Patelia et al., 2:4
http://dx.doi.org/10.4172/scientificreports720

Open Access Scientific Reports

Research Article Open Access

# Determination of HER2 Status in ER Positive Human Breast Cancer

**Emanual Michael Patelia***, **Kunal Patel and Mikul Patel**

*Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, Gujarat, India*

**Abstract**

According to the method (BCA), it is used for the quantitative analysis of proteins. It is used for the determination of unknown sample of protein. The SDS-PAGE is used for the identification of proteins though the separation. According to the gel image the sample of protein is ER-α and HER positive. So, the person needs the therapy for ER-α and HER positive breast cancer. The amino-terminal region was consistently detected in the cell nucleus by specific immunohistochemistry leading to the concept of a potential intranuclear association between ER cleavage products and/or other regulatory proteins. Human epidermal growth factor receptor 2 (HER2) belongs to the family of epidermal growth factor receptors (EGFRs). The availability of specific antibody-based test systems is essential to testing of HER2 protein expression.

**Keywords:** Human breast cancer; Breast malignancy; Vascular disease

## Introduction

1.4 million Reports of women having breast cancer had been projected in 2008 [1]. In all most important region of the world, breast malignancy is the Commonest, or second commonest, cancer in women [2]. Now breast cancer is everywhere in the world. High-income, westernized, industrialized countries have the widespread problem of breast cancer. There is notable increase in life expectation in people of least-income levels in many countries and the augmented propensity to accept a westernized way of life, on the basis of that cancer is a rapidly growing global problem [3]. Of course, in the above statements, 'cancer' could be Same as 'vascular disease' or 'chronic obstructive pulmonary disease' or 'diabetes' or any number of chronic situations coupled with aged populations adopting a westernized lifestyle. Today, every woman in the world has somewhat risk of developing the disease. Clinically, a positive ER-α status correlates with favorable prognostic features, including a lower rate of cell proliferation and histologic evidence of tumor differentiation. ER-α status is also prognostic for the site of gross metastatic spread. For reasons unknown, ER-α-positive tumors are more likely to initially manifest clinically apparent metastases in bone, soft tissue, or the reproductive and genital tracts, whereas ER-α-negative tumors more commonly metastasize to brain and liver. Several studies have correlated ER-α expression with lower Matrigel invasiveness and reduced metastatic potential of breast cancer cell lines [4,5]. Moreover, when ER-α-positive cells are implanted in nude mice, tumors appear only in the presence of estrogens and are poorly metastatic as compared with those developed from ER-α-negative breast cancer cell lines [6,7]. This paradox suggests that ER-α expression could be associated with or involved in pathways that hinder cancer progression. At the transcriptome level, gene expression analysis has revealed that different molecular subtypes exist within ER-α- positive and ER-α-negative breast cancers, and these are associated with different clinical outcomes. ER-α-positive tumors exist in at least two subtypes, luminal A and luminal B, which vary markedly in terms of gene expression and prognosis [8]. Conversely, hormone-receptor-negative breast cancer comprises two distinct subtypes, the Her2 (human epidermal growth factor receptor 2) subtype and the basal-like subtype [8,9], which differ in biology and behavior, and are both associated with a poor outcome. Importantly, a very similar subdivision of breast cancers has been produced based upon immunohistochemistry, conducted to analyze patterns of protein expression in tumor sections, which suggests that a few protein biomarkers can be used to stratify breast cancers into different fundamental groups [10,11].

## Methods

### Method 1: Bicinchoninic acid (BCA) protein or copper-based assay

The bicinchoninic acid (BCA) assay was made-up by Paul K. Smith in 1985 [12] at Pierce Chemical Company, the key point of this assay [13,14]. Mutually BCA assay and Lowry assay are based on the alteration of $Cu^{2+}$ to $Cu^{1+}$ under alkaline environment. This replace is defined as Biuret reaction. This reaction is biased by four amino acid residues (cysteine, cystine, tyrosine, and tryptophan) and also by the peptide backbone.

BCA is a thorough chromogenic reagent for $Cu^{1+}$ and in the next step of the reaction two BCA molecules react through one $Cu^{1+}$ ion. The quantity of $Cu^{2+}$ reduced is a role of protein concentration that can be resolute spectrophotometrically by a color transform of the sample solution into purple, which absorbs at 562 nm. The absorbance is basically relative to the amount of protein in presence in the solution and it can be predictable by consideration with a accepted protein standard, such as bovine serum albumin (BSA) [12,15,16] (Figure 1).

BCA assay is extra intolerant to a diversity of ionic and nonionic detergents such as NP-40, Triton X-100 and denaturating agents similar to urea and guanidinium chloride that be liable to block with additional colorimetric protein assay such like Lowry [15]. Some chemical molecules, for example, reducing sugars, can impede with BCA assay.

**Use of** SDS-PAGE **electrophoresis:** The particular special effects of the interferences can be eliminated or reduced during numerous strategies such as removing the interfering substances throughout

***Corresponding author:** Emanuel M Patelia, Department of Pharmaceutical Chemistry and Analysis, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar - 388121, Gujarat, India, E-mail: ricky.emanual@gmail.com

**Received** June 10, 2013; **Published** June 29, 2013

**Citation:** Emanual MP, Kunal P, Mikul P (2013) Determination of HER2 Status in ER Positive Human Breast Cancer. 2: 720 doi: 10.4172/scientificreports.720

dialysis, gel filtration or if the protein concentration is high enough, via diluting the sample.

**Preparation of the BCA working reagent:** BCA reagents A and B are obtainable commercially from a number of different sources.

1. Mix 50 parts of Reagent A (a solution containing bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1 N NaOH, pH 11.25) with 1 part of Reagent B (4% (w/v) $CuSO_4.5H_2O$), preparing adequate reagent for all the standards and samples. 2ml of working reagent is required for each sample (Figure 2).

2. Mix until the solution is a uniform light green colour. The solution is stable for 1 day.

**Preparation of samples and standards:**

1. Get ready a series of protein standards ranging in concentration from 12.37 to 1000 µg/ml such that the final volume for the assay is 0.1 ml. Examples of dilution standards are given in Table 1.

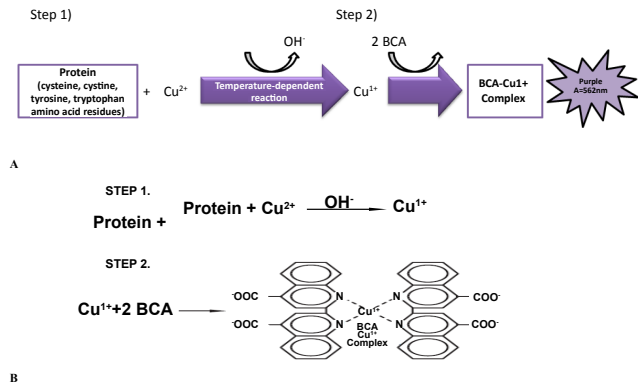


**Figure 1:** Demonstration of mechanism (a) and reaction (b) of protein analysis.

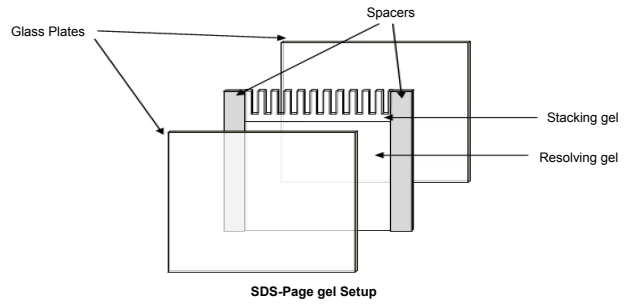


**Figure 2:** Diagram of SDS-gel electrophoresis.

| Standards (µg/ml) | Absorbance at 562 nm |
|---|---|
| 12.37 | 0.008 |
| 37.04 | 0.024 |
| 111.1 | 0.083 |
| 333.3 | 0.247 |
| 500 | 0.41 |
| 1000 | 0.821 |
| Sample | 0.231 |
| Concentration Found | 2400 µg/ml |

**Table 1:** Calibration standards for protein determination.

2. Prepare the unknown samples in analogous way such that the final volume is 0.1 ml.

3. Add 2.0 ml of the BCA working reagent to each sample and standard, vortex gently and follow one of the subsequent incubation parameters:

   a. 60°C for 15 minutes or:

   b. 37°C for 30 minutes or:

   c. 25°C (room temperature) from 2 hours to overnight

4. If required, allow the tubes to cool to room temperature.

5. Transfer the samples to cuvettes and assess the absorbance at 562 nm using the BCA protein mode.

## Method 2: Determinetion of HER2 status in ER positive human breast cancer sample

**Preparation of Sodium Dodecyl Sulphate-Polyacrylamide (SDS-PAGE) gel:** Clean the two glass plates (size: e.g. 10 cm×8 cm), 119 the polytetrafluoroethylene comb, the two spacers and the silicone rubber tubing (diameter e.g. 120 0.6 mm×35 cm) by gentle detergent and wash expansively by water, followed by dehydrated alcohol, and set aside the plates to desiccated at room temperature. Note: drying by a towel or a tissue may initiate stainable corruption, while using air-drying prevents this hazard. Lubricate the spacers and the tubing with non-silicone grease. Apply the spacers alongside each one of the two short sides of the glass plate 2 mm left from the edges and 2 mm away from the long side equivalent to the bottom of the gel. Initiate to set the tubing on the glass plate through using one spacer like a guide. Cautiously bend the tubing at the underneath of the spacer and track the long side of the glass plate. While assenting the tubing by one finger alongside the extensive side twists once more the tubing and place it on the subsequent little surface of the glass plate, with the spacer as a guide. Set the following glass plate in just right position and hold the mould jointly with hand pressure. Apply two clamps on each one of the two short sides of the mould. Cautiously apply four clamps on top of the longer surface of the gel mould consequently forming the underneath of the gel mould. Validate that the tubing is running alongside the edge of the glass plates and has not been extruded while insertion of the clamps. The gel mould is at this instant prepared for pouring the gel.

**Preparation of the resolving gel:** In a conical flask, get ready the proper volume of solution containing the preferred concentration of acrylamide for the resolving gel. Mix up the components in the array exposed. Where suitable, prior to adding up the ammonium persulfate solution and the TEMED, filter the solution if necessary under vacuum

**Figure 3:** A typical example of destained gel.

through a cellulose acetate membrane (pore diameter 0.45 µm). Maintain the solution under vacuum, while swirling the filtration unit, until no additional bubbles are produced in the solution. Add appropriate amounts of ammonium persulfate solution and TEMED swirl and transfer immediately into the gap between the two glass plates of the mould. Leave adequate space for the stacking gel (the length of the teeth of the comb plus 1 cm). Using a tapered glass pipette carefully overlay the solution with water-saturated isobutanol. Leave the gel in a vertical position at room temperature to allow polymerisation.

**Staining/destining:** Dip the gel in a large overload of *staining solution* and permit to stand for at least 1 h. Eradicate the staining solution. Destain the gel with a large excess of destaining solution. Change the de*staining solution* numerous times, awaiting the stained protein bands are clearly apparent on a clear environment. The more carefully the gel is destained, the lesser is the quantity of protein that can be detected by the method. Destaining can be speed up by counting a few grams of anion-exchange resin or a small sponge in the de*staining solution* (Figure 3).

## Results for BCA assay

### Calculation

According to the Chart 1,

$y = 0.001x - 0.009$

Therefore, unknown concentration $x = (0.231 + 0.009)/0.001$

So, $x = 240$ µg/ml

But there is 10 times dilution is there. So the found figure should be multiplied by unit 0f 10.

(Unknown concentration) $x = 240 \times 10 = 2400$ µg/ml = 2.4 mg/ml

## Results for SDS-PAGE Electrophoresis

### Calculation for Finding of Concentration for Loading on SDS PAGE

From BCA method, unknown sample 1000 µl contains 2400 µg,

So, 24 µg needs to be occupied by = $24 \times 1000/2400 = 10$ µl.

## Discussion

This proportional method for determining the concentration of an "unknown" is abstractly simple and simple. However, its completion in an assay protocol is complicated by pipetting and dilution steps, assessment of replicates, blank-corrections and other factors. These steps repeatedly cause mystification with regard to the calculation that is essential to achieve a last determination.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a method anticipated for cataloging out proteins based on their aptitude to move within an electrical current, which is a function of the span of their polypeptide chains or of their molecular weight. The SDS-PAGE separation is just like the high performance thin layer chromatography [17,18] and also like the column chromatography (HPLC) (Figure 4).

### Pharmacological therapy for ERA and HER Positive Breast Cancers

Endocrine therapies for breast cancer are treatments frequently in use following surgery, chemotherapy, and/or radiation are over. They are premeditated to assist stop reappearance of the disease by blocking the special effects of estrogen. They do this in one of numerous ways.

- The drug tamoxifen [19,20], in use by several women for up to five years subsequent to early treatment for breast cancer, helps

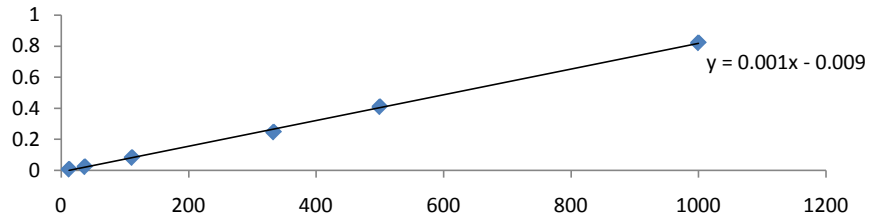


**Chart 1:** Calibration curve of protein standards.

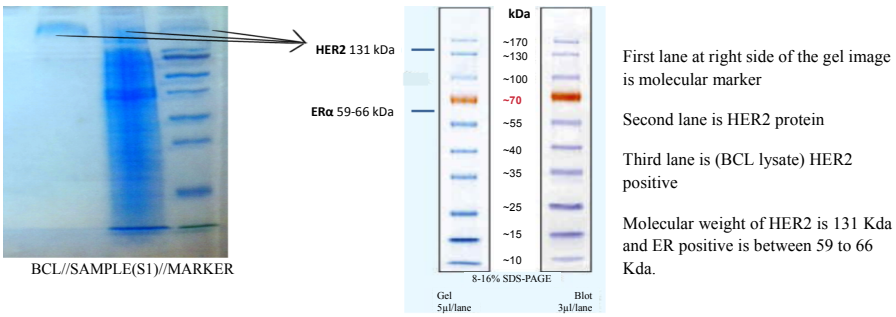


**Figure 4:** A typical photograph of developed gel and comparison with of it with PAGE ruler.

to check reappearance by blocking the estrogen receptors on breast cancer cells and preventing estrogen from binding to them.

A class of drugs called aromatase inhibitors essentially stops estrogen production in post-menopausal women. Women who have not yet gone through menopause cannot take these drugs.

### References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, et al. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127: 2893-2917.
2. Curado MP, Edwards BK, Shin HR, Ferlay J, Storm H (2009) Cancer Incidence in Five Continents, Volume IX. Lyon, France: IARC Press.
3. Boyle P, Levin B (2008) World Cancer Report 2008. IARC Press, Lyon, France.
4. Platet N, Prévostel C, Derocq D, Joubert D, Rochefort H, et al. (1998) Breast cancer cell invasiveness: correlation with protein kinase C activity and differential regulation by phorbol ester in estrogen receptor-positive and -negative cells. Int J Cancer 75: 750-756.
5. Thompson EW, Paik S, Brünner N, Sommers CL, Zugmaier G, et al. (2007) Association of increased basement membrane invasiveness with absence of estrogen receptor and expression of vimentin in human breast cancer cell lines. J Cell Physiol 150: 534-544.
6. Fuqua SA, Cui Y (2004) Estrogen and progesterone receptor isoforms: clinical significance in breast cancer. Breast Cancer Res Treat 87 Suppl 1: S3-10.
7. Price JE, Polyzos A, Zhang RD, Daniels LM (1990) Tumorigenicity and metastasis of human breast carcinoma cell lines in nude mice. Cancer Res 50: 717-721.
8. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, et al. (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 98: 10869-10874.
9. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, et al. (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 100: 8418-8423.
10. Abd El-Rehim DM, Pinder SE, Paish CE, Bell J, Blamey RW, et al. (2004) Expression of luminal and basal cytokeratins in human breast carcinoma. J Pathol 203: 661-671.
11. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, et al. (2004) Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 10: 5367-5374.
12. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, et al. (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150: 76-85.
13. Olson BJ, Markwell J (2007) Assays for determination of protein concentration. Curr Protoc Protein Sci Chapter 3: Unit 3.
14. Yoshida M (1992) Malignant schwannoma arising from the trigeminal nerve in a rat. J Comp Pathol 106: 315-318.
15. Wastell C, MacNaughton J (1975) Letter: Unusual reaction to pentagastrin. Br Med J 1: 334.
16. Walker JM (1994) The bicinchoninic acid (BCA) assay for protein quantitation. Methods Mol Biol 32: 5-8.
17. Patelia E M, Shah DA, Mori A (2013) Simultaneous estimation of pregabaline and methylcobalamine in pharmaceutical formulation by HPTLC-Desitometry Mehtod. J Chormat Seperation Techniq 4: 2-4.
18. Ahir KB, Patelia EM, Shah A (2013) Simultaneous Estimation of Metformin Hydrochloride and Repaglinide in Pharmaceutical Formulation by HPTLC-Densitometry Method. J Chormat Seperation Techniq 4: 166.
19. Craig RC, Stitzel ER (2004) Modern pharmacology with clinical application US: United states press.
20. Horwitz KB, Tung L, Takimoto GS (1995) Progestins, Progesterone Receptors, and Breast Cancer Hormones. Health and Disease 1: 283-306.