INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY
Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).
ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.
Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.
The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the MPL2 microplate luminometer from Berthold Detection System, Lus2 microplate luminometer from Anthos, Centro LB960 microplate luminometer from Berthold Technologies, and Platelumino from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

INTRODUCTION OF FSH IMMUNOASSAY

Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback relationship.

FSH is a glycoprotein secreted by the basophilic cells of the anterior pituitary. Gonadotropin-release hormone (GnRH), produced in the hypothalamus, controls the release of FSH from anterior pituitary. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar structurally, therefore the biological and immunological properties of each are dependent on the unique beta subunit.

In the female, FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The LH produced then binds to the theca cells and stimulates steroidogenesis. Increased intraovarian estradiol production occurs as follicular maturation advances, thereupon stimulating increased FSH receptor activity and FSH follicular binding. FSH, LH, and estradiol are therefore intimately related in supporting ovarian recruitment and maturation in women.

FSH levels are elevated after menopause, castration, and in premature ovarian failure. The levels of FSH may be normalized through the administration of estrogen, which demonstrate a negative feedback mechanism. Abnormal relationships between FSH and LH, and between FSH and estrogen have been linked to anorexia nervosa and polycystic ovarian disease. Although there are significant exceptions, ovarian failure is indicated when random FSH concentrations exceed 40 mIU/ml.

The growth of the seminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH. However, androgens, unlike estrogen, do not lower FSH levels, therefore demonstrating a feedback relationship only with serum LH. For reasons not only fully understood, azospermic and oligospermic males usually have elevated FSH levels. Tumors of the testes generally depress serum FSH concentrations. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis.

PRINCIPLE OF THE TEST

Diagnostic Automation FSH Quantitative Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a polyclonal anti-FSH antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-FSH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies.
After a 60 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Luminometer. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of FSH in the sample. By reference to a series of FSH standards assayed in the same way, the concentration of FSH in the unknown sample is quantified.

**Materials Provided with Test Kit**

1. Antibody-coated microtiter wells, 96 wells per bag.
2. Reference standard set, contains 0, 3, 10, 30, 60 and 120 mIU/ml (WHO, 2nd IRP, 78/549) human FSH, liquid, ready to use.
3. Enzyme conjugate reagent, 12.0 ml.
4. Wash Buffer Concentrate(50X), 15 ml
5. Chemiluminescence Reagent A, 6.0 ml
6. Chemiluminescence Reagent B, 6.0 ml

**Materials Required but not Provided**

1. Distilled water.
2. Precision pipettes: 50 - 200µL, 1.0ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Chemiluminescence Reagents A and B.
5. Luminometer.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

**STORAGE**

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.

**SPECIMEN COLLECTION & PREPARATION**

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

**REAGENT PREPARATION**

1. All reagents should be allowed to reach room temperature (18-22°C) before use.
2. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.
ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50μl of standard, specimens, and controls into appropriate wells.
3. Dispense 100μl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer.
8. Strike the wells sharply onto absorbent paper to remove residual water droplets.
9. Dispense 100 μl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
10. Read wells with a chemiluminescence microwell reader 5 minutes later. (Between 5 and 20 min., after dispensing the substrates).

Important Note:
The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU readings.

CALCULATION OF RESULTS
1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or quadratic regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against FSH concentration in mIU/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of FSH in mIU/ml from the standard curve.

EXAMPLE OF STANDARD CURVE
Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

<table>
<thead>
<tr>
<th>FSH (mIU/ml)</th>
<th>Relative Light Units (RLU) (10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.008</td>
</tr>
<tr>
<td>3.0</td>
<td>1.620</td>
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<tr>
<td>10</td>
<td>6.680</td>
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<tr>
<td>30</td>
<td>23.82</td>
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<tr>
<td>60</td>
<td>44.47</td>
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<tr>
<td>120</td>
<td>76.06</td>
</tr>
</tbody>
</table>

EXPECTED VALUES AND SENSITIVITY:
It is important for each laboratory to establish the normal range limits. The following normal range should be considered as a guideline only:
The minimum detectable concentration of FSH by this assay is estimated to be 2.5 mIU/ml.

**LIMITATIONS**

There are some limitations of the assay.

As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background reading.

**REFERENCES**

2. Cohen K.L. Metabolism 1977; 26:1165