

Turbidimetric method for haptoglobin quantification in bovine serum samples

Haptoglobin (Hp) is a major acute phase protein in cattle, characterized by a low baseline concentration and a prominent increase following the inflammatory stimuli. Hp is a valuable biomarker in general health screening and animal welfare assessment. It can be used to detect inflammatory or infectious diseases (including subclinical conditions), and to monitor disease progression and treatments efficacy. The main function of Hp is to bind free hemoglobin (Hb) blocking its harmful effects. This binding affects Hp quantification in hemolysed samples by some assays, particularly the Hp-Hb binding assay, however our method is not affected by hemolysis.

Main features

- Automated: easy to adapt to different clinical chemistry analyzers
- Species-specific: antibodies and calibrators specific for bovine
- Not affected by hemolysis, differently to the Hp-Hb binding assay
- Sensitive: allows the quantification of baseline serum Hp concentration.

Analytical principle

Hp from serum reacts with anti-Hp antibodies bound to latex particles. The immuneaggregates formed originate an increase of turbidity in the reaction media which is determined by a measurement of absorbance. The increase of turbidity is proportional to Hp concentration in the sample.

| Type of assay | Particle enhanced turbidimetric immunoasay (latex) | |
|--------------------------|---|--|
| Format | 2 liquid reagents, ready to use | |
| Calibration | Standardized to the European reference serum for acute phase proteins (EU Concerted Action QLK5-CT-1999-0153) | |
| Linear range | 0-400 mg/L | |
| Security range (prozone) | > 2000 mg/L | |
| Interferences | No interferences by hemoglobin (20 g/L), bilirubin (0.150 g/L) or triglycerides (10 g/L, intralipid) | |

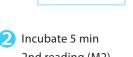
| | Precision* | |
|------------------------|---------------------|----------------------|
| Concentration (g/L) | Within-run CV(%) | Between-day CV(%) |
| 36 | 3.9 | 4.9 |
| 300 | 1.1 | 1.1 |

^{*20} days study in an Olympus AU400 analyzer. Every day samples were analyzed in duplicates, in two runs.

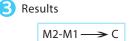
Assay procedure*

1 Add buffer (R1, 240 μl) Add sample (S, 3 μl) Add immunoparticles (R2, 60 μl) 1st reading (M1)

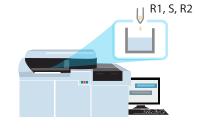
M1: Abs 500nm

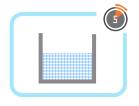


2nd reading (M2)



M2: Abs 500nm







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^{*}Recommended procedure. Volume, time and wavelenght may be adjusted depending on the analyzer features