

## An overview of electrochemiluminescent (ECL) technology in laboratory investigations

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The last years have seen the development and refinement of many new immunoassay measurement principles and systems<sup>1, 2</sup>. The major trend has been away from liquid phase assays with radio isotopic labels, and toward fast solid-phase assays based on monoclonal antibodies<sup>3</sup>. This development is moving further towards precise and reliable non-isotopic, automated or semi automated assays with detection limits measured in the picomolar ( $10^{-12}$ ) and attomolar ( $10^{-18}$ ) range<sup>4</sup>. Electrochemiluminescence (ECL) has been developed as a highly sensitive process in which reactive species are generated from stable precursors (i.e. the ECL – active label) at the surface of an electrode<sup>5</sup>. This new technology has many distinct advantages over other detection systems as shall be discussed later.

### ECL Assay Principles

Electrochemiluminescence (ECL) processes are known to occur with numerous molecules including compounds of ruthenium, osmium, rhenium or other elements. ECL is a process in which highly reactive species are generated from stable precursors at the surface of an electrode. These highly reactive species react with one another producing light<sup>6</sup>. The development of ECL immunoassays is based on the use of a ruthenium chelate as the complex for the development of light. The chemiluminescent reactions that lead to the emission of light from the ruthenium complex are initiated electrically rather than chemically. This is achieved by applying a voltage to the immunological complexes (including the ruthenium complex) that are attached to Streptavidin – coated micro particles. Streptavidin, isolated from *Streptomyces avidinii* is preferred to avidin in this biotin- mediated immunoassay since it has an affinity for biotin comparable to that of avidin, is less basic and had no carbohydrate residues, thus limiting non - specific reactions with acidic groups and lectins<sup>7</sup>. The advantage of electrically initiating the chemiluminescent reaction is that the entire reaction can be precisely controlled.

### Test Principles

Three test principles are used for the estimation of analytes and antibodies in the samples:

- Competitive principle for extremely small analytes
- Sandwich principle for larger analytes and
- Bridging principle to detect antibodies in the sample<sup>8</sup>.

### Competitive Principle

This principle is applied to analytes of low molecular weight, such as FT<sub>3</sub>, FT<sub>4</sub>, Cortisol, Testosterone, Estradiol and others. The sequences of the reactions involved are given below with FT<sub>3</sub> as an example.

- In the first step, sample and a specific anti – T<sub>3</sub> antibody labeled with a ruthenium complex are combined in the assay cup.
- After the first incubation, biotinylated T<sub>3</sub> and Streptavidin- - coated paramagnetic micro particles are added. The still free binding sites of the labelled antibody become occupied with the formation of an antigen – hapten complex. The entire complex is bound to the micro particle via interaction of biotin and streptavidin.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell. The immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by a system buffer.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is indirectly proportionately to the amount of antigen in the patient sample.
- Evaluation and calculation of the concentration of the antigen (FT<sub>3</sub>) are carried out by means of a calibration curve that was established using standards of known antigen concentration.

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### **Sandwich Principle**

The sandwich principle is applied to high molecular weight analytes such as, Thyroid stimulating hormone (TSH), Follicle stimulating hormone (FSH), Luteinizing hormone (LH) and others. The sequence of reactions involved is given below with TSH as an example.

- In the first step, patient sample is combined with a reagent containing biotinylated TSH antibody and a ruthenium- - labelled TSH – specific antibody in an assay cup. During a nine – minute incubated step, antibodies capture the TSH present in the sample.
- In the second step, streptavidin – coated paramagnetic particles are added. During a second nine- minute incubation, the biotinylated antibody attaches to the streptavidin – coated surface of micro particles.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by the system buffer.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of TSH present in the sample.
- Evaluation and calculation of the concentration of the antigen are carried out by means of calibration curve that was established using standards of known antigen concentration.

### **Bridging Principle**

The bridging principle is similar to the sandwich principle, except that the assay is designed to detect antibodies, not antigens (e.g. IgG, IgM and IgA). This is accomplished by including biotinylated and ruthenium – labelled antigens in the reagents for which the targeted antibody has the affinity. The sequence of reactions involved is given below.

- In the first step, serum antibodies bind with the biotinylated and ruthenium – labelled antigens to form an immune complex.
- The immune – complex then reacts with streptavidin – coated micro particles via the biotinylated antigen.

- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but the unbound reagent and the sample are washed away by a system buffer.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of analyte in the sample.
- Evaluation and calculation of the concentration of the antibody are carried out by means of calibration curve that was established using standards of known antibody concentrations.

### **Advantages of ECL Technology <sup>8</sup>**

Electrochemiluminescence (ECL) detection technology promises scientists new “yardsticks” for quantification. It is a highly innovative technology that offers distinct advantages over the other detection techniques <sup>5, 8</sup>.

- Extremely stable non – isotopic label allows liquid reagent convenience
- Enhanced sensitivity in combination with short incubation times means high quality assays and fast result turnaround
- Large measuring range of five orders of magnitude minimizes dilutions and repeats, reduces handling time and repeat costs
- Applicable for the detection of all analytes providing a solid platform for menu expansion.

### **ECL Technology in Nepal**

Roche – Hitachi has launched its fully automated analyzer operating on ECL technology (ElecSys 2010) in the Department of Clinical Biochemistry at the College of Medical Sciences, Bharatpur, Chitwan, Nepal. Hormone assays (FT<sub>4</sub>, FT<sub>3</sub>, TSH, FSH, LH, Prolactin, Insulin, Cortisol), Tumour markers (PSA, CA- 125, CEA, CA-15-3) and cardiac markers are regularly been done. The sensitivity, reliability and reproducibility of the results are found to be excellent.

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