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SPR Biosensor for Early and Rapid Detection of Procalcitonin, D-dimer and Vitamin-D from Human Plasma / Serum by using Mini VIDAS System

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Abstract: **The biomerieux minividas chemiluminescence immunoassay is a modernized, reliable, and unbeatable technique which is used to quantify the Procalcitonin, D-dimer and Vitamin-D from the human serum sample. For most of the patient's symptoms are non specific. The immunoassay (RIA) methods results are showing the significant inconsistency, lead to miscalculation in the immunoassay methods when compared with the reference method. LIA, ELFA, ECLIA and SRP immunosorbent assay methods are highly effective, compared with qualitative immunoassay. Hence, the SPR biosensor is a high sensitivity pipetting device, applied to recognize the sepsis, severe sepsis, thrombosis (DIC, DVT and pulmonary embolism), rickets, deficiency or insufficiency of vitamin D.**

Keywords: **Procalcitonin, sepsis, D-dimer, DIC, pulmonary embolism, Vitamin D, rickets and SPR.**

1. INTRODUCTION

Procalcitonin:

PCT is a prohormone of calcitonin, where as CT (calcitonin) is secreted by C-cells of the thyroid gland and neuroendocrine cells, CALC-1 gene located on chromosome 11 and the mRNA product is known as procalcitonin, these molecule further modified in to 116 amino acid residuces of procalcitonin. Further, it is divided into 3 distinct molecules; active calcitonin (32 amino acids), katacalcitonin (21 amino acids) and N-terminal procalcitonin (57 amino acids).

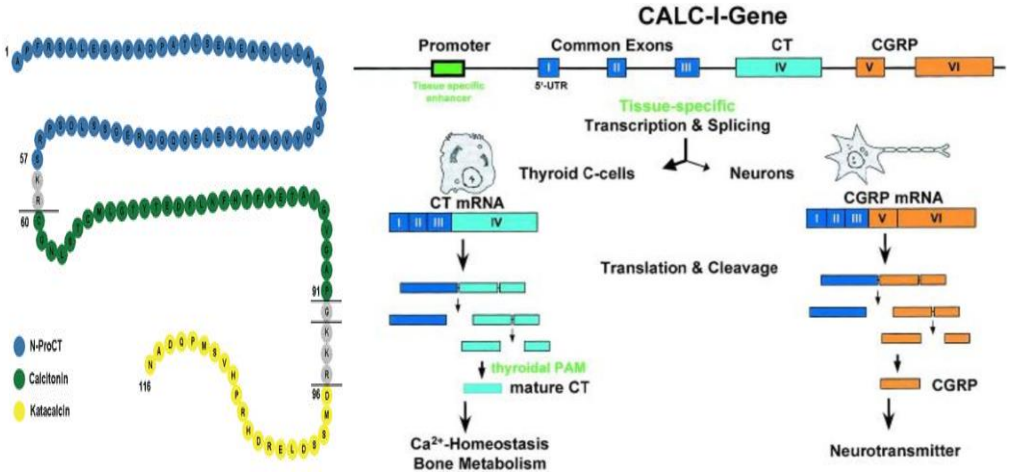
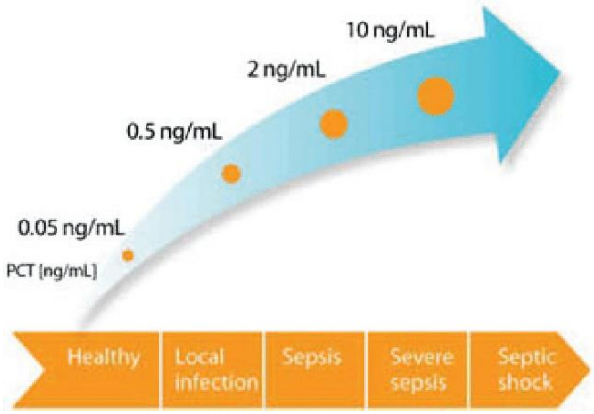


Figure 1: Structure and gene assembly of procalcitonin

The quantification of procalcitonin are based on the immunometricassay principle, luminometric immunoassay (LIA), ELFA (enzyme-linked fluorescent assay) and ECLIA (electrochemiluminescent immunoassay) are used in the automated platforms of BioMérieux VIDAS system. The polyclonal sheep or calf anti-calcitonin antibody is immobilized on a solid phase and a monoclonal gold-conjugated mouse-anti-katacalcin antibody is used as a tracer in the soluble phase. In this study the serum was used. The serum of the sample dispersible the tracer antibody when implemented to the given test area and proceeds into the test area. The antibody-antigen complex becomes detectable when it is bound by the immobilized anti-calcitonin antibody in the area of the test-strip, where it develops a colour change at concentrations above 0.5 ng /ml.

Procalcitonin is secreted mostly by two different process, lipopolysaccharide induced pathway (virulent substance or metabolite from microbes) and inflammatory mediator molecules inducing another pathway. Procalcitonin reference value is generally low in the viral infections, chronic inflammatory disorders or autoimmune processes. Procalcitonin reference values more in sepsis (0.5-2 ng/ml) and frequently influence the values between 10 and 100 ng/mL, or substantially above in individuals. Sepsis is the main reason for the development of multiple organ failure and mortality in Intensive Care Unit (ICU). Sepsis can be difficult to distinguish from non infections condition. Early diagnosis and antibacterial therapy are very important. Patients, who are doubtful to have these conditions, usually undergo blood cultures before the initiation of the therapy. Procalcitonin is a pro peptide of calcitonin, has recently been identified as a marker of severe sepsis, septic shock and non-viral infections. The different types of bacteria, virus, fungi and parasites can cause sepsis, but the bacterial infection is most common. During sepsis, the microorganisms entered in to the blood stream, cells under goes continuous proliferation, increasing the number of microorganisms and releasing the effective virulent factors into the blood stream. Clinical symptoms of sepsis include tachycardia, tachypnea, fever and leucocytosis.



D-Dimer:

D-dimer are particularly degraded the proteolytic components from the peptide molecule and liberated in to free molecule in the circulatory blood system. D-dimer otherwise called as fragment D-dimer or fibrin degradation product, a small protein (subunit or fragment) found in the blood, after blood coagulates is degraded by fibrinolysis. During the coagulation of blood, fibrinogen is converted in to fibrin by thrombin activation. D-dimer indicates the existence of stabilized fibrin. The D-dimer fragment is the end product of this process .Fibrin made up of two subunits (D- and E-units). Fibrin monomers self assembled into fibers, with growth of fibrin via end-to-end and side-to-side association of molecules. These peptide molecules are covalently cross-linked with factor XIIIa (outer D subunit or domains of adjacent fibrin monomers) and the middle E domain (3^{rd} fibrin subunit or monomer molecule). The structure modified within fibrin in which 2 D domains or subunits are covalently linked .Even if FbDP's differ in size, they are identified by the presence of one or more D-dimer subunit. Quantification of D-dimer assays is based on the luminance technology used to identify the disseminated intravascular coagulation (DIC), deep venous thrombosis (DVT) and pulmonary embolism. The assay principle combines a two-step enzyme immunoassay (antigen-antibody–anti antibodies are labeled with enzyme) with a end fluorescent detection **(**ELFA**).** The Solid Phase Receptacle (SPR) is made of polypropylene and contains ten well; it is act as a measuring device. Since the 1960s, clinicians have been measuring the products of plasmin action on fibrin, in the form of fibrinogen degradation products, as an indicator of intravascular fibrinolysis. Initial use of the test was to assist in the evaluation and monitoring of patients with disseminated intravascular coagulation. In the 1990s, it was turned out to be useful in diagnosis of thromboembolism, the present study to evaluate the value of biochemical markers of the acute phase reaction (D-dimers, white blood cell [WBC] count, C-reactive protein [CRP], fibrinogen) in the detection of acute

AD. The clinical conditions associated with elevated levels of D-dimer are numerous. Some of these include thrombosis (arterial or venous), pulmonary embolism, venous thrombosis, disseminated intravascular coagulation, myocardial infarction, stroke, postoperative state, liver disease, malignancy and pregnancy.

Vitamin D:

Vitamin D otherwise called as the Sun Vitamin is a secosteroid prohormone. The prohormone activates the various gene and express the metabolic products to carried out the different function in the cells, those functions are: proliferation, neuromuscular cells development, increasing the immune response, reduction of inflammation, increasing the intestinal absorption of calcium, magnesium and phosphate. The minimal or higher amounts of vitamin D are cause the diseases like rickets, osteoporosis, osteopenia, 17 varieties of cancers are breast, prostate , lung, thyroid, ovarian and colon .etc.**,** heart disease, high blood pressure, obesity, metabolic syndrome , diabetes, autoimmune diseases**,** secondary hyper-parathyroidism, multiple sclerosis**,** erectile dysfunction, dementia and seasonal affective disorder.

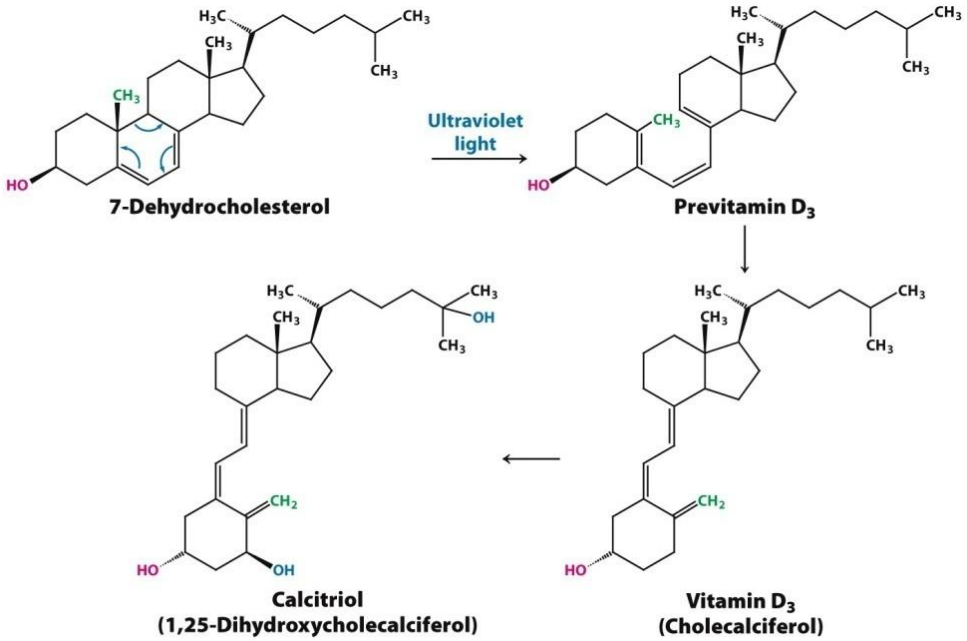


Figure 26.32
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There are mainly two forms of vitamin D. Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D ($C_{27}H_{43}OH$) is produced in the skin of animals when the ultra violet light energy is recognized by a messenger molecule -7-dehydrocholesterol. The enzyme 25-hydroxylase secreted from liver tissue, the substrate cholecalciferal is hydroxylated in to 25-hydroxycholecalciferol, then 1-alpha-hydroxylase is a cytochrome P450 enzyme encoded by the CYP27B1 gene from the kidney cells. This enzyme hydroxylate the substrate 25-hydroxycholecalciferol in to 1, 25-dihydroxycholecalciferol. The intermission time of 25-hydroxycholecalciferol is several weeks, while that of 1, 25-dihydroxycholecalciferol (vitamin D3 biologically) is only a few hours. More amounts of vitamin D3 are present in the fatty fish (cod, Sardines, Salmon, Mackerel, Tuna and Caviar). Small amounts of vitamin D2 are present in eggs, milk, dairy products, orange juice, soy milk, cereals, mushrooms and vegetables. The vitamins D2 and D3 are used for clinical supplementary to human and the molecule are properly metabolized by the body. High-performance liquid chromatography, LC-MS/ MS, radioimmunoassay and chemiluminescent immunoassay which are applied to quantified the amount of vitamin D from the human serum sample.

Principle of a biomerieux Minividas Chemiluminescence Immunoassay:

The Solid Phase Receptacle is invariable accessory material for the test. The chemical substance is freshly prepared and pre allocate in the sealed reagent strips. the test reaction is automatically processed by mini vidas immunological analyzer with user friendly software. In and out of regularly repeated mechanisms of SPR test performed regularly, unbounded chemical ingredients and biological molecules are removed through cleaning buffer and the end of this reaction step is 4-Methyl-umbelliferyl phosphate cycled in and out of the SPR. Then, hydrolysis of this substrate molecule into a chemiluminance product by immobilized enzyme, the 4-Methyl-umbelliferone chemiluminance is detected at 450 nm.

The capability of the chemiluminance based on the absorption of alkaline phosphatase present on the SPR that modify the substrate. At the end, product outcomes are automatically detected by the analyzer. For some assay method have two identification steps, First step for antigen detection, the SPR is commonly coated on the interior with capture antibody or sometimes with byproducts of the chemical substance. For antibody identification, the SPR is labeled with an encapsulated antigen or antibody carried out to the antigen. Depending on the assay methods, the conjugate can be byproducts of the chemical substance or an antibody labeled with alkaline phosphates' enzyme.

2. MATERIALS AND METHODS

Quantification of Procalcitonin, D-dimer and Vitamin D:

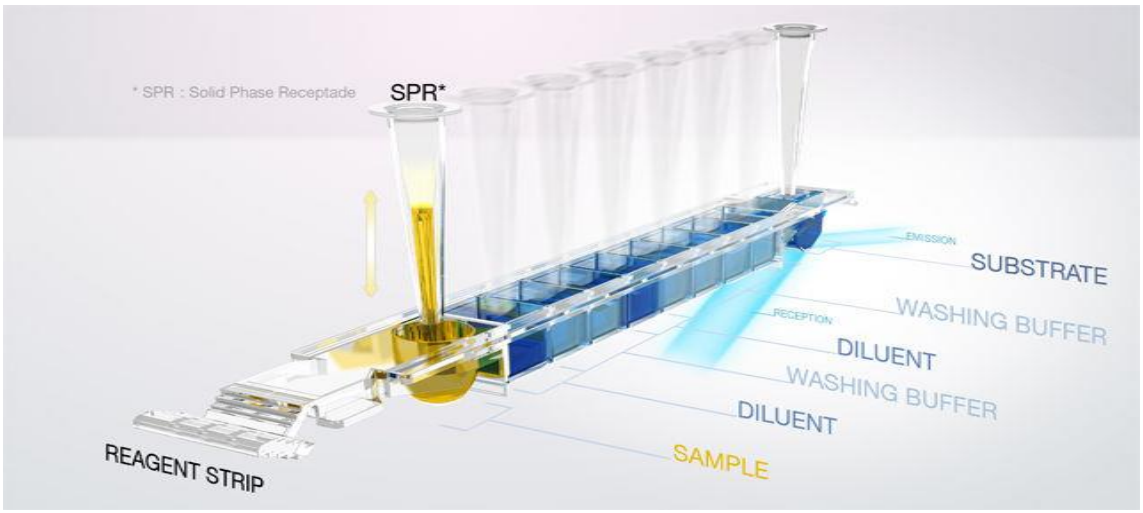
The serum sample collected under the good laboratory practices. The test samples were collected quickly. The collected sample is stable for 5 to 7 days at 2C. If the collected sample unable to quantify within twenty four hours, freeze until the test can be perform. Allow the sample to reach at room temperature before testing. The sample was collected by using micropipette, add 200 µl of sample into the first well of the SRP strip and read the results.

Biomerieux Minividas Chemiluminescence Immunoassay kit:

The biomerieux minividas chemiluminescence Immunoassay kit contains everything required to carry out a specific assay, single or dual reagent strips, SPRs (Solid Phase Receptacle), standard/calibrator(s), controls, diluents.

Single Reagent Strip:

Single reagent strip is made of polypropylene material with ten wells. The sample is loaded in the first well. Conjugate, diluents, and wash buffer solution are already sealed in the second well in to eighth well. The chemiluminance of the substrate is measured finally (10th well), the SRP is properly loaded in the guided channel.



SPR:

The polystyrene device (SPR) is able to grabbing the soluble proteins, viruses and bacteria. It's labeled with a color-coded, bar-coded dot drill in the middle. SPR vial is not re usable. The SPR is invariable for the immunological response. SPR interior walls are labelled with antibody or antigens that encounter the target substance. The target analyte from the sample binds to the SPRs interior coating (antibody or antigen) to form a (sandwich) or antigen -antibody-anti antibody labeled complex. The reactants alter the hydrolysis state of the molecule into a luminous final product .The SPR is used to pipette out the samples and mixed the reagent well and perform the following operations sampling, incubation, mixing, washing. The beveled edge of the SPR allows it to pierce the protective seal that covers the wells in a single reagent strip. The polypropylene strip tray, enters in and out of liquids to be transferred from one well to another well.

Loading reagent strips and SPR:

Lift the reagent strip, and insert it into a proper test position, slide the reagent strip into the section channel, open the SPR block door, place the SPR in the SPR block position straightly over the single reagent strip, continue for the steps for the further strips and SPRs to be loaded, Close the SPR block door and the reagent strip tray cover.

Immunological analyzer -Minividas System:

3. RESULTS AND DISCUSSIONS

The SPR biosensor is a high sensitivity device, which used to quantify the biomarkers procalcitonin, D-dimer and vitamin D from the patient's serum sample by Using Mini VIDAS System, collect the recorded sample data and tabulated to identified the bacterial infections from one patient -sepsis, sever sepsis in two patients, All negative results were observed from the D-dimer test, Vitamin D deficiency was identified from two patients and insufficiency of vitamin D from one patient. The regular standard laboratory parameters and physical investigation in many cases failed to demonstrate the diagnosis of sepsis, particularly in emergency patients (ICU). During the more severe conditions in our study there is very good association between bacteremia and high PCT-serum level. PCT-Q test is useful for early positive result.

Quatification of Procalcitonin by SPR biosensor:

S.No.	Age (Years)	Gender	Procalcitonin, Healthy <0.05ng/ml, Localinfection(0.05-0.5ng/ml), Sepsis (0.5-2.0ng/ml), Sever sepsis (2.0-10ng/ml), Septic shock >10ng/ml
1	34	Female	0.02
2	67	Male	3.09
3	44	Male	0.01
4	16	Male	0.02
5	23	Male	0.01
6	09	Female	0.03
7	11	Male	1.34
8	34	Female	0.01
9	49	Male	0.04
10	71	Female	2.76

Quantification of D-dimer by SPR biosensor:

S. No.	Age (Years)	Gender	D-dimer , (< 500ng/ml –negative), (> 500ng/ml –positive)
1	73	Female	0.25
2	50	Female	0.21
3	41	Female	7.2
4	40	Male	0.09
5	52	Male	0.17
6	65	Male	0.07
7	37	Female	1.3
8	55	Male	0.27
9	72	Male	6.4
10	39	Male	0.13

Quantification of Vitamin D by SPR biosensor:

S. No.	Age (Years)	Gender	Vitamin D, Deficiency < 20ng/ml, Insufficiency 20-29 ng/ml, Sufficiency 30-100 ng/ml, Toxicity >100ng/ml
1	44	Female	43.3
2	38	Male	17.3
3	47	Male	36.8
4	55	Male	30.6
5	27	Female	32.2
6	44	Male	33.8
7	60	Female	27.2
8	52	Male	33.9
9	33	Female	41
10	46	Male	14.7

4. CONCLUSION

Radioimmunoassay methods showed the significant inconsistency in test, it's possible to lead miscalculation in the immunoassay methods when compared with the reference method. The LIA, ELFA, ECLIA and SRP immunosorbent assay methods are highly effective when compared with qualitative immunoassay. Hence, the SPR biosensor is a high sensitivity pipetting device, applied to recognize the sepsis, severe sepsis, thrombosis (DIC, DVT and pulmonary embolism), rickets, deficiency or insufficiency of vitamin D. The analysis of vitamin D and its metabolites is a fast growing field. The result is completely estimates the vitamin D from serum sample and can be used as a immediate identification for vitamin D status and observing the patient's feed back against vitamin D deficiency and response to vitamin D therapy .

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