MUCOPROTEINS

Colorimetric determination of Mucoproteins in serum (Winzler's fraction)

IVD

ORDER INFORMATION

REF	Kit size
GD0650 00	1x120 + 2x100 ml

INDICATION

Mucoproteins are glycoproteins having the principal characteristic to be not precipitated by perchloric acid. The perchloric-soluble fraction represents 1-2% of protein content in serum, mainly constituted by α_1-acid glycoprotein (or orosemucoid). Increased Mucoproteins levels are found during inflammatory, necrotic, and neoplastic processes involving connective-tissues particularly. High concentrations are present in acute articular rheumatism, rheumatoid arthritis, myocardial infarction, tuberculosis, acute glomerulonephritis, several tumors and malignant hemopathyes. Determination of Mucoproteins concentration in serum has a relevant diagnostic significance in case of icterus, consenting to distinguish obstructive icterus, causing increased levels, from hepatocellular icterus causing decreased levels.

METHOD PRINCIPLE

Mucoproteins are separated from other proteins by precipitation with Perchloric acid and then detected with Coomassie colouring.

Mucoproteins + Coomassie → Blue dye

COMPOSITION

REAGENT A:

Perchloric acid	750 mmol/l
Irritant	**R 36-38; S(1/2-)23-26-36-45**

REAGENT C:

Brillant Blu R Coomassie	0.5%

STANDARD:	1x5 ml
Stabilized proteic solution	125 mg/dl (5 mg/dl Tirosine)

Preparation

Reagents are liquids ready to use.

Storage and stability

Store at room temperature (15-25 °C). Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Centrifuge tubes
- Centrifuge (5000 RPM)
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution 9 g/l

SAMPLES

Serum, stable 1 day at 2-8 °C or 15 days at -20 °C (freeze only one time).

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial control sera with known proteins concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Mucoproteins separation

1. Pipette in a centrifuge tube 100 µl of serum and 400 µl of Reagent A, shake until complete homogenization.
2. Incubate for 20 minutes at room temperature.
3. Centrifuge at 5000 rpm for 20 minutes.
4. Recover the supernatant.

Determination of mucoproteins in the supernatant

Allow the reagents to reach working temperature before using.

	Blank	Standard	Sample
Reagent A	100 µl	-	-
Standard	-	100 µl	-
Supernatant	-	-	100 µl
Reagent C	1000 µl	1000 µl	1000 µl

Mix and incubate for **5 minutes** at **room temperature** (20-25 °C). Read the absorbance (A) of the standard and supernatants at **620 (600-640) nm** against Blank. Colour is stable for 60 minutes.

CALCULATION OF RESULTS

Utilize the following formula:

$$\text{Mucoproteins, mg/dl} = \frac{\text{A sample}}{\text{A standard}} \times 125$$

$$\text{Mucoproteins in Tirosine, mg/dl} = \frac{\text{A sample}}{\text{A standard}} \times 5$$

Conversion factor

Mucoproteins (mg/dl) : 25 = Tirosine (mg/dl)
Tirosine (mg/dl) x 25 = Mucoproteins (mg/dl)

Note:

1. To keep the appropriate variability of the method, it is suggested to accurately respect the assay times indicated in the present insert.
2. The obtained values are not comparable with those obtained measuring α_1-acid glicoprotein.

REFERENCE VALUES

Mucoproteins	< 125 mg/dl
Tirosine	< 5 mg/dl

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of two in house controls at different Mucoproteins concentrations. The obtained results are reported in the following tables:

Within-run

Control	n	Mean (mg/dl)	SD	CV%
Level 1	10	37.12	1.55	4.2
Level 2	10	68.70	3.70	5.4

Between-run

Control	n	Mean (mg/dl)	SD	CV%
Level 1	5	36.60	2.40	6.6
Level 2	5	70.00	5.83	8.3

Linearity

The assay is linear up to 350 mg/dl.

Sensitivity

Test sensitivity, in terms of limit of detection, is 5 mg/dl.

Interferences

No interferences have been found.

PRECAUTIONS IN USE

Reagent A is irritant (Xi). Refer to Safety Data Sheet.
Reagent C and Standard are not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

If glassware and apparatus are lightly blue due to the Reagent C, clean them with acidified water.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

1. PASQUINELLI F., Diagnostica e Tecniche di Laboratorio, Rosini Ed. (1979)
2. HENRY R.J., Clinical Chemistry, Principles and Technics, Harper and Ron Pub. (1974)
3. WINZLER R.J., J. Clin. Invest. 27:609 (1948)
4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
5. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC