Title: Tear Based Breast Cancer Detection

Principal Investigator: V. Suzanne Klimberg MD. PH.D.

Funding Sponsor: Namida Lab, Inc.

Version: 2

Date: 4/13/2021

1. Introduction and Purpose:

Namida Lab, Inc. is a biotechnology company located in Fayetteville, AR, with a certified high-complexity CLIA lab. In January of 2021, Namida Lab, Inc. completed Melody® analytical and clinical validation, a screening test for breast cancer based on proteins from tears. The validation process was carried out manually and now needs to be conducted on the Hamilton StarPlus automated ELISA system. To do this, Namida Lab, Inc. needs fifty tear samples collected from women recently diagnosed with breast cancer but who have not undergone any treatment.

2. Background:

What is the Problem?

Most women recognize screening mammography as the current gold standard in breast cancer screening. However, disparities are present, leaving groups of women without adequate access to screening. Gaps in access to screening exist due to geographic location, socio-economic status, race, and ethnicity (references). In addition, studies show women who have received a false-positive result are more likely to lose trust in the current breast health continuum of care. Therefore less likely to return for follow-up screening.

How can Melody® help?

Currently, Melody® is not intended to be a replacement for screening mammograms but an additional tool to facilitate discussion between providers and patients. Tear sample collection can be done during any routine office visit and does not require specially trained personnel. The simple collection process allows patients to be tested in a clinic they are already familiar with by personnel they already know and trust. Melody® early iteration goals are one additional piece of information gathered. One more conversation about breast health may be the key to increasing participation in annual or biennial screening mammograms.

Key terms

- The Clinical Laboratory Improvement Amendment (CLIA) passed in 1988 was established to provide quality standards, strengthen Federal oversight of clinical laboratories, and ensure the reliability and accuracy of patient test results. This program allows clinical labs to develop and validate the testing protocols, which can only be run in the lab where the protocol was validated.
- Namida Lab, Inc. is a CLIA certified high complexity lab.
- The FDA is responsible for developing and assigning CLIA test complexity categorization rules and guidance.
- Melody® is a high complexity lab-developed test that has been developed and validated by Namida Lab, Inc. and will be run only by Namida Lab, Inc.
- A clinical utility study evaluates the usefulness, benefits, and drawbacks of an intervention (Smart, 2006).

What is the Validation process for an LDT?

CLIA clearly outlines requirements for validation of a lab-developed test. In short, each assay must meet guidelines for precision, accuracy, sensitivity and specificity (both analytical and clinical), linearity and assay range. Each laboratory drafts an umbrella standard operating procedure (SOP) to conduct validation

of new assays. This SOP is then customized to each specific test the lab intends to offer. The medical director must approve all SOPs and subsequent changes to SOPs in the CLIA lab.

After completion of validation experimentation, all results are compiled in a validation report and then reviewed and approved by the medical director. All CLIA labs are under the jurisdiction of their respective state CLIA licensing boards and undergo an inspection every two years. At the time of inspection, the lab must provide validation reports for all assays available on the testing menu for review.

What was the outcome for the validation of Melody®?

Designing the validation of Melody® was unique because there are no lab developed tests using protein biomarkers in tears to Namida Lab, Inc.'s knowledge. The nature of the sample alone provided a new level of challenge that made the validation process more stringent. Both analytical and clinical validation of Melody® was completed in January 2021. A summary of parameters analyzed and the results for the validation can be found in tables 1-2. All performance criteria were successfully achieved, and clinical validation resulted in a sensitivity of 92% and a specificity of 54%. These values were in line with previously observed sensitivity and specificity values from development work.

Table 1: Summary of Analytical Validation

Study Parameter	Sample Description (Name, Number, Replicates)	Results	Comparison to Acceptance Criteria (Pass/Fail)
Accuracy	20 spiked LGF samples	Percent recovery between 80-120% for all samples	Pass
Intra-Assay Precision	24 replicates of 3 concentrations per analyte	All %CV less than or equal to 15%	Pass
Inter-Assay Precision	Duplicates of 3 concentrations 1xday for 5 days.	All %CV less than or equal to 15%	Pass
Sensitivity	20 replicates of blank per protein	S100A8 11% 21.10 pg/ml S100A9 14% CV 24.32 pg/ml	Pass
Linearity	7 unknown concentrations	Percent difference less than 15% for all samples	Pass

Table 2: Summary of Clinical Validation

Study Parameter	Sample Description (Name, Number, Replicates)	Results	Comparison to Acceptance Criteria (Pass/Fail)
Accuracy	26 samples 3 replicates each sample	92%	Pass
Sensitivity	52 LGF samples in duplicate	92%	Pass
Specificity	50 LGF samples in duplicate	54%	Pass
Stability of LGF samples	1 pooled disease LGS 1 pooled non-disease LGS	N/A	Refer to Deviations and Section B, Number 4

3. Concise Summary of Project:

What exactly is Melody®?

Part 1: Tear sample collection

Tear samples are collected using a Schirmer strip, a class I medical device, typically used to test for dry eye. The filter paper strips have been repurposed to collect proteins from the ocular cavity following protocols outlined in the literature (references).

Tear collection is conducted through the following steps (figure 1):

1. The strip is placed in the participants' lower eyelid, and having a foreign object in the eye will cause the eye to water. The fluid released washes the inner surface of the eyelid and the ocular lens itself, allowing proteins to adhere to the strip.
2. The strip is left until the liquid reaches the 25 mm mark or 5 minutes has passed.
3. The strip is then placed in a tube containing a buffered solution and is shipped overnight to Namida Lab, Inc.

Figure 1: Collection of tear samples.

Part 2: Clinical Lab assay

During shipment, the proteins leave the strip and go into the solution in the tube. It is this solution that is evaluated using standard ELISA techniques in the high-complexity CLIA lab. Namida Lab, Inc. is outfitted with a Hamilton Star Plus automated ELISA system. The data generated from the ELISA assay is used together with demographic information to generate a Melody® score. The score is then compared to the diagnostic threshold, and classification of High and Low will be reported.

A reference range has been developed using the distribution of Melody® scores from the clinical studies previously conducted (figure 2). In the future, this scale will be further optimized and provided to clinicians to guide their breast health discussions with patients.

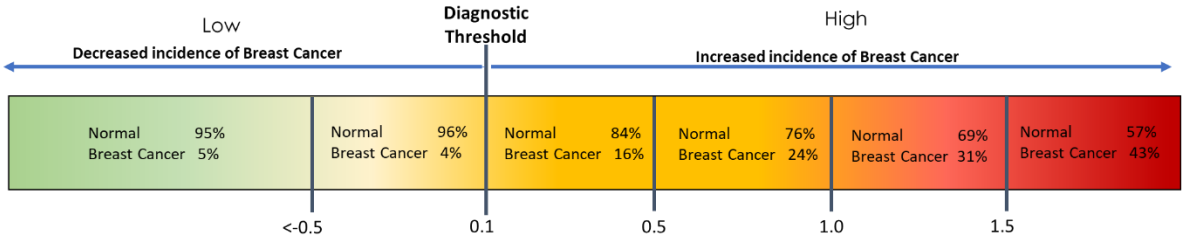

Figure 2: The Melody® scale was generated using 705 samples evaluated during development and validation. Percentages in each range show the distribution of normal and breast cancer patients that fall within each range.

With validation manually completed, Namida Lab, Inc. needs to do an equivalency study on the automated robot to demonstrate consistency between manual and automated results. This analysis will

be carried out using an equal number of samples. The samples are collected from normal (i.e., recent mammogram came back with no new findings) and recently diagnosed breast cancer patients.

Namida Lab, Inc.'s Clinical Laboratory Supervisor has defined qualification criteria of 100% agreement between manual and automated validation for the validation to be considered successful.

4. Study Procedures:

Fifty participants will be enrolled following the timeline in Table 3. The participation is limited to a one-time sample collection.

Table 3: Study Timeline

Objective	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12
Namida Lab clinical team to train UTMB clinic staff on tear collection procedures.												
Begin Study enrollment and sample acquisition												
Conduct study enrollment and sample acquisition												
Enroll last patient												
Finalize any outstanding clinical forms												
Close study												

i. Data acquisition: Each participant will be provided with a questionnaire to be filled out at the time of sample collection. The study team will assist participants in answering questions such as type of breast cancer, staging information, and tumor size. If known at the time of sample collection or after the biopsy results are received. In addition, a clinical questionnaire will be filled out for each participant with specific information relating to their breast health and current diagnosis.

ii. Clinic: Visit #1
- Participant is seen
- Study eligibility is assessed
- Informed consent process
- Registration
- Questionnaires completion
- Tear sample collection

The tear sample, study participant questionnaire, and clinical info questionnaire will be shipped overnight to Namida Lab, Inc.

5. Sub-Study Procedures:

N/A

6. Criteria for Inclusion of Subjects:

Subjects may be invited to participate in this study if the individual is an adult woman and has recently been diagnosed with breast cancer.

7. Criteria for Exclusion of Subjects:

Subjects must be excluded from this study if they have already started treatment for the breast cancer diagnosis.
Age less than 18 or more than 100 years.

8. Sources of Research Material:

Enrolled participants will be asked to complete a brief questionnaire with questions around reproductive health, personal history of cancer, and family history of cancer. In addition, a clinical questionnaire will be filled out for each participant with specific information relating to their breast health and current diagnosis. Both questionnaires can be found in the appendix section of this document.

Tear specimens will be collected from each participant.

9. Recruitment Methods and Consenting Process:

Potential participants will be recruited from the patient population of the study's principal investigator (PI). Suppose there are potential study participants who are not patients of the principal investigator. In that case, the potential participant will not be contacted by the study staff. Unless the potential participant has been informed of the study by their medical provider, has expressed interest in receiving more information, and/or enrolling in the study.

Individuals will be informed of the study and the opportunity to participate after diagnosis and before any treatment(s). Potential participants will be provided the information about the study, given time to review the informed consent form (IFC) and ask any questions before signing.

10. Potential Risks:

Possible risks from the collection of tears are rare but include conjunctivitis or scleral or corneal abrasion. There are no known occurrences of any of these risks from the previous IRB-approved study.
Any time information is collected, there is a potential risk for loss of confidentiality. Every effort will be made to keep the information confidential; however, this cannot be guaranteed.

11. Subject Safety and Data Monitoring:

The Principal Investigator will monitor this study.

Due to the minimal risk to the participants and short duration of participation, this study will be reviewed on an as necessary basis from the time the IRB approves it. Suppose abnormal values are found or the testing fails in some manner. In that case, all investigators and collaborators will be informed immediately, and enrollment halted until the matter can be resolved. The patients will not be informed of their results, and the tests will not be used to decide their treatment in any way. Any unexpected or adverse events that occur, the information on events will be sent to the IRB within 30 working days.

12. Procedures to Maintain confidentiality:

After sample collection, the sample will be placed in a tube with a three-to-four-digit code generated by Namida Lab, Inc. (For example, ADX-001). Corresponding labels will be provided for the study participant questionnaire, informed consent, and HIPPA form to ensure the sample and paperwork can be matched.

Completed paperwork will remain locked in the Namida Lab, Inc. facility. It will only be accessible by the clinical study staff at Namida Lab, Inc.

Any information obtained in this study will be kept confidential. Representatives from the UTMB Institutional Review Board, the United States Food and Drug Administration (FDA), or other institutional oversight offices may review records at any time. If the results of this study are published in medical literature, participant's names will not be identified.

13. Potential Benefits:

Each study participant will receive a $25.00 gift card provided by Namida Lab, Inc. for participation.

The results of the tear evaluation will not be disclosed to the patient or be used for their medical assessment or treatment.

14. Biostatistics:

Clinical acceptance criteria are defined as 100% agreement between the manual and automated classification of each sample.

Namida Lab, Inc.'s CLIA Lab Medical Director established the sample size.

15. Appendix

Tear Based Breast Cancer Detection Participant Questionnaire

Sample ID:

Thank you for taking the time to participate in our study. Please answer the following questions to the best of your knowledge.

Initials: _____

Age: _____ Sex: ☐ Male ☐ Female

Height _____ Weight _____

Race:

☐ African/African American
☐ Arab/Persian
☐ Asian
☐ East Indian
☐ Hispanic/Latino/Latina
☐ Native American
☐ White/Caucasian
☐ Multiracial
☐ Prefer not to answer

Section 1: In section 1 we need you to answer a few questions about your eye health:

1. Have you been diagnosed by a physician with any of the following ocular disorders?
   - ☐ Glaucoma
   - ☐ Macular Degeneration
   - ☐ Cataracts
   - ☐ Dry Eye
   - ☐ None

2. Have you had Lasik surgery? ☐ Yes ☐ No

3. Are you currently wearing contact lenses?
   ☐ No ☐ Yes ☐ right eye only ☐ left eye only

4. Have you used of the following types of eye drops today? ☐ Red eye reducing
   - ☐ OTC for allergies
   - ☐ Prescription steroid for allergies
   - ☐ General hydration
   - ☐ Saline
   - ☐ Drops for Glaucoma
   - ☐ Other (Please describe):
   - ☐ None

Section 2: In section 2 we will cover a few questions about your reproductive health:

We are asking the next few questions to gather information about additional hormones that you may be taking.

5. Are you currently using any of the following types of Birth Control?
   - ☐ Pill Name if known: _____
   - ☐ Implant
   - ☐ Contraception Injection
   - ☐ Contraceptive Patch
   - ☐ Intrauterine device (IUD)
   - ☐ Vaginal Ring
   - ☐ None

6. Are you currently using any type of Hormone replacement therapy? ☐ Yes ☐ No

7. Please select what hormones you are taking and/or the name of your medication:
   - ☐ Testosterone
   - ☐ Estrogen
   - ☐ Progesterone
   - ☐ Thyroid
   - ☐ Other:

8. Medication Name if known: _____

9. At what age did your periods begin? ☐

10. Do you still have regular periods? ☐ Yes ☐ No

11. If you answered No on question 10, please select one of the following reasons:

☐ Perimenopause
☐ Menopause
☐ Hysterectomy
☐ Response to Birth Control
☐ Uterine Ablation
☐ Unknown
☐ Other (please describe):

12. Do you still have one or both ovaries?
☐Both ☐One ☐None

Section 3: In section 3 you will find questions about medications you may have taken today.

13. Have you taken any over the counter anti-inflammatory medication in the past 8 hours?
☐No ☐Yes Describe: _____

14. Have you taken any prescription anti-inflammatory medication in the past 24 hours?
☐No ☐Yes Describe: _____

15. Have you taken any immune suppressant medication today?
☐No ☐Yes Describe: _____

Section 4: In section we need information on your history and your family history of cancer.

16. In the chart below please list any cancers you have had.

Cancer Type	Year of diagnosis	Treatment	Treatment ongoing
		☐Chemo ☐Surgery ☐Radiation	☐Yes ☐No Further details:

17. Please list below, any members of your family that have or have had: Breast, Ovarian, Prostate, Melanoma, Pancreatic, Stomach, Uterine, Thyroid, Colon, and/or Sarcoma.

☐No Family history of cancer ☐Family history unknown

Immediate Family	
Mother	
Father	
Sibling	
Maternal Family	
Grandmother	
Grandfather	
Aunt	
Uncle	
Cousin	
Paternal Family	

Grandmother	
Grandfather	
Aunt	
Uncle	
Cousin	

18. What Procedure/s are you here for today?
    - ☐Screening Mammogram
    - ☐Diagnostic Mammogram
    - ☐Screening Ultrasound
    - ☐Diagnostic Ultrasound
    - ☐Biopsy
    - ☐Screening MRI
    - ☐Diagnostic MRI
    - ☐Other: _____

Study Participant Stop Here

Collection Date	Collection Time	Collectors Initials	Distance for sample A	Distance for Sample B
Sample Collection Notes:				

For lab use only:

Date Received		Date Stored	
Time Received		Time Stored	

Tear Based Breast Cancer Detection Clinical Information

Sample ID: **Collection date:** **Collection time:**

Initials: _____

Age: _____ **Patient Height: _____** **Patient Weight: _____**

1. **Please select Patient's Breast Density Category**:
   - ☐ Fatty
   - ☐ Scattered Fibroglandular Densities
   - ☐ Heterogeneously Dense
   - ☐ Extremely Dense

2. **Has patient had previous biopsies?**
   - ☐ Yes
   - ☐ No

3. **Has the patient had at least one biopsy with atypical hyperplasia?**
   - ☐ Yes
   - ☐ No

4. **For the procedures below, if conducted at your clinic, indicate procedure outcome:**

☐ Screening Mammogram		☐ Normal	☐ Called back	Date:
☐ Screening Ultrasound		☐ Normal	☐ Called back	Date:
☐ Screening MRI		☐ Normal	☐ Called Back	Date:
☐ Diagnostic Mammogram		☐ Normal	☐ Called Back	Date:
☐ Diagnostic Ultrasound		☐ Normal	☐ Called Back	Date:
☐ Diagnostic MRI		☐ Normal	☐ Called Back	Date:
☐ Breast Biopsy	☐ Benign	☐ Positive	☐ Atypical	Date:

5. **BiRads Score**
   - ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

6. **Which of the following was recommended for patient?**
   - ☐ 6 month follow up (not high risk)
   - ☐ 6 month follow up (high risk screening protocol)
   - ☐ Returned to normal screening
   - ☐ Clinical Exam follow-up (patient to young for yearly mammogram screening)

7. **Were calcifications identified?**
   - ☐ Yes
   - ☐ No

8. **If patient received a biopsy with Atypical results please indicate type of atypia:**
   - ☐ LCIS
   - ☐ Atypical Ductal Hyperplasia
   - ☐ Atypical Lobular Hyperplasia
   - ☐ Other (describe):

9. **If Benign please select or describe**:

- ☐ Hyperplasia of usual type
- ☐ Benign Calcifications
- ☐ Papilloma
- ☐ Sclerosing Adenosis
- ☐ Fat Epithelial Atypia
- ☐ Fibroadenoma
- ☐ Radial Scar
- ☐ Stromal Fibrosis
- ☐ Calcifications
- ☐ Fibrocystic Change

10. **The following information is requested for positive diagnosis:**

Breast Cancer type and location				**Receptors:**
☐ DCIS	☐ left	☐ right	☐ bilateral	☐ ER+
☐ IDC	☐ left	☐ right	☐ bilateral	☐ ER-
☐ ILC	☐ left	☐ right	☐ bilateral	☐ HER2 +
☐ Inflammatory	☐ left	☐ right	☐ bilateral	☐ HER2 –
☐ IMC	☐ left	☐ right	☐ bilateral	☐ PR +
☐ Other:				☐ PR-

11. **Metastasis:**

- ☐ Yes: _____
- ☐ No

12. **Tumor Grade**

- ☐ GX
- ☐ G1-low grade
- ☐ G2-intermediate grade
- ☐ G3-high grade
- ☐ G4-high grade

13. **Stage if known:**

- ☐ 0
- ☐ Ia ☐ Ib
- ☐ IIa ☐ IIb
- ☐ IIIa ☐ IIIb ☐ IIIc
- ☐ Iva ☐ IVb

14. **Ki67**

- ☐ High
- ☐ Borderline
- ☐ Low

15. **Tumor/s Size**