

Development of a Chemiluminescent Assay (CIA) for the Receptor of Alpha Fetoprotein (RECAF) to Separate Cancer from Normal Sera

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Abstract

Aims: A radioimmunoassay for the receptor to alpha fetoprotein (RECAF) from BioCurex separates normal specimens from wide variety of cancers. Our aim was to develop a non-radioactive immunoassay for the RECAF for the early detection and screening of cancer.

Methods: The RECAF CIA assay is a competitive assay that utilizes a IgM monoclonal antibody to RECAF (1.4G11) on the solid phase and an acridinylated Human Milk Protein RECAF conjugate. We perform the RECAF CIA assay by mixing diluted human sera with acridinylated RECAF, and adding the mixture to a 1.4G11-coated microtiter plate. Following incubation, the plates are washed and read in a chemiluminometer. The initial development of the assay focused on the blocking buffer's composition and pH. BioCurex performed initial experiments and confirmation testing. Then, BioCurex shipped the RECAF CIA assay to Abbott Laboratories for confirmatory testing.

Results: Our experiments demonstrated that the separation of cancer from normals were most affected by pH and by Tween 80 concentration. Verification studies using breast, gastric, and other types of cancers (n = 68) and normal samples (n = 52) had an area under the curve (AUC) for the ROC curve of 0.954 with a cancer/normal (C/N) ratio of 1.7. Initial experiments at Abbott Laboratories with prostate cancer (n = 8) and non-cancer samples (n = 16) had an AUC for ROC curve of 0.95 with a C/N ratio of 1.3.

Conclusions: We developed a non-radioactive RECAF CIA assay that separates multiple types of cancer from normal sera with a C/N ratio ranging from 1.3 to 1.7. Our future studies will focus on increasing the cancer/normal ratio to create a manufacturable RECAF CIA assay.

Introduction

AFP is one of the main circulating proteins in the fetus and can be internalized by fetal and malignant cells^[1,2]. Hence, RECAFTM behaves like a widespread oncofetal antigen^[3]. Therefore, RECAF in tissue and blood can be used for cancer diagnosis^[4]. Currently, RECAF is measured with the RECAF radioimmunoassay (RIA) from BioCurex, Vancouver, BC. However, a RECAF RIA assay is difficult to automate. On the other hand, a non-isotopic RECAF assay would allow the assay to be placed on automated instruments and increase the availability of the assay to clinical laboratories.

Objectives of this study were to develop and evaluate the performance of a competitive RECAF chemiluminescent immunoassay (CIA) using acridinium-labeled RECAF antigen. This is a first step toward automating RECAF determination in sera.

Materials and Methods

Assay format: Ninety six (96) well microtiter plates were coated with the 1.4G11 monoclonal anti-RECAF antibody. The plates were lyophilized and stored at room temperature for later use. Affinity purified RECAF antigen was coupled to acridinium (Ac RECAF). Before testing, serum samples were diluted 1:5 in assay diluent. The competitive RECAF CIA assay was carried out as follows: 50 uL of Ac RECAF at 400 ng/mL was mixed with 50 uL of cord serum calibrators or diluted serum samples. The mixture was then transferred to anti-RECAF-coated microtiter plates and incubated for 2 hours at 37°C. The RECAF CIA plates were washed, trigger was added and chemiluminescence was determined. **Figures 1 and 2** demonstrate the assay principle.

Statistics: The RECAF CIA assay results were analyzed using Analyze-It (v 1.73, 2000) for ROC curve analysis and C/N ratio. The MedCalc (v 9.3.2.0, 2007) statistical software was used to generate box and whisker plots.

Assay Diluent Optimization: Our experiments demonstrated that separation of cancer from normal RECAF values were most affected by pH and by detergent concentration.

Experiment 1: In BioCurex facilities (**Figure 3**), the RECAF CIA assay with optimized assay diluent was used to quantitate RECAF values in sera from patients with breast, ovarian, kidney, stomach, lung, thyroid, stomach, intestine, uterus, prostate, cervix, and testicular cancer from Russia (n = 68) and in normal serum samples from Canada and Russia (n = 52).

Experiment 2: At Abbott Laboratories (**Figure 4**), the RECAF CIA assay with optimized assay diluent was used to quantitate serum RECAF levels in stage III prostate cancer (n = 8), benign prostatic hyperplasia (BPH; n = 8), and normal specimens (n = 8). The comparison of Abbott and BioCurex ROC curves are shown in **Figure 5**.

Figure 1: RECAF CIA Competitive Assay Principle with No RECAF Present

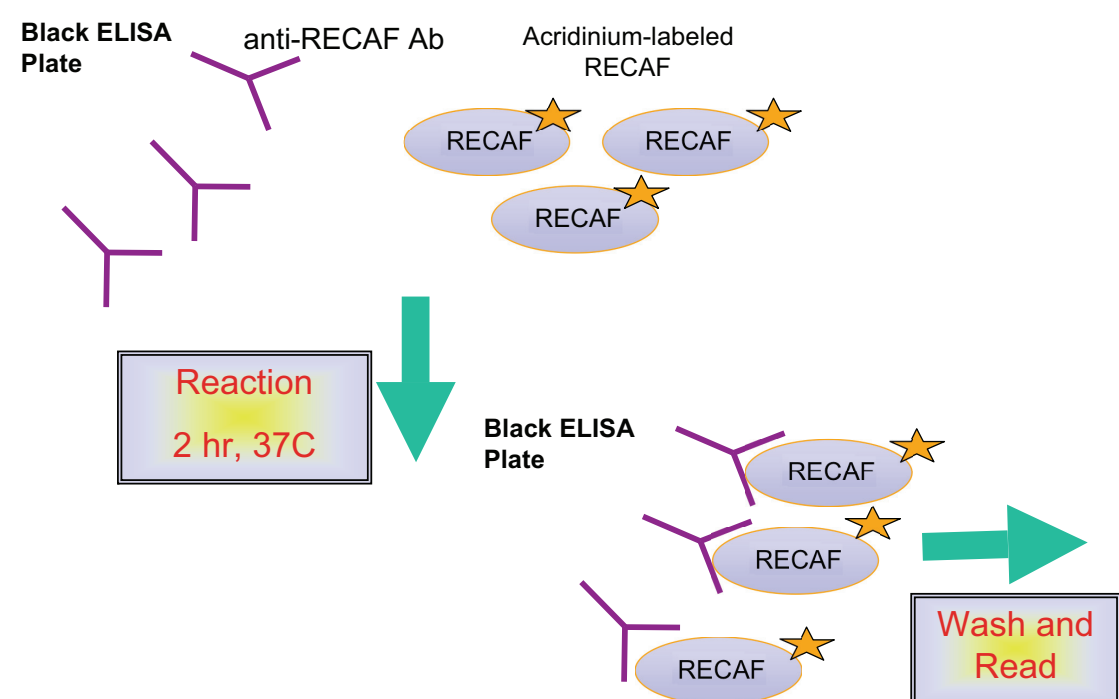
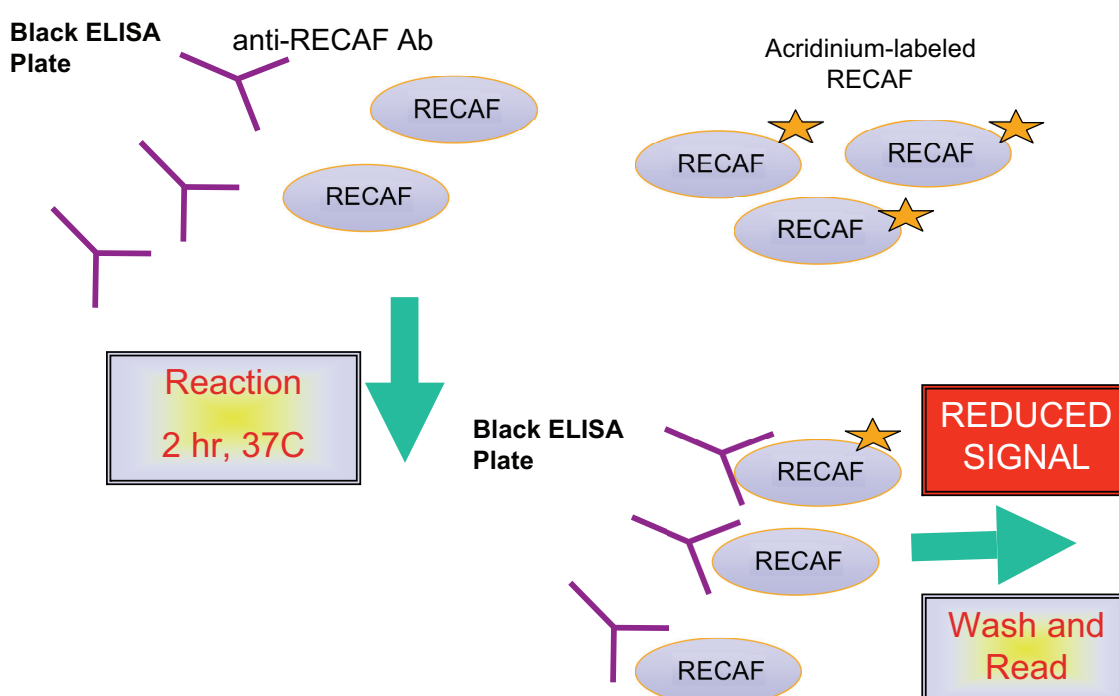
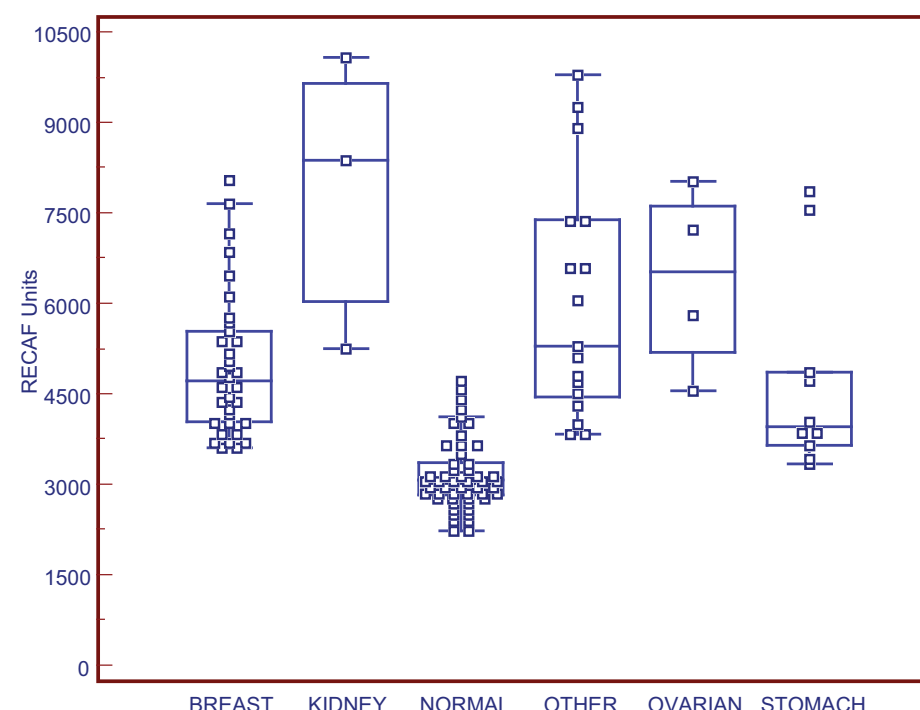


Figure 2: RECAF CIA Competitive Assay Principle with RECAF in Sample



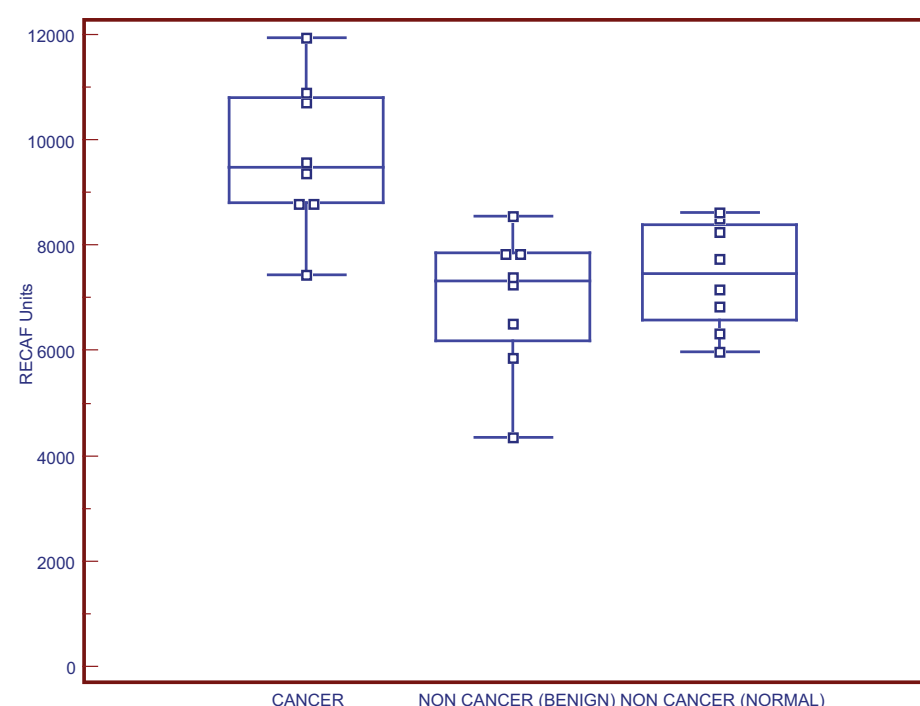
Results

Figure 3: RECAF CIA Assay at BioCurex: Cancer vs Normal Sera



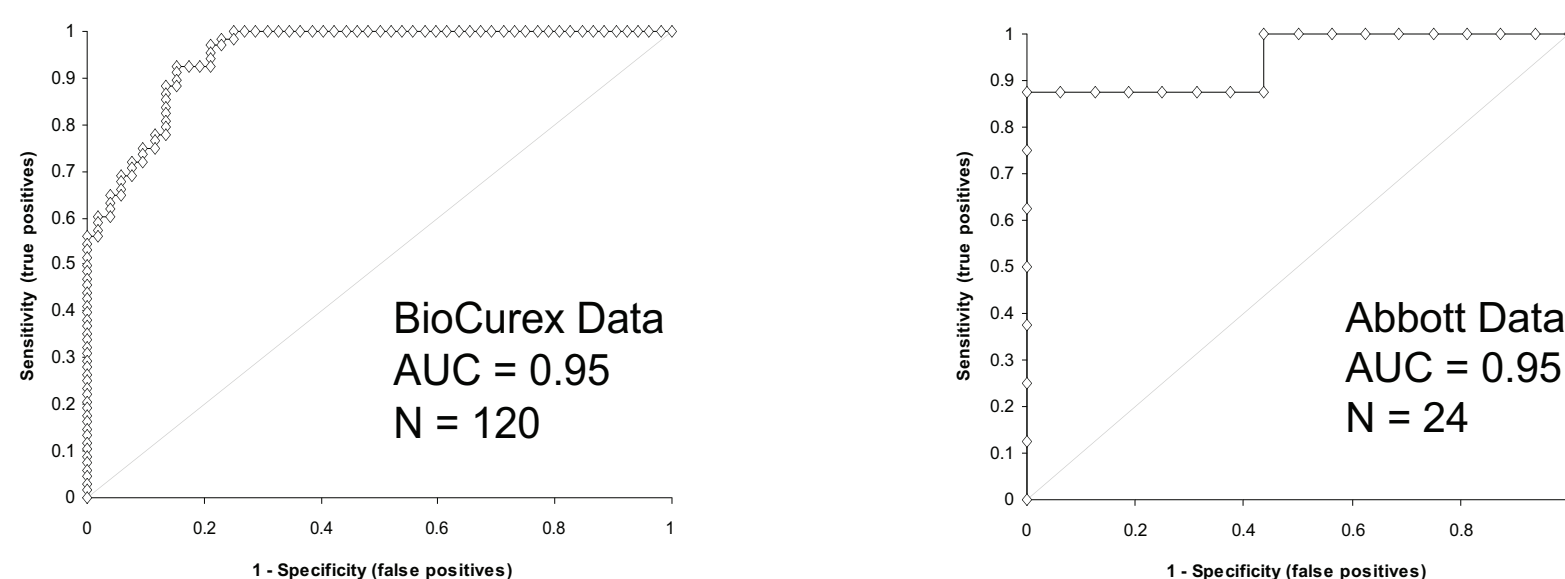
Multiple solid tumor cancers have elevated RECAF values compared to normal samples with a C/N ratio is 1.7. Other cancers include lung, thyroid, stomach, intestine, uterus, prostate, cervix, and testicular cancer.

Figure 4: RECAF CIA Assay at Abbott: Prostate Cancer, BPH, and Normal Specimens



RECAF units for Prostate Cancer samples are elevated compared to BPH and normal specimens. The C/N ratio is 1.3.

Figure 5: ROC Curves from RECAF CIA Assay from BioCurex and Abbott Laboratories



Similar AUC for ROC curves were obtained at both BioCurex and Abbott Laboratories.

Conclusions

The results obtained in both BioCurex and Abbott facilities are consistent. The RECAF CIA assay can discriminate normal from cancer specimens with:

- AUC of 0.95
- C/N ratio ranging from 1.3 to 1.7

The availability of a non-isotopic assay:

- Makes the transfer of the RECAF CIA assay to other sites easier
- Allows for independent validation of our results
- Facilitates the automation of a RECAF immunoassay

References

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