6. Discussions
CA15-3 (also known as MUC1) is the most widely used serum marker in BC and the reference range of serum CA15-3 is less than 30 U/ml. CA15-3 is a large transmembrane glycoprotein with high molecular weight belongs to 250 and 350 kDa or more than 400 kDa. CA15-3 is a soluble form of MUC1, and its elevation may be detected in the serum samples of BC patients, especially those with metastatic disease. However, an increased level of CA15-3 can also be associated with colorectal and ovarian cancer. Nowadays, CA15-3 is most commonly used in detecting BC and monitoring the treatment in BC-oriented patients. CA15-3 has been approved for monitoring BC by US Food and Drug Administration (Joseph and John, 2005). It is an independent predictor of reappearance, and a powerful prognostic sign in patients with advanced BC (Keshaviah et all, 2007). However, BC is still the main cause of cancer death among women, with approximately 1.4 million new cases and nearly 0.5 million deaths worldwide (American Cancer Society, 2011). CA15-3 There is increasing demand for this protein to be used for kit calibrators and standard in different research and diagnostic procedures. Unfortunately, very little knowledge about the synthesis of CA15-3 with BC Cell lines is available.

In the present study, we have developed a sandwich assay by utilizing two monoclonal antibodies raised against CA15-3 protein purified from culture supernatant of BC cell line T47-D.

To achieve these goals we screened cell lines T47-D, MCF-7, Zr75-30 for their ability to secrete CA15-3 in tissue culture based system, their PDT, and expression of CA15-3 on the surface of the membrane. It was found that PDT of MCF-7, T47-D, and Zr75-30 was near to 30, 38, 116 Hrs. respectively. CA15-3 secretion at 90% confluency was estimated 5, 20, 25 IU/ml. With the immunofluorometric staining of cell lines T47-D and MCF-7 we narrowed
down T47-D for process development. T47-D is a well-characterized breast carcinoma cell line which is utilized for studies related to BC and its therapy. The cell line was adapted to low serum, and augmentation of CA15-3 secretion was performed by testing variety of hormones, interleukin, chemical, media supplements. The present study has demonstrated a correlation between Glucose and insulin concentrations, estrogen and progesterone, PC-1 and IL-6 in CA15-3 augmentation/secretion by T47-D cell line. The effect on CA15-3 secretion was observed with combinatorial use of estrogen and progesterone in media. The commercial defined PC-1 media supplement (Lonza) showed the best result in terms of CA15-3 secretion. Whereas IL-6, D-glucose, and Insulin showed very little effect on secretion. The upscale of tissue culture was performed in roller bottle apparatus. The best revolution was near to 3 revolutions in 5 minute.

The reported process available for the purification of CA15-3, have been very suggestive and shown to be producing part pure CA 15-3 from biomedical fluids, tissue macerate. All procedures have produced CA 15-3 along with another cancer antigen e.g. CA125, CA 19-9, CA 72-4 with high level of contamination. Whereas immunoaffinity columns yield high pure CA15-3 with very poor recovery. To our knowledge; here we report the first detailed protocol for purification of high pure CA15-3 from tissue culture supernatant of T47-D BC cell line. The purified protein was characterized by SDS-PAGE, western blotting, Indirect and sandwich ELISA and comparative FTIR of native and cell-derived CA15-3.

With this purified protein we raised more than 300 hybridoma clones. Out of these 300 clones, 30 parents were screened for cross-reactivity and frozen for future use. Clone 8E04, 4b054B09 along with three others were upscaled and purified for characterization. Further characterization of antibody revealed that these antibodies were capable of detecting
1IU/100ul of CA15-3 during indirect ELISA. Side by side these were also capable of detecting native as well as a cell derived CA15-3 in western blot.

We established an immunoassay, by our own antibodies developed against, cell-derived CA15-3 purified from culture supernatant of T47-D cell line. The assay can detect CA15-3 present in any fluid very efficiently. The assay shows no reactivity towards another cancer marker ie highly specific for CA15-3. The purified cell derived CA15-3 shows a higher affinity towards commercial antibodies which predicts the presence of more exposed epitopes other than native protein. Synthesis of cell culture based CA15-3 shows a great future of producing homogenous protein, which may utilize for making standards, antibodies and even for vaccines. These purified antigens may have glycosylation-related to original cancer cell because of their cancerous origin and can be handled easily under normal laboratory conditions. The essential media conditions and growth factor requirement for optimal secretion can easily achieve. The purification process for these proteins (cell culture based) are very easy and the cross contaminants are negligible or very few(less than 1%) in most of the cases have been tested. The biological activity of these proteins is much more than native hence a very good Immunogen for monoclonal antibody generation. The use of this purified antigen in Balb-c mice as Immunogen produced a good immunological reaction and a good titer of antisera was achieved with secondary immune response in early days of the 4th month after first immunization. On fusion of splenocytes with myeloid partner approximate 300 clones with higher OD were screened. Out of 300 hybridoma clones raised against cell derived CA15-3, 30 clones with high reactivity against CA15-3 were selected and a sandwich ELISA was performed. The comparison of results of sandwich ELISA designed with newly synthesized antibodies and commercially available kit shows resemblance in the detection of CA15-3. However, Cancer marker is a big glycoprotein. Secondary, tertiary structure it adapts, it becomes difficult to develop antibodies or assay on the whole, which
can detect all these epitopes. Therefore, antibodies, assay develop; specific epitopes, only these get detected. Cell-derived antigen, antibodies, one pattern detection is possible. Further investigations and development are called for.

The developed in-house immunoassay shows sensitivity of 94.20%, specificity of 100%, a positive predictive value of 100 along with same score of 100 for negative predictive values.