Soluble Transferrin Receptor (sTfR) - Test System

Intended Use: The Quantitative Determination of sTfR Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Chemiluminescence.

2.0 SUMMARY AND EXPLANATION OF THE TEST

The soluble transferrin receptor (sTfR) has been introduced as a promising new diagnostic tool for differentiating between iron deficiency anemia (IDA) and anemia of chronic disease (ACD). The circulating sTfR concentration is proportional to cellular iron stores and increases with increased cellular iron needs and cellular proliferation. Furthermore, sTfR levels may be a good estimate of body iron compared with the sTfR to ferritin ratio. Distinguishing between IDA and ACD is a key step for determining whether iron supplementation would be beneficial.

As well as helping identify iron deficiency, sTfR is useful for monitoring erythropoiesis in malignancy and chronic renal disease. Development of erythropoiesis following bone marrow or stem cell transplantation is correlated with the overall marrow recovery, and which can be monitored with sTfR. Further, during the aplastic period prior to transplantation, sTfR levels decline. Once erythropoiesis has been restored, sTfR levels increase. In anemia of chronic renal failure, the early increase in sTfR level is independent from the suppression of iron absorption. However, concurrent sTfR and ferritin values, and has been suggested as a good estimate of body iron stores. In anemia of chronic renal failure, the early increase in sTfR level is independent from the suppression of iron absorption.

4.0 REAGENTS

Materials Provided:
- A. sTfR Calibrators – 0.5ml/vial - Icons A-F
- B. sTfR Tracer Reagent – 12.0 ml/vial
- C. Signal Reagent A – 7.0ml - Icon C
- D. Signal Reagent B – 7.0ml - Icon C
- E. Wash Buffer – 40 ml - Icon F
- F. Signal Reagent C – 0.1ml - Icon C
- G. H2O2 – 1.0ml - Icon F
- H. Microplate (8 x 125 well)
- I. User-supplied reagents

Precautions:
- Avoid extended exposure to heat and light.
- Note 1: Do not use reagents beyond the kit expiration date.
- Note 2: Avoid extended exposure to heat and light. Opened reagents are for 60 days after storing at 2-8°C. Kit and component stability are identified on the label.
- Note 3: Above reagents are for a single 96-well microplate.

4.1 Required but not provided:
- 1. Microplate reader for delivering 0.010ml (10µl) with a precision of better than 1.5%.
- 2. Dispenser(s) for repetitive deliveries of 0.10 and 0.350ml (100 & 350µl)
- 3. Microplate washer or a squeeze bottle (optional).
- 5. Absorbent Paper for cleaning the microplate wells.
- 6. Plastic wrap or microplate cover for incubation steps.
- 7. Vacuum aspirator (optional) for wash steps.
- 8. Timer.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HIV Antibody, by FDA licensed testing. A slight excess of solution is made. Discard the unused portion if not used within 36 hours after mixing. If complete utilization of the reagents is not achieved, the kit components must be discarded.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, calibrators and controls to room temperature (20 ± 2°C).

1. Format the microplates’ wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate (low and high). Using an unshielded microwell strips into the aluminum bag, seal and store at 2-8°C.
2. Weigh 0.010ml (10µl) of the appropriate serum reference calibrator into the assigned well.
3. Add 0.100ml (100µl) of the sTfR Biotin Reagent A to all wells.
4. Add 0.200ml (200µl) of the sTfR Biotin Reagent B to all wells.
5. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times.
8. Add 0.100ml (100µl) of Anti-sTfR Tracer Reagent to all wells.
9. Incubate 30 minutes at room temperature.
10. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times.
12. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section). Always reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE PLATE AFTER SIGNAL ADDITION

The relative light units (RLUs) in each well will be read within 3 minutes (180 seconds) of signal addition. The results should be read within thirty (30) minutes of signal addition.

14. Read the relative light units (RLUs) in each well for 0.2 – 1.0 seconds. The results should be read within thirty (30) minutes of signal addition.

15. The results should be read within thirty (30) minutes of signal addition.

0.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of sTfR in unknown samples.

1. Record the RLUs obtained from the printout of the luminometer.

2. Plot the RLUs for each duplicate serum sample versus the corresponding sTfR concentration in nmol/L on linear graph paper.

3. Draw the best-fit curve through the plotted points.

4. The concentration of sTfR in the unknown is the point on the curve which is equal to the concentration of sTfR in the unknown.

5. Locate the average of the RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the sTfR concentration in nmol/L on the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average 0.0221 of the unknown intersects the calibration curve at 17.3 nmol/L sTfR concentration (See Figure 1).
2. Four out of six quality control pools should be within the established criteria should be met:

In order for the assay results to be considered valid the following must be strictly followed to ensure compliance and proper device usage.

11.0 PERFORMANCE CHARACTERISTICS

14.3 Specificity

The % cross reactivity of the sTfR antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of sTfR needed to displace the same amount of labeled analog.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. Highly lipemic, hemolized or grossly contaminated specimen(s) should not be used.

4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

5. If the within and between assay precision of the sTfR AccuLite® CLIA Test System were determined by analyses on three different laboratories, the expected ranges for the sTfR AccuLite® CLIA Test System are detailed in Table 1.

6. Plate readers measure vertically. Do not touch the bottom of the wells.

7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

8. Use components from the same lot. No intermixing of reagents from different batches.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established ranges.

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. ‘Heterophilic antibodies: a problem for all immunoassays’ Clin. Chem. 1988;3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.

6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 15% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a “normal” adult population, the expected ranges for the sTfR AccuLite® CLIA Test System are detailed in Table 1.

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of “normal” persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and reasons, each laboratory should depend upon the range of values estimated by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.4 Precision

The within and between assay precision of the sTfR AccuLite® CLIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

15.0 REFERENCES


Revision: 2 Date: 2019-Jul-16 DCO: 1353 MP8675 Product Code: 8675-300

TABLE 1

<table>
<thead>
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<th>Substance</th>
<th>Cross Reactivity</th>
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<tbody>
<tr>
<td>Human Differictransferrin</td>
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</tr>
<tr>
<td>Human Apotransferrin</td>
<td>ND</td>
</tr>
<tr>
<td>Human Heart Ferritin</td>
<td>ND</td>
</tr>
<tr>
<td>Human Liver Ferritin</td>
<td>ND</td>
</tr>
<tr>
<td>Human Spleen Ferritin</td>
<td>ND</td>
</tr>
<tr>
<td>Trockin</td>
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</tr>
<tr>
<td>Human Serum Albunin</td>
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</tr>
<tr>
<td>Bilubin</td>
<td>ND</td>
</tr>
<tr>
<td>Hemoglobin</td>
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</tr>
</tbody>
</table>

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<th>Sample</th>
<th>96(A)</th>
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TABLE 4

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
</tr>
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<tbody>
<tr>
<td>Human Differictransferrin</td>
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