Poster 5

Analytical validation of novel point-of-care assay kit for estrogen receptor status based breast cancer determination

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Purpose

We have identified Nw-hydroxy L-Arginine (NOHA) as a highly sensitive and specific blood-based biomarker to distinguish estrogen receptor (ER) negative from ER positive (i.e., levels <4nM indicative of ER negative disease) in breast cancer patients (U.S. Utility Patent 10,073,099). In this study we examine the comparative analytical validity of our novel point-of-care NOHA assay kit to that of our validated ELISA in predicting breast cancer ER status.

Methods

NOHA assay kit analytic validity testing was assessed by testing buffer solutions with known ELISA-assessed NOHA concentrations (0-15nM) to both assay kit's sensitivity and selectivity in predicting ER status. Dried buffer samples on Telimmune card (North Webster, IN), were soaked for 30 min with 300µl 1X phosphate buffered saline from a pre-measured syringe in tube-1 containing lyophilized 5ng/ml anti-NOHA monoclonal antibody (mAB) of ~150KDa. 250µl of tube-1 were transferred into tube-2 containing lyophilized 5ng/ml Goat F(ab')2 Anti-Mouse IgG fragment cross-adsorbed-antibody HRP (Abcam, Waltham, MA) of ~87KDa, and incubated 30 min. 200µl from tube-2 got extracted through 100 KDa cut-off PCTE membrane filter (Sterlitech, Auburn, WA) into light protected tube-3, containing 50 µl 2.5 mM tetramethylbenzidine (TMB) substrate, and incubated for 5 min before removing light protection to observe blue-scale color intensity. Assay kit color intensity results were compared with ELISA NOHA measurement for experimental and color chart guide validation. Statistical difference was set at $p<0.05$.

Results

Analytic selectivity and selectivity testing (in 75 samples, with known NOHA of 0-15 nM, at 0.2 nM increments) revealed strong correlation between assay kit color intensity estimates vs. ELISA-determined NOHA levels, with 95% confidence intervals (CI = 0.991-0.998, $r2 > 0.99$, and $p < 0.05$) at almost all tested concentrations.

Conclusion

This study provides the first evidence suggesting NOHA assay kit utility with near 100% correlation to our validated ELISA in predicting breast cancer ER status. Subsequent validation of this assay kit for analytic precision, dilution linearity, and percent recovery in predicting ER status, will broaden its potential as a low-cost less-invasive alternate to breast cancer prognosis and in real-time therapy response determination, both in US, and in resource constrained global settings.

Keywords: Point-Of-Care assay kit, NOHA, blood-based cancer biomarker