottle labels.

REAGENT STABILITY

Opened reagents can be used for 1 month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate matter in solution is cause to discard.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 700 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and/or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

After drawing blood, allow it to completely coagulate. Centrifuge the coagulated blood and collect the supernatant. The supernatant can be directly used for testing without dilution. Samples may be stored for up to 1 week refrigerated. Serum samples stored for extended periods should be frozen at -20°C.

For storage of samples for more than a few days, use of plastic tubes is recommended instead of glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent 4 x 20 mL
Reagent 2 (R-2) Antiserum Reagent 4 x 20 mL

Materials Required But Not Supplied

Calibrators: K-ASSAY® Multi-Analyte Calibrator, Cat. No. KAI-016C. (6 calibrators containing human serum with known levels of IgG).

Two Reagent Clinical Chemistry Analyzer:

Capable of accurate absorbance readings at 700 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

ASSAY CODE

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 700 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and/or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

After drawing blood, allow it to completely coagulate. Centrifuge the coagulated blood and collect the supernatant. The supernatant can be directly used for testing without dilution. Samples may be stored for up to 1 week refrigerated. Serum samples stored for extended periods should be frozen at -20°C.

For storage of samples for more than a few days, use of plastic tubes is recommended instead of glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent 4 x 20 mL
Reagent 2 (R-2) Antiserum Reagent 4 x 20 mL

Materials Required But Not Supplied

Calibrators: K-ASSAY® Multi-Analyte Calibrator, Cat. No. KAI-016C. (6 calibrators containing human serum with known levels of IgG).

Two Reagent Clinical Chemistry Analyzer:

Capable of accurate absorbance readings at 700 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

ASSAY PROCEDURE

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 717):

Sample 4 µL

• ← R1 (Buffer Reagent) 250 µL

• ← 37°C, 5 min.

• ← R2 (Antiserum Reagent) 250 µL

• ← 37°C, 5 min.

2-point endpoint, 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

<table>
<thead>
<tr>
<th>INSTRUMENT</th>
<th>Roche / Hitachi 717</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPERATURE</td>
<td>37°C</td>
</tr>
<tr>
<td>TEST</td>
<td>(IgG)</td>
</tr>
<tr>
<td>ASSAY CODE</td>
<td>(2 POINT) : (24) - (50)</td>
</tr>
<tr>
<td>SAMPLE VOLUME</td>
<td>(4)</td>
</tr>
<tr>
<td>R-1 VOLUME</td>
<td>(250)</td>
</tr>
<tr>
<td>R-2 VOLUME</td>
<td>(250)</td>
</tr>
<tr>
<td>WAVELENGTH</td>
<td>(700)</td>
</tr>
<tr>
<td>CALIB. METHOD</td>
<td>(NONLINEAR) (1) (6)</td>
</tr>
<tr>
<td>STD.(1) Conc.-POS.</td>
<td>(*1) - (1)</td>
</tr>
<tr>
<td>STD.(2) Conc.-POS.</td>
<td>(*2) - (2)</td>
</tr>
<tr>
<td>STD.(3) Conc.-POS.</td>
<td>(*3) - (3)</td>
</tr>
<tr>
<td>STD.(4) Conc.-POS.</td>
<td>(*4) - (4)</td>
</tr>
<tr>
<td>STD.(5) Conc.-POS.</td>
<td>(*5) - (5)</td>
</tr>
<tr>
<td>STD.(6) Conc.-POS.</td>
<td>(*6) - (6)</td>
</tr>
<tr>
<td>SD LIMIT</td>
<td>(999)</td>
</tr>
<tr>
<td>DUPLICATE LIMIT</td>
<td>(10000)</td>
</tr>
<tr>
<td>SENSITIVITY LIMIT</td>
<td>(0)</td>
</tr>
<tr>
<td>ABS. LIMIT (SLOPE)</td>
<td>(32000)</td>
</tr>
<tr>
<td>PROZONE LIMIT</td>
<td>(320000)</td>
</tr>
<tr>
<td>EXPECTED VALUE</td>
<td>(99999)</td>
</tr>
<tr>
<td>PANIC VALUE</td>
<td>(-99999)</td>
</tr>
<tr>
<td>INSTRUMENT FACTOR</td>
<td>(1.00)</td>
</tr>
</tbody>
</table>

*1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that IgG levels be determined using a multi-point calibration curve prepared using the K-ASSAY® Multi-Analyte Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be performed each day.

QUALITY CONTROL

A quality control program is recommended for all clinical testing and laboratories. It is recommended that control sera, both normal and abnormal, be run with each batch of samples to monitor the procedure.

The values obtained for controls should fall within the
RESULTS / CALCULATIONS

IgG levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The measurable range for this IgG test kit is between 100 mg/dL and 4,500 mg/dL. If IgG concentrations are greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and re-assay. Multiply the result by 5 to compensate for the dilution.

PERFORMANCE

Precision

Precision Assay: Within Run

<table>
<thead>
<tr>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 20</td>
<td>N = 20</td>
<td>N = 20</td>
</tr>
<tr>
<td>Mean = 901</td>
<td>Mean = 1,883</td>
<td>Mean = 2,927</td>
</tr>
<tr>
<td>SD = 16.9</td>
<td>SD = 30.0</td>
<td>SD = 78.1</td>
</tr>
<tr>
<td>CV = 1.9%</td>
<td>CV = 1.6%</td>
<td>CV = 2.7%</td>
</tr>
</tbody>
</table>

Precision Assay: Between Runs

<table>
<thead>
<tr>
<th>Sample I</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 7</td>
<td>N = 7</td>
<td>N = 7</td>
</tr>
<tr>
<td>Mean = 437</td>
<td>Mean = 1,500</td>
<td>Mean = 2,002</td>
</tr>
<tr>
<td>SD = 13.4</td>
<td>SD = 20.9</td>
<td>SD = 28.2</td>
</tr>
<tr>
<td>CV = 3.1%</td>
<td>CV = 1.4%</td>
<td>CV = 1.4%</td>
</tr>
</tbody>
</table>

Accuracy / Correlation

A comparison of this K-ASSAY® IgG Assay and an INCSTAR IgG Test Kit was performed on a Hitachi 704 automated analyzer and a COBAS Mira. The test results provided the following data:

\[
\begin{align*}
\text{y} &= 1.0789x - 88.01 \\
\text{r} &= 0.99322 \\
\text{n} &= 46 \\
\text{x} &= \text{INCSTAR IgG Test Kit} \\
\text{y} &= \text{K-ASSAY® IgG assay} \\
\text{x min} &= 476 & \text{y min} &= 500 \\
\text{max} &= 3,957 & \text{max} &= 4,507 \\
\text{mean} &= 1,542 & \text{mean} &= 1,576 \\
\end{align*}
\]

Assay Range

100 - 4,500 mg/dL

INTERFERENCE

<table>
<thead>
<tr>
<th>Substance</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>No interference up to 20 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>No interference up to 500 mg/dL</td>
</tr>
<tr>
<td>Intralipid</td>
<td>No interference up to 500 mg/dL</td>
</tr>
</tbody>
</table>

EXPECTED VALUE

The expected value is between 806 - 1,813 mg/dL. These values were determined using normal serum from 89 healthy donors. Each laboratory should establish its own expected values using this kit.

REFERENCES


LABELING SYMBOLS

LOT Lot Number

Reagent

Expiration or “Use By” Date

Catalog Number

For In Vitro Diagnostics Use

Temperature Limitation, Store between 2 and 8 degrees C

Manufacturer

Consult Package Insert for Instructions for Use

Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

Advena Ltd.
Tower Business Centre, 2nd Flr., Tower Street, Swatar, BKR 4013 Malta

ORDERING / PRICING / TECHNICAL INFORMATION

KAMIYA BIO MEDICAL COMPANY
12779 Gateway Drive
Seattle, WA 98168 USA
TEL: (206) 575-8068 / (800) 526-4925
FAX: (206) 575-8094