

### Quantitative determination of Haptoglobin (HAPTO) IVD

Store 2 - 8°C.

#### PRINCIPLE OF THE METHOD

HAPTO is a quantitative turbidimetric test for the measurement of haptoglobin in human serum or plasma.

Anti-haptoglobin antibodies when mixed with samples containing haptoglobin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the haptoglobin concentration of the patient sample, that can be quantified by comparison from a calibrator of known haptoglobin concentration.

#### CLINICAL SIGNIFICANCE

The haptoglobin is an  $\alpha_2$ -glycoprotein synthesized in the liver that binds hemoglobin irreversibly. The hapto-hemoglobin complexes, as well free haptoglobin itself, play significant roles in the iron storage and prevents of possible renal damage as a consequence of hemoglobin excretion. As an acute-phase protein, haptoglobin is increased in the presence of acute inflammatory process, tissue necrosis or malignancy.

Haptoglobin deficiency in plasma is a consequence of hemolysis "in vivo", presence of estrogens in pregnancy and oral contraceptive therapy, as well as most forms of acute or chronic hepatocellular disease, including viral hepatitis.

Haptoglobin test is mainly used for the determination and monitoring the hemolytic disorders. Under normal circumstances, approximately 1% of circulating red blood cells are destroyed from circulation. An increase of only 2% destruction per day will completely deplete plasma haptoglobin in the absence of a stimulus to production, such acute inflammation or corticosteroids therapy.

#### REAGENTS

<b>R1</b>	Tris buffer 20 mmol/L, PEG 8000, pH 8.2. Sodium azide 0.95 g/L.
<b>R2</b>	Goat serum, anti-human haptoglobin pH 7.5. Sodium azide 0.95 g/L.
<b>Saline Solution</b>	NaCl 9% (for samples predilution)

#### CALIBRATION

The assay has been standardized against the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM).

#### PREPARATION

**Reagents:** Ready to use.

**Samples and Controls Pre-Dilution:** Before the use, samples and/or controls must be diluted in this way:

- 25  $\mu$ L sample or control + 250  $\mu$ L NaCl 9 g/L(1:11)

#### STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use reagents over the expiration date.

**Reagent deterioration:** The presence of particles and turbidity.

Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

#### ADDITIONAL EQUIPMENT

- Pipette 100 - 1000  $\mu$ L
- Pipette 10 - 100  $\mu$ L
- Cuvettes and microstirrers (ref. code ACC16-037)

#### SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 7 days at 2-8°C or 3 months at -20°C.

The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.

#### TEST PARAMETER

Filter: set filter on position B  
Reading time: 120 seconds

#### PROCEDURE

1. Pipette into an appropriate number of cuvettes:

kind of reagent	$\mu$ L
R1	400
Pre-diluted sample or control	50

2. Incubate the cuvettes in positions 1-4 for at least 1 minute
3. Transfer each cuvette in the reading channel and, when requested on the display, add 100  $\mu$ L of R2
4. Read the result which will appear automatically on the reader's display after 120 sec

#### QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Vital Diagnostics SERUM PROT CONTROL BILEVEL (Cod.: ACC16-057) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

#### REFERENCE VALUES<sup>2</sup>

Between 30 - 200 mg/dL. Each laboratory should establish its own reference range.

#### PERFORMANCE CHARACTERISTICS

1. Linearity: Up to 250 mg/dL (Nota 1), under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample / reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. Detection Limit: Values less than 1.3 mg/dL give non-reproducible results.
3. Prozone effect: No prozone effect was detected upon 1200 mg/dL
4. Sensitivity:  $\Delta$  4.69 mA / mg/dL (100 mg/dL).
5. Precision:

	Mean	SD	CV%
Intra-assay (n=10)	95.5	2.54	2.65
Inter-assay (n=10)	95.5	3.48	4.02

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained using the method Immage from Beckman. 73 samples ranging from 10 to 400 mg/dL of Haptoglobin were assayed. The correlation coefficient (r) was 0.95 and the regression equation  $y = 1.086x + 13$ .

The results of the performance characteristics depend on the used analyzer.

#### INTERFERENCES

Hemoglobin (50 g/L), bilirubin (50 mg/dL), rheumatoid factors (950 IU/mL), do not interfere. Lipemia ( $\geq$  6 g/L), interfere. Other substances may interfere <sup>6,7</sup>.

#### NOTES

1. Linearity depends on the calibrator concentration.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

#### BIBLIOGRAPHY

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#### PACKAGING

Ref.: ACC16-008	Cont.	: 1 x 40 mL R1
		: 1 x 10 mL R2
		: 1 x 50 mL Saline Solution