i-CHROMA™ TSH

ImmunoAssay for Quantitative Measurement of TSH in Human Serum/Plasma with i-CHROMA™ Reader System

INTENDED USE

i-CHROMA™ TSH test along with the i-CHROMA™ Reader is a fluorescence immunoassay that quantifies Thyroid Stimulating Hormone (TSH) concentration in human serum or plasma. The test is useful in the diagnosis of thyroid or pituitary disorders.

INTRODUCTION

The determination of serum or plasma levels of thyroid stimulating hormone (TSH or thyrotropin) is recognized as an important measurement in the assessment of thyroid function. Thyroid stimulating hormone is secreted by the anterior lobe of the pituitary gland, and induces the production and release of thyroxine (T4) and triiodothyronine (T3) from the thyroid gland. It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta. Although the concentration of TSH in the blood is extremely low, it is essential in the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The opposite action will occur when there are decreased levels of thyroid hormones in the blood. This process, known as a negative feedback mechanism, is responsible for maintaining the proper blood levels of these hormones.

The i-CHROMA™ TSH Test measures quantitatively TSH concentration in human Serum/plasma.

PRINCIPLE

The test uses a sandwich immunodetection method, such that the detector antibody in buffer binds to TSH in blood sample and antigen-antibody complexes are captured to another TSH antibody that has been immobilized on test strip as sample mixture migrates nitrocellulose matrix. Thus the more TSH antigen in blood, the more antigen-antibody complexes accumulated on the test strip. Signal intensity of fluorescence on detector antibody reflects the more TSH antigen captured and is processed by i-CHROMA™ Reader to show TSH concentration in specimen. The working range of i-CHROMA™ TSH test is 0.1 ~ 100 µIU/mL.

<table>
<thead>
<tr>
<th>Adults</th>
<th>TSH(µIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-54 years</td>
<td>0.4-4.2</td>
</tr>
<tr>
<td>55-87 years</td>
<td>0.5-8.9</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
</tr>
<tr>
<td>1st Trimester</td>
<td>0.3-4.5</td>
</tr>
<tr>
<td>2nd Trimester</td>
<td>0.5-4.6</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td>0.8-5.2</td>
</tr>
</tbody>
</table>

* Reference Value

MATERIALS PROVIDED

BodiTech Med Incorporated i-CHROMA™ TSH Catalog [REF] Catalog No. 13017

kit contains:
- Test Device: 25T/box
- Detection Buffer: 1vial (2ml)
- Sample Mixing Tube: 25T/box
- ID Chip: 1ea/box
- Insert sheet: 1

MATERIALS REQUIRED BUT NOT PROVIDED

i-CHROMA™ Reader [REF] Catalog No. FR-203
- Thermal Printer
- Transfer pipette (75µL size)

COMPOSITION OF REAGENTS

The i-CHROMA™ TSH consists of Test Device, Detection Buffer, and ID Chip. Test Device is individually sealed with a desiccant in aluminum pouch. Detection Buffer is packed and delivered separately from Test Device in a styrofoam box filled with ice pack.

- Test Device contains a test strip in which mouse anti-TSH antibody and streptavidin have been immobilized on the test and the control line of strip, respectively.
- Detection Buffer, dispensed in a vial, contains fluorescencelabeled anti-human TSH antibody, fluorescence-biotin labeled BSA, gelatin as a stabilizer, and sodium azide as a preservative in PBS.

WARNINGS AND PRECAUTIONS

- IVD For In Vitro Diagnostic Use.
- Carefully follow the instructions and procedures described in this insert. [REF] Catalog No. 13017
- Don’t use Test Device if its lot # does not match with ID chip # that is inserted onto the instrument.
- The i-CHROMA™ TSH is only operational in the i-CHROMA™ Reader. And tests should be applied by trained staff working in the laboratories where the sample(s) is taken by qualified medical personnel.
- Neither inter-change materials from different product lots nor use beyond the expiration date. The use of medical device beyond expiration date may affect on test result.
- The i-CHROMA™ TSH Test Device should remain in its original sealed pouch until ready to use. Do not use the Test Device if the pouch is damaged or the seal is broken. Discard after single use.
- The i-CHROMA™ TSH Test Device and Reader should be used away from vibration and magnetic field. During normal usage, i-CHROMA™ TSH may introduce minute vibration, which should be regarded normal.
- Use separate clean pipette tips and sample vials for different specimens. The pipette tips and sample vials should be used for one specimen only. Discard after single use.
**STORAGE AND STABILITY**

- Store the Detection Buffer in a refrigerator at 2°C - 8°C. The Detection Buffer is stable up to 20 months if stored in a refrigerator.
- Once removed from refrigerator, allow the Detection Buffer for 20 minutes to return to room temperature before testing.
- Store i-CHROMA™ TSH Device at 4°C-30°C in its sealed pouch. i-CHROMA™ TSH Device is stable for 20 months (while in the sealed pouch) if stored at 4°C-30°C.
- If stored in a refrigerator, allow a minimum of 20 minutes for the Test Device to reach room temperature while it is in the sealed pouch.
- Do not remove the device from the pouch until ready to use. The Test Device should be used immediately once opened.
- The storage and shipping of test kit should be complied as indicated in manual. However, it is remotely possible that only part of test kit is affected by stability problems.

**SAMPLE COLLECTION AND PREPARATION**

The test can be performed with either serum or plasma.

- For serum sample, collect the blood in a tube without anticoagulant and allow to be clotted. Remove the serum from the clot as soon as possible to avoid hemolysis. For plasma sample, collect the blood in a tube treated with EDTA. Anticoagulants other than EDTA for plasma specimen have not been evaluated. If testing cannot be conducted within an hour after preparation of specimen, the serum/plasma should be stored at -20°C until tested. In case of use, apply it immediately after specimen was taken.
- The specimen must be at room temperature and be homogeneous before testing. Frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing. If specimens are to be shipped, they should be packed in compliance with regulations.
- It is recommended to avoid using severely hemolyzed specimens whenever possible. If a specimen appears to be severely hemolyzed, another specimen should be obtained and tested.

**ASSAY PROCEDURE**

- Image of the test kit

**RESULT**

The i-CHROMA™ Reader calculates TSH test results automatically and displays concentration of TSH in blood sample on the LCD as form of µIU/mL. For further information, refer to the Operation Manual for the i-CHROMA™ Reader.

**QUALITY CONTROL**

- A quality control test should be performed as a part of good testing practice, to confirm the expected QC results, to confirm the validity of the assay, and to assure the accuracy of patient results. QC specimens should also be run whenever there is any question concerning the validity of results obtained. Upon confirmation of the expected results, the test device is ready to use with patient specimens. Control standards are not provided with this test kit. If you want to perform QC of test kit, we recommend using BodiTech Med’s control reagent. For information about obtaining the controls, contact BodiTech Med Incorporated’s Technical Services for assistance.

- i-CHROMA™ TSH contains internal procedure control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was properly inserted and read by i-CHROMA™ Reader. An invalid result from the internal control causes an error message on i-CHROMA™ Reader indicating that the test should be repeated.

**LIMITATIONS OF THE PROCEDURE**

- Blood specimens, used test devices, pipette tips and sample vials are potentially infectious. Proper laboratory safety techniques, handling and disposal methods should be followed in accordance with standard procedures and relevant regulations observed by microbiological hazard materials.
- The test will be applied on a routine basis and not in emergency situations.
- Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.

**i-CHROMA™ TSH**

- Set a Test Device on a dust-free clean place.
- Check/insert ID Chip onto the instrument. Make sure that the Test Device lot # matches with ID Chip #.
- Take out the vial of Detection Buffer from refrigerator and leave it at room temperature.
- Take 150 µL(75 µL * 2) of serum/plasma or control with a transfer pipette and put it into Sample Mixing Tube.
- Add 75µL Detection Buffer to the Sample Mixing Tube containing serum/plasma or control.
- Mix well the specimen with Detection Buffer by pipette.
- Take 75 µL of sample mixture and load it onto the well of disposable Test Device.
- Leave the Test Device at room temperature for 12 min before inserting the device into the holder.
- Insert Test Device onto the holder of i-CHROMA™ Reader. And press “SELECT” button. Make sure direction of Test Device and push the device back all the way.
- Read the results on the display screen of i-CHROMA™ Reader.

➤ Refer to i-CHROMA™ Reader Operation Manual for the complete instructions on use of the Test.
The results of \textit{i-CHROMA}™ TSH should be evaluated with all clinical and laboratory data available. If TSH Test results do not agree with the clinical evaluation, additional tests should be performed.

The false positive results include cross-reactions with some components of serum from individual to antibodies; and non-specific adhesion of some components in human blood that have similar epitopes to capture and detector antibodies. In the case of false negative results, the most common factors are: non-responsiveness of antigen to the antibodies by that certain unknown components are masking its epitope, such that antigen cannot be seen by the antibodies; instability of TSH antigen, resulting in degradation with time and, or temperature, such that they become no longer recognizable by antibodies; and degraded other test components. The effectiveness of the test is highly dependent on storage of kits and sample specimens at optimal conditions.

Plasma using anticoagulants (e.g. heparin or citrate) other than EDTA has not been evaluated in \textit{i-CHROMA}™ TSH and thus should not be used.

Other factors may interfere with \textit{i-CHROMA}™ TSH and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.

\section*{PERFORMANCE CHARACTERISTICS}

\subsection*{1. Analytical Sensitivity}
Analytical sensitivity was calculated by Average of 10 specimens (0 at value) ± 3SD. Analytical sensitivity of \textit{i-CHROMA}™ TSH Test was determined by testing 10 times each using 3 lots of reagents. Analytical sensitivity of \textit{i-CHROMA}™ TSH was 0.1 µIU/mL.

\subsection*{2. Specificity}
Other bio-molecules, such as LH (300mIU/mL), FSH (200mIU/mL), and hCG (200,000 mIU/mL) were added to test specimen with much higher level than their physiological level in normal blood. There was no significant interference with the TSH measurement, nor was there any significant assay cross-reactivity with those bio-molecules tested.

\subsection*{3. Hook Effect}
No high dose hook effect is observed in this assay at TSH concentrations up to 500 µIU/mL.

\subsection*{4. Precision}
For the intra-assay imprecision, 10 replicates were tested at each control sample. For the imprecision evaluation, tests were conducted on 10 sequential days with 5 replicates and for 3 persons at each TSH concentration.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Sample (µIU/ml) & Intra-assay Mean (µIU/ml) & CV(%) & Inter-assay Mean (µIU/ml) & CV(%) \\
\hline
0.25 & 0.27 & 17.9 & 0.21 & 18.7 \\
0.5 & 0.46 & 11.0 & 0.45 & 12.8 \\
2 & 2.02 & 5.7 & 2.05 & 2.5 \\
5 & 5.20 & 3.4 & 4.91 & 2.8 \\
20 & 19.76 & 3.4 & 19.95 & 2.7 \\
50 & 50.01 & 3.7 & 50.20 & 4.9 \\
\hline
\end{tabular}
\caption{Intra- and inter-assay precision}
\end{table}

\subsection*{5. Comparability}
TSH concentrations of 65 clinical specimens were quantified independently with \textit{i-CHROMA}™ TSH test system and Beckman coulter Access2 automatic analyzer according to established standard test procedure. Test result was compared and their compatibility was investigated with linear regression and correlation of coefficient ($R$). Linear regression and correlation of coefficient were $Y=1.0086X + 0.5462$ and $R=0.983$, respectively.

\section*{REFERENCES}

BodiTech Med’s express and implied warranties (including implied warranties of merchantability and fitness) are conditional upon observance of BodiTech Med’s published directions with respect to the use of BodiTech Med’s products.

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