TRANSFERRIN

Diagnostic reagent for determination of Transferrin concentration.
Liquid. Dual reagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-432	80 mL
MH-433	54 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is for the quantitative determination of Transferrin analyte in human serum and plasma.

GENERAL INFORMATION

Transferrin (originally named siderophilin) is the major plasma transport protein for iron (Fe^{+3}). It has a molecular weight of 79.6 kDa including 5.5% carbohydrate. It is a single polypeptide chain with two N-linked oligosaccharides and two homologous domains, each with a Fe^{+3} binding site. It is essentially synthesized in the liver and circulates with a half-life of 8 to 10 days. Transferrin reversibly binds two ferric (Fe^{+3}) ions with high affinity at physiological pH but lower affinity at low pH, which allows the release of iron in intracellular compartments. After cellular delivery of iron via receptor-mediated endocytosis, apotransferrin is recycled back into the circulation. There are few clinical indications for direct measurement of transferrin. Indirect assessment of transferrin concentration can be done by total iron binding capacity (TIBC).[1] Since only one-third of the iron-binding sites of transferrin in normal subjects are occupied by Fe^{+3}, serum transferrin has a significant iron-binding capacity. Serum transferrin or TIBC changes in iron metabolism disorders. Transferrin or TIBC is decreased in types of anemia with iron overload and in those with hereditary hemochromatosis. Transferrin is also decreased in conditions of impaired synthesis, such as chronic liver disease and malnutrition, and in conditions of increased losses, such as nephrotic syndrome. As transferrin is increased in people with iron deficiency, it is decreased in those with chronic inflammatory diseases or malignancies as it is a negative acute phase protein.[2] For this reason, some consider transferrin to be the best test to distinguish between iron deficiency anemia and anemia of chronic disease.[3] However, it is not very sensitive in detecting iron deficiency in hospitalized patients.[4,5]

TEST PRINCIPLE

Immunoturbidimetric assay

Transferrin in the sample precipitates in the presence of anti-human transferrin antibodies.

This precipitation formed by the antigen-antibody complex depends on the concentration of transferrin and is measured turbidimetrically at a wavelength of 540 nm.

REAGENT COMPONENTS

Reagent 1:

Imidazole buffer	:< 0.12 mol/L,
Goat anti-human transferrin antibodies	: ≤ %50
Sodium azide	: % 0.1

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[6]

SAMPLE REQUIREMENTS

Collected by standard procedure serum, lithium heparin plasma or potassium EDTA plasma can be used. Multiple sample freezing and thawing should be avoided.

Transferrin activity stability in serum and plasma:

8 hours +20/+25ºC
7 days +2/+8ºC
1 month -20ºC

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Protein Calibrator.

Protein Calibrator- Lyophilized
Ref.No: VT-012

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Protein Control Level I- Lyophilized
Ref.No: VT-013

Protein Control Level II- Lyophilized
Ref.No: VT-014

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

The expected value range is 200 - 360 mg/dL.[16]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[7]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[8]

The determined analytic measuring interval for Transferrin is 10-700 mg/dL.

Detection Capability

Limit of Detection (LoD): 5 mg/dL

Limit of Quantitation (LoQ): 10 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[9]

Linearity

This method shows measurement linearity in the activities up to 700 mg/dL.

Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[10]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[11]

Repeatability (Within Run) and Repeatability (Day to Day) CV% values of Transferrin have been given in the table 1 and 2 respectively.

Table 1. Transferrin Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
167 mg/dL	3.01	1.80	80
394 mg/dL	5.10	1.29	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[12]

Table 2. Transferrin Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
167 mg/dL	6.01	3.60	80
394 mg/dL	9.46	2.40	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[12]

Interference

Endogenous interferant and analyte concentrations that have been used in the Transferrin scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[13,14]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Transferrin interference scanning test is appropriate, is determined as ±10%.[15]

In Transferrin test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Bilirubin	: ≤ 20 mg/dL
Rheumatoid Factors	: ≤ 300 IU/mL
Lipemia	: ≤ 7.5 g/L
Hemoglobin	: ≤ 10 g/L

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[14]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD**:** For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests