Reactivos GPL

Barcelona, España

CE

- HAPTO -

HAPTOGLOBIN
Turbidimetry

Store at: +2+8ºC.

Presentation:
Cod. IT110 CONT: R1 1 x 40 mL. + R2 1 x 10 mL .

Procedure

Diagnostic reagent for quantitative determination of human haptoglobin (HAPTO).

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
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 know haptoglobin concentration.

COMPOSICIÓN DE LOS REACTIVOS

Diluente (R1)	Tris buffer 20 mmol/L, PEG 8000, ph 8.3 Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human haptoglobin, ph 7.5. Sodium azida 0.95 g/L.
Optional	PROT-CAL. Cod: IT210

REAGENT PREPARATION AND STABILITY
Antibody and diluent are ready to use and stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-*8ºC.*
Do not freeze; frozen reagents could change the functionality of the test.
Signs of reagent deterioration:
- Particles and turbidity indicates contamination or reagents deterioration.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Stored at tightly closed at 2-8ºC. Do not use reagents over the expiration date.

CALIBRATION
The assay is calibrated to the Reference Material CRM 470/RPPHS. It must be used the PROT CAL 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.

SPECIMEN
Fresh serum or plasma. EDTA or heparin should be used as anticoagulant.
Stable 7 days at 2-8ºC or 3 months at –20ºC.
The samples with presence of fibrin should be centrifuged.
Do not use highly hemolized or lipemic samples.

Discard contaminated specimen

MATERIAL REQUIRED BUT NOT PROVIDED
- Thermostatic bath at 37ºC.
- Spectrophotometer capable of accurate absorbance readings at 340 nm (320-360)
- Cuvettes with 1 cm light path.

General laboratory equipment

PROCEDURE
CALIBRATION CURVE:
Prepare the following PROT CAL 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
ClNa 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0,25	0,5	0,75	1,0

MANUAL 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.

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 dilution. haptoglobin concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL
GENERAL PROTEIN CONTROLS Ref.: IT220.

Serum Controls are recommended for internal quality control. 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 and 200 mg/dL.

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE
The haptoglobin is an α-glycoprotein , synthesized in the liver, and it is able to bind irreversibly to hemoglobin. The haptoglobin complexes, and also the free haptoglobin, play an important role in the iron conservation and in the prevention of possible renal damages produced by hemoglobin excretion. Haptoglobin concentration, as an acute phase protein, increases as a result of inflammation, tissue necrosis or neoplasia.Levels of haptoglobin are decreased in plasma as a consequence of *in vivo* hemolysis, presence of estrogens in the pregnancy, the contraceptive therapy, as well as many acute or cronic liver diseases included the acute viral hepatitis.
The haptoglobin assay is mainly used for the determination and monitoring of hemolytic alterations. Under normal circumstances, the 1% of hematies are destroyed. An increase of just 2% of destruction will reduce completely the haptoglobin concentration in absence of production stimulus as inflammations or corticoid therapy.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
- *Linearity:*Up to 300 mg/dL[(note 1,2)].Under the described assay conditions.
- *Limit detection*: Values less than 1.3 mg/dL give non-reproducible results.
- *Prozone effect*: No prozone effect was detected upon 1200 mg/dL.
- *Sensitivity*: Δ 4.96 mA/mg/dL. (100 mg/dL.),
- *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%

- *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.

INTERFERING SUBSTANCES
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. 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, as well the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
3. GPL have instructions for many automatic instruments, available on request.
4. 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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