Auria: ELISA Validation Study

The goal of this study was to develop a diagnostic algorithm that could distinguish breast cancer samples as a group with high enough sensitivity and specificity to warrant clinical validation. Three proteins were selected from the biomarker discover study for evaluation by ELISA. Statistical analysis produced three potential diagnostic models which were evaluated at two points, with Sensitivity ranging from 52 - 90% and Specificity ranging from 31 - 79%, depending on the model and threshold under evaluation.

Given the nuances in various screening modalities and patient journeys within the breast health continuum of care, the samples in this study were further stratified beyond simply "normal" and "breast cancer". Included in the categories of analysis were "call-backs" defined as normal diagnostic mammogram following a screening mammogram, "BiRads Category 3" where the patient's screening and diagnostic imaging is not confirmatory and they are recommended for follow-up imaging and some recommended interval of time (normally 3-6 months) to determine if the area of suspicion has changed at all, and "Benign" where the patient under went a biopsy and the pathology report indicated findings were benign. Break down of sample types are provided in Table 1. Each scenario was evaluated by all models which is why there is a range in specificity and sensitivity.

Table 1: Samples for ELISA Validation Study

Category	Training Set (n=391)	Test Set (n=456)
Normal	223	145
Call-Back	81	83
Breast Cancer	87	21
Benign	–	121
BiRads Cat 3	–	86

Table 2: Breast Cancer Subtype for ELISA Validation Study

Breast Cancer Subtype	Training Set	Test Set
IDC	48	14
ILC	4	3
DCIS	20	2
Multiple	4	0
Other	11	2

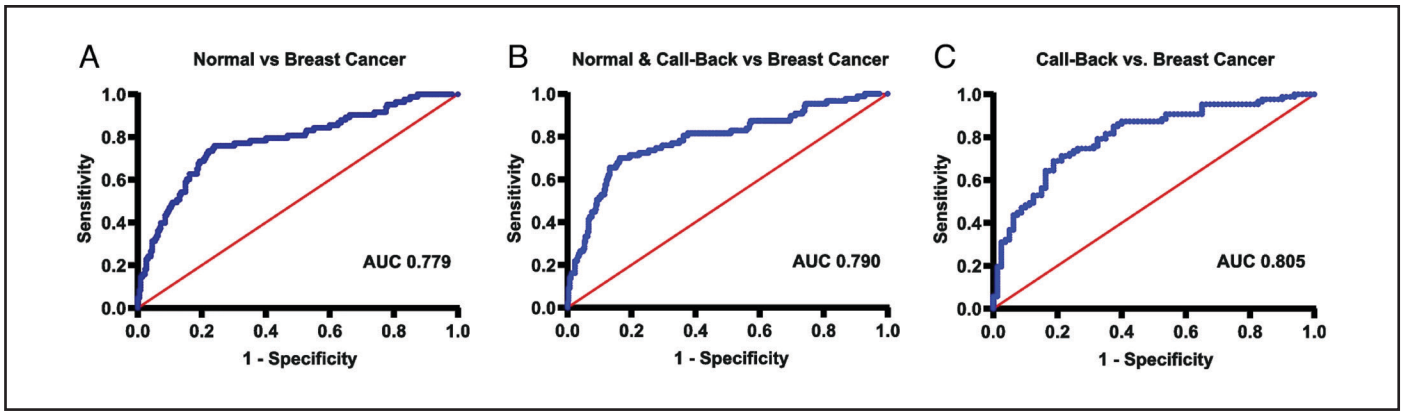

Figure 1: Receiver operator characteristics (ROC) curves comparing (A) control vs breast cancer (B) call-back vs breast cancer (C) control + call-backs vs breast cancer.

In summary, a range of diagnostic parameters were observed based on the three models identified Sensitivity 52 - 90% Specificity 31 - 79%. The nature of model has the best potential for cut off optimization, therefore that model was selected for clinical validation moving forward.

Reference: Daily, A., Ravishankar, P., Wang, W. et al. **Development and validation of a short-term breast health measure as a supplement to screening mammography.** Biomark Res 10, 76 (2022). https://doi.org/10.1186/s40364-022-00420-1