Haptoglobin MonlabTest®

Turbidimetry

Quantitative determination of Haptoglobin (HAPTO)

IVD Only for professional in vitro diagnostic use
Store at 2-8ºC

INTENDED USE

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

PRINCIPLE OF THE METHOD

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 know haptoglobin concentration.

CLINICAL SIGNIFICANCE

The haptoglobin is an α_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 hemolisys "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.
Hepatoglobin test is meanly used for the determination and monitoring the hemolytic disorders. Under normal circumstances, approximately 1% of circulating red blood cells are destroyed every day. If this increases to 2%, it will completely deplete plasma haptoglobin in the absence of production stimulus such as acute inflammation or corticosteroids therapy.

REAGENTS

Diluent (R1)	Tris buffer 20 mmol/L, PEG 8000, pH 8.3. Sodium azide 0.95 g/L..
Antiserum (R2)	Goat serum, anti-human haptoglobin pH 7.5. Sodium azida 0.95 g/L.
Optional	Mutlicalibrator Protein Serum (MO-165044).

CALIBRATION

The assay has been standardized against the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements, IRMM). It must be used the Mutlicalibrator Protein Serum Calibrator to calibrate the reagent. The reagent (both monoreagent and bireagent) should be recalibrated every month, when the controls are out of specifications, and when changing the reagent lot or the instrument settings.

PREPARATION

- Reagents: Ready to use.
- Calibration Curve: Prepare the following Multicalibrator Protein Serum dilutions in ClNa 9 g/L as diluent. Multiply the concentration of the haptoglobin calibrator by the corresponding factor stated in table bellow to obtain the haptoglobin concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC 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

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 340 nm filter (320 – 360 nm).

SAMPLES

Fresh serum. Stable 7 days at 2-8ºC or 3 months at –20ºC.
The samples with presence of fibrin should be centrifuged before testing.
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Do not use highly hemolized or lipemic samples.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength: 340 nm
   Temperature: 37 ºC
   Cuvette ligth path: 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1 (µL)	800
Sample or Calibrator (µL)	10

5. Mix and read the absorbance (A_1) after the sample addition.
6. Immediately, pipette into de cuvette:

Reagent R2 (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

MONLAB has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCULATIONS

Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the haptoglobin concentration of each calibrator dilution. Haptoglobin concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. MONLABTEST Multicontrol Protein Serum (MO-165045) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[2]

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

PERFORMANCE CHARACTERISTICS

1. **Measurement range:** Up to 300 mg/dL 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.

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3. **Prozone effect:** No prozone effect was detected upon 1200 mg/dL
4. **Sensitivity:** Δ 4.96 mA / mg/dL (100 mg/dL).
5. **Precision:** The reagent has been tested for 20 days, using three levels of serum in a EP5-based study.

EP5	CV (%)		
	39.25 mg/dl	97.35 mg/dl	191.5 mg/dl
Total	8%	3.2%	2.3%
Within Run	1.5%	0.9%	1.2%
Between Run	6.7%	2.3%	1.2%
Between Day	4%	2%	1.5%

6. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained using the method from Beckman (System Array 360 CE). 35 samples ranging from 10 to 400 mg/dL of Haptoglobin were assayed. The correlation coefficient (r) was 0.98 and the regression equation y = 0.88x + 4.8.

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 (≥ 6 g/L), interfere. Other substances may interfere [6,7].

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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5. Tarukosky PH et al. Scand J Clin Lab Invest 1966; 18: 80-86.
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PACKAGING

Ref.: MO-165041	R1: 1 x 40 mL R2: 1 x 10 mL

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	Manufacturer	IVD	For i*n vitro* diagnostic use only
	Don't re-use		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by

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