**INTENDED USE**

For the quantitative determination of human haptoglobin in human serum by immunoturbidimetric assay. Haptoglobin is a protein that binds hemoglobin, the oxygen-carrying pigment in red blood cells. Measurement of haptoglobin may aid in the diagnosis of hemolytic diseases (diseases in which the red blood cells rupture and release hemoglobin) relating to the formation of hemoglobin-haptoglobin complexes and certain kidney diseases. For in vitro diagnostic use.

**INTRODUCTION AND SUMMARY**

The K-ASSAY® Haptoglobin assay is intended for the quantitative determination of human haptoglobin by immunoturbidimetric assay. The antiserum used in the kit was produced against purified human haptoglobin. The haptoglobin antibody interacts with the haptoglobin in the serum forming immune complexes. The immune complexes cause an increase in light scattering which correlates with the concentration of serum haptoglobin.

Haptoglobin is a serum protein which binds hemoglobin, the oxygen-carrying pigment in red blood cells. Measurement of haptoglobin may aid in the diagnosis of hemolytic diseases related to the formation of hemoglobin-haptoglobin complexes and certain kidney diseases. Haptoglobin, an acute phase protein, is a potential reporter molecule for glycosylation changes in the serum which may increase in inflammation and liver disease.

Haptoglobin has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay. The K-ASSAY® Haptoglobin assay uses an immunoturbidimetric assay format.

**PRINCIPLE OF TEST**

The K-ASSAY® Haptoglobin assay quantifies the haptoglobin in the patient's serum based on immunoturbidimetric assay.

**PROCEDURE**

**Materials Supplied**

| Reagent 1 (R-1) Buffer Reagent | 3 x 18 mL |
| Reagent 2 (R-2) Antiserum Reagent | 2 x 7 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 haptoglobin).
- Two Reagent Clinical Chemistry Analyzer: Capable of accurate absorbance readings at 600 nm. Capable of accurately dispensing the required volumes.

**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**: 3 µL
- **R1 (Buffer Reagent)**: 250 µL at 37 °C, 5 min.
- **R2 (Antiserum Reagent)**: 70 µL at 37 °C, 5 min.

**Automated Method (Example)**

**Chemistry Parameters for Automatic Analyzer**

**INSTRUMENT**

Roche / Hitachi 717

**TEMPERATURE**

37°C

**TEST**

| ASSAY CODE | (2 POINT) : (24) - (50) |
| SAMPLE VOLUME | (3) |
| R-1 VOLUME | (250) (NO) |
| R-2 VOLUME | (70) (NO) |
| WAVELENGTH | (1600) |
| CALIB. METHOD | (NONLINEAR) (4) (6) |
| STD.(1) Conc.-POS. | (1) |
| STD.(2) Conc.-POS. | (2) |
| STD.(3) Conc.-POS. | (3) |
| STD.(4) Conc.-POS. | (4) |
| STD.(5) Conc.-POS. | (5) |
| STD.(6) Conc.-POS. | (6) |
| SD LIMIT | (999) |
| DUPLICATE LIMIT | (10000) |
| SENSITIVITY LIMIT | (0) |
| ABS. LIMIT (SLOPE) | (32000) (INCREASE) |
| PROZONE LIMIT | (32000) (LOWER) |
| EXPECTED VALUE | (99999) (99999) |
| FANG VALUE | (99999) (99999) |
| INSTRUMENT FACTOR | (1.0) |

*1-6: Input concentration of calibrators.
Parameters for other automated analyzers are available.
CALIBRATION

It is recommended that haptoglobin 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 number / type of other assays being performed. Initially, calibration should be performed each day.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the stated recovery range.

LIMITATIONS OF PROCEDURE

The measurable range for haptoglobin is between 20 to 220 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution.

If the haptoglobin concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 1 part isotonic saline or filtered to compensate for the dilution.

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PERFORMANCE

Precision

The precision for the K-ASSAY® Haptoglobin assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

Precision Assay: Within Run

Sample I | Sample II
---|---
N = 20 | N = 20
Mean = 68.15 | Mean = 366
SD = 0.88 | SD = 5.5
CV = 1.3% | CV = 1.5%

Precision Assay: Between Runs

Sample I | Sample II
---|---
N = 8 | N = 8
Mean = 85.9 | Mean = 225.6
SD = 0.8 | SD = 1.4
CV = 0.972% | CV = 0.624%

Accuracy / Correlation

A comparison of the K-ASSAY® Haptoglobin assay and an Incstar Haptoglobin Test Kit was performed using a Hitachi 717. The test results provided the following data:

\[
\begin{align*}
   y &= 0.937x - 9.25 \\
   r &= 0.993 \\
   n &= 60 \\
   x &= \text{Incstar Haptoglobin Test Kit} \\
   y &= \text{K-ASSAY® Haptoglobin assay} \\
   \min x &= 34 \\
   \min y &= 4 \\
   \max x &= 266 \\
   \max y &= 267 \\
   \text{mean} x &= 109 \\
   \text{mean} y &= 93
\end{align*}
\]

Assay Range

20 - 220 mg/dL (multi-point calibration)

INTERFERENCE

Bilirubin F and C No interference up to 20 mg/dL
Lipemia No interference up to 5%

Haptoglobin binds tightly to free hemoglobin. Use of hemolyzed samples is not recommended as will have a negative effect on this assay's results.

EXPECTED VALUE

The expected value as reported is between 30 to 178 mg/dL. Each laboratory should establish its own expected values using this kit.

REFERENCES


EU AUTHORIZED REPRESENTATIVE

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