

I. GENERAL INFORMATION

Device Generic Name: RT-PCR multigene expression test, sentinel lymph node, cancer metastasis detection

Device Trade Name: GeneSearch Breast Lymph Node (BLN) Test Kit

Applicant's Name and Address: Veridex, LLC
33 Technology Drive
P.O. Box 4920
Warren, NJ 07059

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II. INDICATIONS FOR USE

The GeneSearch™ Breast Lymph Node (BLN) Assay is a qualitative, in vitro diagnostic test for the rapid detection of greater than 0.2 mm metastases in nodal tissue removed from sentinel lymph node biopsies of breast cancer patients.

Results from the assay can be used to guide the intra-operative or post-operative decision to remove additional lymph nodes. Post-operative histological evaluation of permanent sections of the tissue specimen, in accordance with usual diagnostic practice and using the Veridex lymph node cutting scheme, is required.

III. CONTRAINDICATIONS

There are no known contraindications for the GeneSearch™ BLN test kit

IV. WARNINGS AND PRECAUTIONS

Warnings, precautions, and limitations can be found in the labeling.

V. DEVICE DESCRIPTION

The presence of metastases in axillary lymph nodes is one of the most important prognostic indicator in breast cancer. H&E histology remains the preferred method to identify the

appropriate cancer type and other tumor characteristics and to measure the size of breast cancer metastases in lymph node tissue. Metastases are often categorized based upon size of the observed clusters into several categories, > 2 mm, between 0.2 and 2 mm, less than 0.2 mm and in isolated clusters of at least 10 cancer cells, or less than 0.2 mm and observed as isolated cells. The GeneSearch™ BLN Assay has been designed to detect the presence of metastases >0.2 mm in lymph nodes.

When SLN dissection (SLND) is conducted, typically the patient undergoes complete ALN dissection (ALND) only when one or more sentinel lymph nodes are positive for the presence of metastases. Patients with negative sentinel lymph nodes are spared the morbidity associated with complete ALND. Patients who undergo ALND have significantly higher rates of increased swelling in the upper arm and forearm (lymphedema), pain, numbness, and motion restriction about the shoulder when compared with patients who undergo only SLND. Rapid assessment of the cancer status of sentinel lymph nodes permits the completion of lymphadenectomy of the nodal basin during the same operative procedure, if required, thus avoiding a second surgery.

The GeneSearch™ BLN Assay provides intra-operative (or post-operative) information additionally provided by post-operative formalin-fixed paraffin-embedded Hematoxylin and Eosin (H&E) histology and immunohistochemistry, i.e., the absence or presence of lymph node metastases in patients that have been diagnosed with breast cancer.

A. Principles of the Procedure

The GeneSearch™ BLN Assay is a real time RT-PCR assay that detects the presence of breast tumor cell metastasis in lymph nodes through the detection of gene expression markers present in breast tissue, but not in nodal tissue (cell type specific messenger RNA). This assay employs real time RT-PCR utilizing the Cepheid SmartCycler® system to generate expression data for these genes. The expression results are then applied against predetermined criteria to provide a qualitative (cancer positive/cancer negative) result. Results of the assay correlate with detection of metastasis by permanent section H&E histology.

B. Real Time RT-PCR Reaction

The GeneSearch™ BLN Assay qualitatively detects the expression of two genes, Mammaglobin (MG) and Cytokeratin 19 (CK19), which are expressed at a high level in tissue of breast origin but only at low expression levels in normal lymph node tissue. The specificity of MG for breast tissue and its usefulness in the detection of metastatic disease in lymph nodes, blood and marrow has been reported.^{1,2} CK19 is an epithelial cell marker that has been frequently associated with breast cancer in lymph nodes, bone marrow and blood, and is expressed at levels a million-fold higher in cells associated with cancer compared to normal cells.³ Using tissue RNA from all available sentinel lymph nodes of 254 subjects, the combination of MG and CK19 was found to be optimal for the detection of breast cancer metastasis to lymph nodes.⁴

In order to maximize the uniformity of distribution of tissue sampling, lymph nodes are divided into sections and alternating sections are combined and processed using the GeneSearch™ BLN Assay. The remaining sections are used for routine histologic evaluation.

Using the GeneSearch™ RNA Sample Preparation Kit, the nodal tissue is homogenized to release RNA molecules. The RNA is purified from the tissue homogenate and RT-PCR is performed on the RNA specimen.

The real time RT-PCR reaction is performed in a homogeneous, one-step, fully contained reaction. Three gene markers (MG, CK19 and an internal control gene [IC; porphobilinogen deaminase, PBGD]) are included in this reaction. A complementary DNA (cDNA) strand is produced from messenger RNA (mRNA) using the reverse transcriptase function of a thermostable DNA polymerase. The reaction containing marker-specific DNA primers and probes, deoxyribonucleoside triphosphates [dNTPs] and DNA polymerase in a buffer is heated to activate the DNA polymerase and then cooled to allow specific annealing of the target-specific reverse (antisense) primers to the target mRNAs. The annealed primers are extended by the DNA polymerase in the presence of excess dNTPs to form cDNA strands.

Following production of cDNAs, the reaction mixture containing the cDNA:RNA hybrid is again heated to denature the strands. The reaction mixture is cooled, allowing the target-specific forward (sense) primers to anneal, and allowing the DNA-dependent DNA polymerase activity to extend the sense strand through to the reverse primer regions. This amplification process results in double-stranded DNA sequences called amplicons. Subsequent cycles of denaturation and annealing/extension exponentially increase the amounts of these amplicons, which are subsequently detected utilizing sequence-specific DNA probes.

C. Detection of Gene Markers

Production of target amplicon is detected using a probe that contains a DNA sequence specific for part of the target amplicon. This probe is linked to a fluorescent molecule and a molecule that quenches fluorescence. The probe initially anneals to the target sequence and then is cleaved by the exonuclease activity of the DNA polymerase as extension from the primer proceeds past the probe region. As a result of this cleavage, the fluorescent molecule is separated from the quencher, leading to an increase in fluorescence. By measuring fluorescence, the presence of target amplicon can be detected.

Each gene marker is detected using fluorescent molecules with different excitation and emission wavelengths. Fluorescence for each of the gene markers is measured following each temperature cycle. Amplification of the gene markers is detected through increased fluorescence due to release of the fluorophore from the proximity of the quencher. The Ct value is determined when the fluorescent signal exceeds a pre-defined threshold limit. If the external controls are valid, then the Ct value for each gene marker in the patient sample is compared to marker-specific Ct cutoff values. If the Ct value for either or both gene markers is less than the cutoff value then the sample is determined to be positive. All amplification, detection of fluorescence, and the interpretation of the signals is done automatically by the SmartCycler® instrument.

VI. ALTERNATIVE PRACTICES OR PROCEDURES

Currently, excised sentinel lymph nodes are evaluated post-operatively by the method of formalin-fixed paraffin-embedded Hematoxylin and Eosin (H&E) (permanent section) histology and immunohistochemistry, i.e., the absence or presence of lymph node metastases in patients that have been diagnosed with breast cancer.

Intraoperative techniques commonly available during sentinel lymph node biopsy surgery include frozen sections (FS) and imprint cytology (IC). The performance of FS as an intraoperative test depends both on the skill of the pathologist, as well as of the type of breast cancer, lobular cancers generally being more difficult to determine.¹⁵ Although the specificity of both FS and IC methods is high, there is considerable evidence to support that both methods suffer from poor accuracy.¹⁵ Based on recent reviews,^{7,16,17} IC has an accuracy ranging from 79-98% and a false-negative rate from 9-52%; whereas the accuracy of FS ranges from 77-99% with a false negative rate from 5 to 70%.

VII. MARKETING HISTORY

The Veridex GeneSearch™ BLN Assay has not yet been marketed as an *in vitro* diagnostic device in the USA, Canada, Japan or Australia. The product was recently CE-marked in Europe as an *in vitro* diagnostic device (Declaration of Conformity signed June 7, 2006). The product has been in clinical trials as an investigational use only (IUO) device in the United States and was available as a performance evaluation only device in Belgium and Italy. The device has not been withdrawn for any safety issues.

The Cepheid SmartCycler® instrument system and core software have been in use in the United States as a device for use with IVD assays since Nov. 18, 2002. Cepheid conducted a recent field correction of instrument software anomaly when used with a different *in vitro* diagnostic device. This software anomaly did not effect the GeneSearch™ BLN Assay software. The software anomaly has been corrected by Cepheid.

VIII. ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The GeneSearch™ Breast Lymph Node (BLN) Assay may be used in conjunction with sentinel lymph node biopsy for a patient who has been counseled on use of this test and has been informed of its performance. False positive results may be associated with increased morbidity, usually due to effects of axillary node dissection surgery. Patients who undergo ALND have significantly higher rates of increased swelling in the upper arm and forearm (lymphedema), pain, numbness, and motion restriction about the shoulder when compared with patients who undergo only SLND. False negative and inconclusive test results may be associated with delayed axillary node dissection. Clinical studies so far are inconclusive about a benefit from treatment based on findings of breast cancer micrometastases in sentinel lymph nodes.

Refer to the patients' and physicians' brochures, which are available on the Veridex Web Site or by contacting Veridex Customer Technical Services.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Precision

The precision of the GeneSearch™ BLN Test Kit was determined using a protocol similar to that recommended in the Clinical Laboratory Standards Institute Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (EP05-A2). Two samples and two assay controls were tested on the GeneSearch™ BLN Test Kit using three operators testing both samples and controls on three lots of GeneSearch™ BLN Test Kits each day for eight days with two runs per day and with two

replicates of each sample and control tested on each run. The Ct values obtained for each applicable marker in each sample were analyzed to determine the standard deviation of the measurements. Lot-to-lot and operator-to-operator variability were also considered in the reproducibility study design.

The reproducibility results are provided in Table 1.

Table 1. Total Run Precision Including Lot and Operator

Total Precision (%CV) Including Lot and Operator				
	Positive Sample	Negative Sample	Negative Control (NC)	Positive Control (PC)
PBGD	5.6%	5.2%	2.5% (3.4%) c	NA b
MG	5.5%	NA a	NA b	1.8% (6.1%) c
CK19	2.5%	NA a	NA b	1.9% (5.6%) c

- MG and CK19 were not analyzed as these markers are not expressed at appreciable levels in a negative sample. Negative results were obtained as expected in samples not containing these markers.
- MG and CK19 are not present in the Negative Control. PBGD is not present in the Positive Control. Negative results were obtained as expected in samples not containing these markers.
- Values, if results from samples where control was not added (user error), to the reaction are included in the analysis.

As part of the main clinical study, two operators from each of three sites participated in a reproducibility study. All operators tested a sponsor-provided reproducibility panel composed of human axillary lymph node tissue homogenate supplemented, when needed, with in vitro transcript of high or low levels of Mg and/or CK-19. There were a total of four panel members, one being negative for either marker. Starting from the RNA isolation step, each operator tested panel samples in duplicate in each run using three different lots of the GeneSearch™ BLN test kit. Samples were tested with the same lot of reagents on two separate days by each operator. The study design resulted in a total of 72 planned replicate results for each of the four panel members across all lots, sites, days, and operators. The GeneSearch™ BLN test kit results were in 100% agreement with the known presence or absence of target for all individual markers (PBGD, MG and CK19). Percent Coefficient of Variations (CVs) for all marker Ct values were ≤ 6.82 % for intra-run, inter-run, inter-site, inter-operator and inter-lot analyses. Standard deviations (SDs) were ≤ 1.88 in all cases.

These data show that GeneSearch™ BLN test kit results are reproducible across sites, operators, lots, days and within runs.

Variability of Ct Values by Variability Sources. Ct values of invalid MG and CK19 results due to IC failure are not included in the analysis.

Panel		Agreement		Intra-Run		Inter-Run		Inter-Site		Inter-Oper		Inter-Lot	
		%	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
A (n=74)	MG: Neg	100	39.92	0.45	1.12	0 ^a	0	0 ^a	0	0.14	0.36	0	0
	CK19: Neg	100	37.44	1.88	5.03	1.60	4.28	1.11	2.96	0 ^a	0	0.87	2.31
	PBGD: Pos	100	28.47	0.90	3.15	0.41	1.45	0 ^a	0	0.39	1.37	0.94	3.31

Panel		Agreement		Intra-Run		Inter-Run		Inter-Site		Inter-Oper		Inter-Lot	
		%	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
B (n=72)	MG: Low Pos	100	23.19	0.51	2.18	0.17	0.73	0	0	0.29	1.26	0.71	3.05
	CK19: Neg	100	39.54	1.02	2.57	1.06	2.69	0.48	1.22	0.72	1.82	0.18	0.44
C (n=74)	MG: Neg	100	37.98	1.11	2.93	0.30	0.79	0.31	0.82	0 ^a	0	1.18	3.11
	CK19: Low Pos	100	27.96	0.30	1.06	0.25	0.90	0 ^a	0	0.25	0.88	0.74	2.63
D (n=72)	MG: High Pos	100	17.66	0.56	3.19	0.16	0.91	0.37	2.11	0.42	2.36	0.61	3.47
	CK19: High Pos	100	20.03	1.37	6.82	0.95	4.76	0.28	1.38	0.83	4.12	0.57	2.85

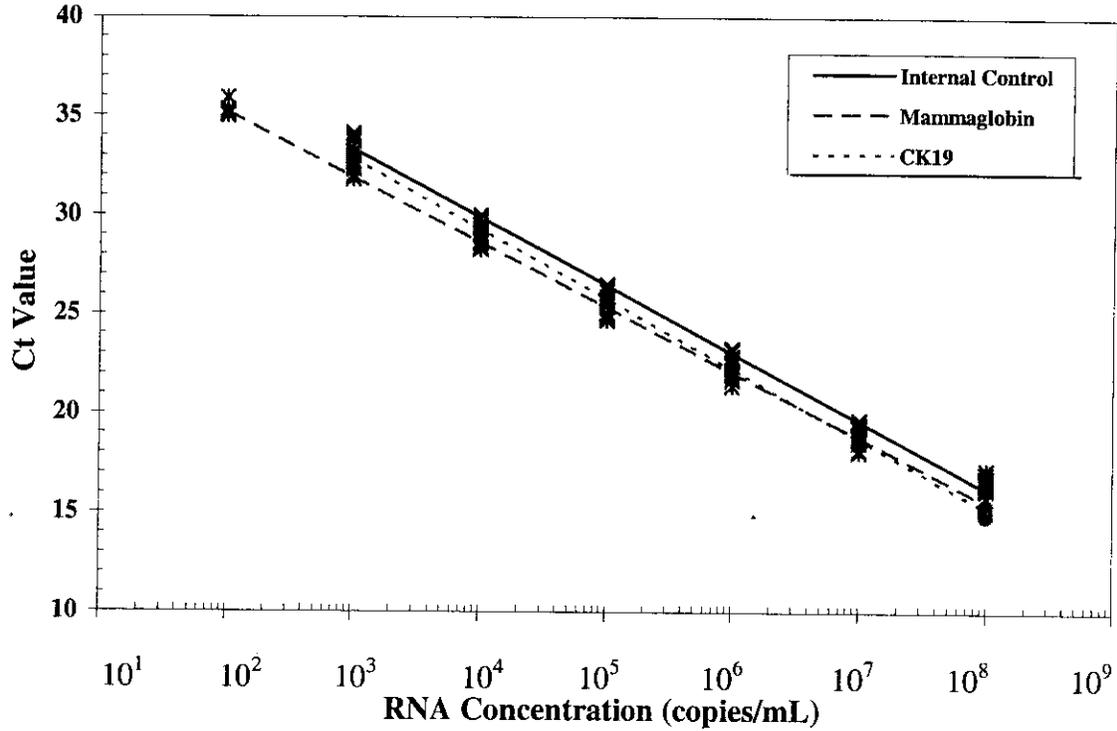
a. According to NCCLS (CLSI) guideline EP05-A2, variance components less than 0 are recorded as 0.

B. Linearity

The linearity of the GeneSearch™ BLN Test Kit was assessed by preparing samples containing known amounts of *in vitro* transcript (IVT) RNA for each marker, testing these samples on the GeneSearch™ BLN Test Kit, and directly comparing the Ct values obtained for each marker in each sample to the concentration of each marker in each sample by regression analysis. The regression line expresses the best prediction of the Ct value based on the concentration.

Samples were prepared by adding IVT RNA in log-fold increments (increases of 10 copies of IVT RNA/ μ L buffer) and tested on the GeneSearch™ BLN Test Kit. Samples were prepared with IVT RNA for one marker at a time (individual IVT RNA) and in combination with each other (IVT RNA Mix). The assay met the criteria for a linear response between the lowest level detected and 10⁸ (8 log) copies per μ L for all markers tested.

Figure 1: Linearity and Detection Limits



C. Limits of Detection

A theoretical limit of detection was calculated using the equation generated by regression analysis during linearity testing. The limit of detection is defined as the number of copies of the target sequence detected at 35.9 Ct, the highest value that can be obtained with the GeneSearch™ BLN Assay thermal cycling protocol. Data is shown in Table 2 below.

Table 2. Detection Limits

Individual IVT RNA

	Equation of the Line $Y = b - (m * (\log_{10}(\text{copies}/\mu\text{L})))$	Analytical Detection Limit
IC Ct	PBGD Ct = $44.50 - 3.571 * \log_{10}(\text{copies}/\mu\text{L})$	$10^{2.4}$
MG Ct	MG Ct = $41.62 - 3.265 * \log_{10}(\text{copies}/\mu\text{L})$	$10^{1.8}$
CK19 Ct	CK19 Ct = $43.62 - 3.559 * \log_{10}(\text{copies}/\mu\text{L})$	$10^{2.2}$

IVT RNA Mix

	Equation of the Line $Y = b - (m * (\log_{10}(\text{copies}/\mu\text{L})))$	Analytical Detection Limit
IC Ct	PBGD Ct = $43.58 - 3.419 * \log_{10}(\text{copies}/\mu\text{L})$	$10^{2.3}$
MG Ct	MG Ct = $41.7 - 3.269 * \log_{10}(\text{copies}/\mu\text{L})$	$10^{1.8}$
CK19 Ct	CK19 Ct = $43.38 - 3.521 * \log_{10}(\text{copies}/\mu\text{L})$	$10^{2.1}$

D. Amplification efficiencies

Using the data from the line equations presented above in conjunction with the following equation for efficiency, $Efficiency (E_x) = 10^{(-1/slope)} - 1$, the following amplification efficiencies are obtained:

Table 3: Amplification Efficiencies

Marker	Slope from Individual IVT Sample (\pm standard deviation)	Slope from Mix IVT Sample (\pm standard deviation)	Efficiency from Individual IVT Sample	Efficiency from Mix IVT Sample
PBGD	3.571 (± 0.025)	3.419 (± 0.029)	90.6%	96.1%
MG	3.265 (± 0.044)	3.269 (± 0.040)	100%*	100%*
CK19	3.559 (± 0.019)	3.521 (± 0.015)	91.0%	92.3%

*NOTE: All efficiency values calculated to be >100% were reported as 100%.

All average efficiencies are greater than 90%. Amplification efficiencies were not substantially different when amplified individually or as a mixture.

E. Guard band studies

To evaluate the potential limitations to the assay resulting from procedural variations (user changes to assay step-by-step procedure), the sponsor performed a series of experiments to evaluate items in the RNA preparation kit and assay test kit such as

- variations in the concentration of various general purpose reagents
- order of addition of reagents, the length of time for tissue homogenization
- stability of RNA after homogenization prior to addition to separation columns
- amount of tissue by weight yielding suitable quality and quantity of RNA
- stability and maximum hold times of purified RNA prior to PCR setup
- volume of PCR reagents utilized to obtain a suitable signal

Such information was used to make recommendations in the package insert. See the test procedure steps, limitations, warnings and precautions, as well as interfering substances section of the labeling for information.

X. SUMMARY OF CLINICAL STUDIES

A. Overview

Two prospective, multi-site U.S. clinical studies were conducted with the investigational device, a Cutoff study and a Pivotal Study. The purpose of the Cutoff study was to determine the final assay cutoff values for calling patients either assay positive or assay negative by the assay software. The purpose of the Pivotal study was to validate the cutoffs used and provide data on safety and effectiveness of the investigational device. The two studies were nearly identical in design and execution, but the Cutoff study used a subset of clinical sites and assay operators used in the Pivotal Study.

B. Cut-off Study

1. Study Objective

The primary objective was to gather data necessary to determine the cutoff values for the assay markers porphobilinogen deaminase (PBGD), MG, and CK19. Marker performance was evaluated against permanent section hematoxylin and eosin results and immunohistochemistry of lymph nodes removed during sentinel lymph node biopsy surgery from subjects aged 18 years or older having a previous diagnosis of breast adenocarcinoma.

2. Secondary Objectives

A secondary objective was to collect long-term clinical outcome data to evaluate the assay and other markers as prognostic markers for long term survival (either overall survival or progression-free survival).

3. Study Endpoints

Sensitivity and Specificity of assay was evaluated as a function of assay marker cut-offs.

4. Study Hypothesis

None

5. Study Methodology

A prospective multi-site clinical trial (Beta Study) was conducted in which assay data on 76 subjects were evaluated to obtain preliminary cut-off values for the three assay markers. An additional 198 subjects sequestered from the Pivotal Study were included in the cutoff analysis, for a total of 274 subjects evaluated.

6. Study Results

Twelve study sites enrolled 338 subjects into this study. Twenty-six subjects were initially excluded. Of the 312 remaining subjects a further 38 were removed as a result of invalid assay results (external or internal control failures). A total of 274 subjects were included in the final analysis.

The Beta dataset provided the basis for the external control QC ranges and the internal control cut-off of PBGD > 36 Ct. The internal control was re-evaluated with the inclusion of the additional 198 subjects sequestered from the Pivotal Study. After investigating potential internal control failures and sources of variability in internal control results, a decision was made to maintain the PBGD cut-off at > 36 Ct.

In choosing cut-offs for CK-19 and MG to determine assay positivity or negativity, the goals were to achieve specificity of least 95% for patient safety and sensitivity of at least 90% for effectiveness. The cut-offs $CK-19 \leq 30$ Ct or $MG \leq 31$ Ct were ultimately chosen because they maximized specificity at 95.9% while achieving sensitivity 91.1% using the lowest cutoffs possible to maximize specificity in independent data. Therefore, for subsequent validation of the assay, a subject was defined to be assay positive for SLN metastasis if $CK-19 \leq 30$ Ct or $MG \leq 31$ Ct, provided internal and external controls give valid results.

C. Pivotal Study

1. Study Objective

The primary objective was to gather the data necessary to support safety and effectiveness of the GeneSearch BLN Assay to determine metastatic status of the nodes of subjects with invasive breast cancer. The cutoffs $CK-19 \leq 30$ Ct or $MG \leq 31$ Ct and $PBGD > 36$ Ct selected in the Cutoff Study were used by the assay software to define subjects as assay positive or negative or having an invalid result.

2. Secondary Objectives

A secondary objective was to collect long-term clinical outcome data to evaluate the assay and other markers as prognostic markers for long term survival (either overall survival or progression-free survival).

3. Study Endpoints

The primary endpoints were sensitivity and specificity of the assay.

4. Study Hypothesis

The primary hypotheses were that assay sensitivity was 70% or better and assay specificity was 90% or better. These hypotheses were to be shown with statistical significance, that is, by indication that the target values were greater than the lower limit of the two-sided 95% confidence interval for the parameter in question.

5. Study Methodology

The study was prospectively conducted at 11 clinical sites on 421 evaluable subjects aged 18 years or older having a previous diagnosis of invasive adenocarcinoma of the breast and previously scheduled for sentinel lymph node dissection. Sentinel lymph node tissue identified by standard locating techniques was removed using each site's intra-operative procedure. Patient node tissue was processed intra-operatively in the proposed assay. The clinical site used alternating tissue slabs for histology and the proposed assay. Each site made a determination of the lymph node status (i.e. breast cancer metastases and further full lymph node dissection) independent of the proposed assay using site-specific criteria and pathological methods. Patient node tissue was also evaluated by permanent section staining histopathology after intra-operative tissue removal. Permanent section histopathology of nodes was evaluated by site pathologists and by a panel of at least 2 of 3 central pathologists. Cancer metastasis > 0.2 mm in size was considered as histologically and clinically significant. Performance characteristics of the proposed assay were compared with permanent section histopathology. Assay results were not used to make subsequent treatment or surgical decisions.

The combination of permanent section hematoxylin and eosin staining (H&E) and immunohistochemistry (IHC using site specific techniques) was used as the comparator test method in these studies to determine the performance of the GeneSearch™ BLN Assay. All histology positive samples in the study data set were confirmed by two independent pathologists. The Pathologists were blinded to all GeneSearch™ BLN Assay results. An "Overall Histology" result was derived from all permanent section H&E and IHC results obtained on the subjects' nodes. The node (and patient) was considered histology positive if

either Site Slides or Central Slides were confirmed positive. For either Site Slide or Central Slide evaluation, if only two pathologists' results were available with one being positive and one negative, the final result was considered undetermined (UND).

For assay performance calculations, histology was divided into two discrete categories of positive or negative, with positive being a metastasis > 0.2 mm, and negative being no detectable metastasis or metastases no larger than 0.2 mm. However, for more in depth analyses, histological results were divided into the following six categories in order of increasing levels of positivity:

N – negative, no evidence of tumor cells;

N(ITC) - isolated tumor cells only;

N(CL) – tumor cell clusters < 0.2 mm;

P(MI) - micrometastasis > 0.2 – 2 mm;

P - metastasis > 0.2 but of unknown specific size; and

P(MA) - macrometastasis > 2.0 mm.

The first three categories were considered negative from a clinical perspective, the last three were considered positive. When either the final Central Slide and/or final Site Slide histology result was positive, the Overall Histology result for the node was positive. If one set of slides had a final result of negative for a node, and the other was UND, the final Overall Histology result for that node was considered UND. For P(MA), P(MI), N(CL), N(ITC), or N, the Overall Histology result was always the more positive of the final Central or final Site Slide result.

Though not required by the clinical protocol, the sites' intraoperative frozen section or touch preparation results were collected to compare the performance of these intraoperative methods to that of the GeneSearch™ BLN Assay when each was compared against permanent section histology. There were no Central Pathologist readings of the intraoperative histology slides. The clinical trial sites collected intraoperative results for patient management either on a rare basis on special request from a given surgeon, or as standard practice for all patients undergoing SLND. Frozen sections were taken only from the node slabs being used for histology. Intraoperative touch preparations could be taken from any node slab, including those to be tested in the GeneSearch™ BLN Assay. Data analysis did not plan to provide support for the use of these other intra-operative techniques in conjunction with the GeneSearch™ BLN assay. There has been no systemic study of the combined use of the GeneSearch™ BLN test kit with other intraoperative techniques to manage patients with some combination of tests.

a) Patient inclusion/exclusion criteria

Inclusion criteria:

- previous diagnosis of invasive adenocarcinoma of the breast
- patient scheduled for sentinel lymph node dissection
- 18 years or older

- female or male, and
- able and willing to give consent to participate in the study.

Exclusion criteria:

- Patients taking part in other research studies that would interfere with their full participation in this study, or
- Prior sentinel or partial axillary lymphadenectomy on the same body side as the scheduled SLN dissection.

b) Sample handling and testing

All sentinel nodes were bisected along the short axis. Nodes 6.0 mm or less in length were bisected to produce two (2) node tissue slabs. Larger nodes were cut along the short axis into an even number of node tissue slabs of approximately the same thickness. All node slabs were between 1.5 - 3.0 mm in thickness. There was an equal number of tissue slabs for histology and the GeneSearch™ BLN Assay. Approximately half of the node was immediately processed as fresh tissue in the BLN Assay. The other half was used for standard site pathology and for additional H&E and IHC (for this clinical study).

After any desired tissue preparation had been performed (if any), alternating node tissue slabs from the same node were combined and subjected in total to processing and testing in the GeneSearch™ BLN Assay. Remaining tissue slabs not used for the GeneSearch™ BLN Assay were processed for permanent section H&E for patient management using standard site procedures (Site Slides).

Additional slides were also prepared from the fixed tissue for shipment to the study Central Pathologists for H&E evaluations (Central Slides). Central Slide sections were 4 to 6 µm thick, and three (3) sections were taken from each 1.5 mm to 3.0 mm fixed node slab. The three (3) sections were taken from levels approximately 150 µm apart. This sectioning plan is equivalent or exceeds recommendations of the American Society for Clinical Oncology for sentinel lymph node biopsy sectioning procedures. Each site determined the number and levels of H&E sections to be evaluated by the site for patient management. IHC evaluations were done when H&E sections were found negative. For each subject there were two separate sets of H&E slides (Site and Central) and for H&E negative subjects, one set of IHC slides.

c) General patient demographics and results

The total of 423 subjects were enrolled aged 18 years or older having a previous diagnosis of invasive adenocarcinoma of the breast and previously scheduled for sentinel lymph node dissection. Two subjects had no assay or histology data available since the removed nodes were too small to share and insure adequate patient management. For the validation study, 421 subjects were utilized.

Subject age ranged from 27 to 92 years with a mean age of 60. Nine subjects had chemotherapy and one had radiation therapy. The majority of subjects (80.4%) were diagnosed with invasive ductal cancer either alone or in combination with other breast cancer types. There were 13.9% of subjects with invasive lobular cancer but no invasive ductal cancer, and 5.7% with invasive cancer other than lobular or ductal. The majority of the subjects had either Stage I breast tumors (62.3%) or Stage II (32.0%). There were 5.3% with

Stage III and 0.5% with Stage IV. Often, subjects were estrogen receptor positive (79.2%), progesterone receptor positive (67.8%), and HER-2 negative (74.2%).

The mean and median numbers of nodes removed were 2.9 and 2, respectively. Two of the 423 subjects had no assay or study histology data available due to their nodes being too small to share tissue for investigative purposes without compromising patient management.

Of 421 subjects remaining, 5 patients had Overall Histology results listed as undetermined and were not included in performance calculations. The overall prevalence of cancer metastases to lymph nodes detected by histology was 29.1% (121 positive subjects in 416 subjects). The prevalence of lymph nodes with metastatic cancer ranged from 14.3% to 45.5% by clinical site. There were 120 subjects positive by H&E and one subject positive by IHC alone (metastasis size classified as 0.2 to 2 mm). Among the 121 histological positives, 94 (78%) were P(MA) (macrometastasis > 2 mm), 23 (19%) were P(MI) (metastases between 0.2 and 2 mm), and 4 (3%) were classified as P (positive without size of metastases specified beyond > 0.2 mm). Among the 295 histology negative subjects, 275 (93%) were classified as N (completely negative), 14 (5%) were N(ITC) (negative with isolated tumor cells), and 6 (2%) were N(CL) (negative with tumor clusters).

Of the 421 subjects evaluable, the assay result was invalid in 34 (8.1%). The invalid result was due to external control or subject sample failure. The sponsor states that these invalid results were not excluded. For the purpose of statistical analysis, these subjects were classified as assay negative since the results do not provide a clinician with evidence of nodal metastases.

The average Ct values for each marker in all study subjects with valid assay results are as follows:

Overall Histology	mean Ct value		
	MG	CK-19	IC
Positive	28.7	24.0	31.3
Negative	39.0	37.2	29.9
All	35.9	33.2	30.3

Of 94 subjects with macrometastases, 97.9% (95% confidence interval 92.5% to 99.7%) were positive in the assay (i.e. sensitivity for macrometastases). Of 322 subjects without macrometastases, 94.0% (95% confidence interval 86.6% to 93.4%) were negative in the assay (i.e. specificity for macrometastases). Of 23 subjects with micrometastases, 56.5% (95% confidence interval 34.5 – 76.8%) were assay positive. Of 299 subjects without micrometastases or macrometastases 94.0% (95% confidence interval 90.7% to 96.4%) were assay negative.

6. Primary Overall Analysis (Intent to Diagnose)

The primary analysis of performance of the assay was based on the 416 subjects with defined Overall Histology results. (Five subjects with Overall Histology results of UND were not

included.) These included 34 subjects for whom the assay result was invalid due to external control or subject sample failure. For the purpose of an intent-to-diagnosis statistical analysis, these subjects were classified as assay negative since the results do not provide a clinician with evidence of nodal metastases.

Using Overall Histology as the reference (derived from all site and central permanent section H&E and IHC results), the following 2 x 2 contingency table comparing the assay to Overall Histology is obtained:

GeneSearch result	positive	negative	total
Positive	106	17	123
Negative	15	278	293
Total	121	295	416

The prevalence of node positive subjects was 123/416 (29.1%). The 95% confidence interval (CI) was 24.8-33.7%.

The sensitivity of the assay, i.e., proportion of histology positive subjects that were assay positive, was 106/121 (87.6%, 95% CI 80.4-92.9%). Because the lower limit of the 95% CI is 80.4%, the primary hypothesis of sensitivity > 80% was demonstrated. The corresponding proportion of assay false negative results among histology positive subjects was 15/121 (12.4%, 95% CI 7.1-19.6%).

The sensitivity of the assay among 94 subjects with histologic macrometastases was 92/94 (97.9%, 95% CI 92.5-99.7%). The sensitivity of assay among 23 subjects with histologic micrometastases was 56.5% (95% CI 34.5 – 76.8%). Among four subjects classified as P, the assay was positive in one.

The specificity of the assay, i.e., proportion of histology negative subjects that were assay negative, was 278/295 (94.2%, 95% CI 90.9-96.6%). Because the lower limit of the 95% CI is 90.9%, the primary hypothesis of specificity > 90% was demonstrated. The corresponding proportion of assay false positive results among histology negative subjects was 17/295 (5.8%, 95% CI 3.4-9.1%).

The specificity of the assay among 275 subjects who were histologically completely negative (N, no tumor seen) was 263/275 (95.6%, 95% CI 92.5-97.7%). The specificity of assay among 14 who were histologically negative but with isolated tumor (N(ITC)) cells was 71.4% (95% CI 41.9 – 91.6%). Among six subjects classified as histologically negative but with clusters (N(CL)), the assay was negative in five.

The positive predictive value (PPV) of the assay, i.e., proportion of assay positive subjects that were histology positive, was 106/123 (86.2%, 95% CI 78.8-91.7%). The corresponding proportion of assay positive subjects who were false positive (histology negative) was 17/123 (13.8%, 95% CI 9.3-21.2%).

The negative predictive value (NPV) of the assay, i.e., proportion of assay negative subjects that were histology negative, was 278/293 (94.9%, 95% CI 91.7-97.1%). The corresponding proportion of assay negative subjects who were false negative (histology positive) was 15/278 (5.1%, 95% CI 2.9-8.3%).

The differences in results between the GeneSearch™ BLN Assay and Overall Histology could be due to tissue sampling since the assay evaluated different portions of the lymph node than did histology. This conclusion is supported by differences between the histological evaluation of the two sets of H&E slides collected; a comparison of site pathologist results on Site H&E Slides to the central pathologist results on Central H&E Slides described below. This is a comparison of H&E evaluations of anatomically close but different sections from the same portions of the lymph node.

Pooled results for 416 subjects were categorized by site and central H&E histology and stratified by GeneSearch assay result without removing subjects with invalid assay results as shown in the following:

Table 5		GeneSearch Assay positive				
		Central pathology				
Site pathology	> 2 mm	0.2-2 mm	<0.2 mm	negative	Total	
>2 mm	79	1	0	3	83	
0.2-2 mm	4	4	0	7	15	
<0.2 mm	0	1	0	2	3	
negative	2	0	0	15	17	
Result not available	2	3	0	0	5	
Total	87	9	0	27	123	

		GeneSearch Assay negative				
		Central pathology				
Site pathology	> 2 mm	0.2-2 mm	<0.2 mm	negative	Total	
>2 mm	1	2	0	1	4	
0.2-2 mm	1	5	0	2	8	
<0.2 mm	0	1	0	4	5	
negative	0	1	1	266	268	
Result not available	0	0	0	8	8	
Total	2	9	1	281	293	

		All assayed samples				
		Central pathology				
Site pathology	> 2 mm	0.2-2 mm	<0.2 mm	negative	Total	
>2 mm	80	3	0	4	87	
0.2-2 mm	5	9	0	9	23	
<0.2 mm	0	2	0	6	8	
negative	2	1	1	281	285	
Result not available	2	3	0	8	13	
Total	89	18	1	308	416	

When the assay is used intra-operatively, a positive assay result would indicate an 86% risk of metastatic breast cancer in the subject. When the assay result is negative, the risk of no metastatic breast cancer would be 95% (no worse than 91% based upon the lower 95% confidence interval of the negative predictive value). Therefore when a negative assay result occurs intra-operatively there is a high degree of confidence (at least 95%) that the subject is absent loco-regional breast cancer metastases and there is little need for complete axillary lymph node dissection to occur during the sentinel lymph node biopsy surgery. This high

degree of confidence when assay negative provides a reasonable clinical rationale for concluding that the subject does not yet have breast cancer metastatic by lymphogenous spread to the local axillary region. It further supports the conclusion that there is sufficiently little risk of metastatic cancer (at most 8% risk of metastatic cancer though assay negative) to not proceed to level I axillary node dissection and the associated morbidity from that operation. Thus the patient could be reasonably spared that surgical intervention without serious risk of metastatic breast cancer from lymphogenous spread of the disease.

The risk of metastatic breast cancer when assay positive is 86%; at least 79% and as much as 92%. The risk of lymphogenous spread of breast cancer is sufficiently high that there could be a justifiable clinical rationale for level I axillary node dissection and histopathological evaluation of other lymph nodes than sentinel lymph nodes. Balancing that risk is the absence of metastatic breast cancer though the assay is positive. In this study the rate of non-metastatic breast cancer though assay positive is estimated to be 14% and as much as 21% (1- lower 95% confidence limit of positive predictive value). This fact suggests that when assay positive that 21% of subjects at most would be subjected to an unnecessary level I axillary node dissection even though absent metastatic spread of breast cancer to the regional lymph nodes. The fact that at most 5% of breast cancer patients with metastatic disease would fail to have a needed surgical intervention because of a negative GeneSearch assay must also be clinically weighed with the fact that at most 21% of subjects would undergo an unnecessary surgical intervention because of a positive GeneSearch assay.

The sponsor chose to classify subjects with invalid assay results as assay negative. If the assay is invalid because the external controls or internal control were outside their specification limits, then the assay result would be classified as no test result. Since some of the invalid assay results could include histologically positive or negative results for the subject node status, a clinical decision would likely be made based on the final histology result when other intra-operative histology procedures have not been performed. In addition, some of the invalid assays could be assay positive as well as assay negative on subsequent re-testing of a clinical sample and so it appears appropriate for the laboratory to classify the assay results as a no test result. In some cases, there may be sufficient RNA homogenate to re-isolate RNA for re-testing of the specimen. If an invalid assay result would occur intra-operatively then it makes clinical sense to make no node determination based upon the proposed assay result in the absence of other intra-operative histology results but defer a clinical decision until a final H&E histology determination can be made. Therefore, it would not be unreasonable clinically to lack an assay result intra-operatively. It would clinically result in a deferred decision on the node status of the patient in the absence of other intra-operative histology results.

7. Evaluable Subjects Overall Analysis

In the primary overall analysis, subjects with assay negative results were treated as assay negative for the purpose including them in the analysis. When these subjects are excluded from analysis, the following 2 x 2 contingency table comparing the assay to Overall Histology is obtained:

Table 6 Central and site pathology (either or both)

GeneSearch result	positive	Negative	Total
Positive	106	17	123
Negative	11	249	260
Total	117	266	383

Sensitivity of the assay was 106/117 (90.6%, 95% CI 83.8-95.2%). The corresponding proportion of assay false negative results among histology positive subjects was 11/117 (9.4%, 95% CI 4.8-16.2%).

Specificity of the assay was 249/266 (93.6%, 95% CI 90.0-96.2%). The corresponding proportion of assay false positive results among histology negative subjects was 17/266 (6.4%, 95% CI 3.8-10.0%).

The positive predictive value of the assay was 86.2% ± 3.1%. The negative predictive value of the assay was 95.8% ± 1.2%.

PPV of the assay was the same as before, 106/123 (86.2%, 95% CI 78.8-91.7%). NPV of the assay was 249/260 (95.8%, 95% CI 92.6-97.9%).

The following shows the categorization of results by site and central H&E histology stratified by GeneSearch assay result when removing subjects with invalid assay results from calculation:

Table 7 Valid GeneSearch Assay positive

Site pathology	Central pathology				Total
	> 2 mm	0.2-2 mm	<0.2 mm	negative	
>2 mm	79	1	0	3	83
0.2-2 mm	4	4	0	7	15
<0.2 mm	0	1	0	2	3
Negative	2	0	0	15	17
#N/A	2	3	0	0	5
Total	87	9	0	27	123

Valid GeneSearch Assay negative

Site pathology	Central pathology				Total
	> 2 mm	0.2-2 mm	<0.2 mm	negative	
>2 mm	0	2	0	1	3
0.2-2 mm	0	3	0	2	5
<0.2 mm	0	1	0	3	4
negative	0	1	1	239	241
#N/A	0	0	0	7	7
total	0	7	1	252	260

All valid GeneSearch samples

Site pathology	Central pathology				Total
	> 2 mm	0.2-2 mm	<0.2 mm	negative	
>2 mm	79	3	0	4	86
0.2-2 mm	4	7	0	9	20
<0.2 mm	0	2	0	5	7
negative	2	1	1	254	258

#N/A	2	3	0	7	12
total	87	16	1	279	383

8. Comparison of GeneSearch BLN test kit results with two current intraoperative histological evaluations

A comparison of the proposed assay with 2 other intra-operative histology procedures, frozen section H&E staining and touch imprint cytology staining was performed. Frozen section H&E staining was performed at several clinical sites, though not all, and each site used its own procedure to section tissue and stain slides intraoperatively. In this comparison, 319 subjects had intra-operative frozen section results and 29 subjects had touch imprint cytology staining results along with GeneSearch test kit results. The results of the GeneSearch assay and frozen section histology (or touch imprint cytology) were compared with overall H&E histology and directly with each other. When compared with overall H&E histology (where final results are categorized as positive or negative by site or central pathologists or both), frozen section histology had a sensitivity of $85.6\% \pm 3.7\%$ (95% confidence interval 76.6% to 92.1%) and a specificity of $97.8\% \pm 1.0\%$ (95% confidence interval 95.0% to 99.3%). The calculations are derived from data as follows:

Table 8 Central and site pathology (either or both)

Frozen section pathology	positive	negative	total	
Positive	77	5	82	Sensitivity = 85.6%
Negative	13	224	237	Specificity = 97.8%
Total	90	229	319	

Note from the table that the prevalence of node positive disease (i.e. metastases to regional node) was $28.2\% \pm 2.5\%$ (95% confidence interval 23.3% to 33.5%). The probability that the positive predictive value of frozen section histology ($93.9\% \pm 2.6\%$) is equivalent with the prevalence of metastases to the regional node was less than 0.001 (Chi square value 230.807 for 1 degree of freedom). Therefore, it is possible to conclude that results from frozen section histology are significantly associated with results of final permanent section H&E histology.

When compared with overall H&E histology (where final results are categorized as positive or negative by site or central pathologists or both), the GeneSearch test kit had a sensitivity of $95.6\% \pm 2.2\%$ (95% confidence interval 89.0% to 98.8%) and a specificity of $93.9\% \pm 1.6\%$ (95% confidence interval 90.0% to 96.6%). The calculations are derived as follows:

Table 9 Central and site pathology (either or both)

GeneSearch result	positive	negative	Total	
positive	86	14	100	Sensitivity = 95.6%
negative	4	215	219	Specificity = 93.9%
total	90	229	319	

The prevalence of node positive disease (i.e. metastases to regional node) was $28.2\% \pm 2.5\%$ (95% confidence interval 23.3% to 33.5%). The probability that the positive predictive value of frozen section histology ($86.0\% \pm 3.5\%$) is equivalent with the prevalence of metastases to the regional node was less than 0.001 (Chi square value 236.026 for 1 degree of freedom).

Therefore, it is possible to conclude that results from the proposed assay are significantly associated with results of final permanent section H&E histology.

Table 10. Comparison of the GeneSearch™ BLN Assay performance to that of other intraoperative methods used in Pivotal Study sites.

Test	N	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)	PPV	NPV
BLN Test kit	319 ⁺	95.6 (89.0-98.8)	94.3 (90.5-96.9)	86.9	98.2
Frozen section histology	319 ⁺	85.6 (76.6-92.1)	97.8 (95.0-99.3)	93.9	94.5
Touch imprint cytology	29	45.5 (16.7-76.6)	100 (81.5-100)	100	75.0

Due to the limited sampling involved in current intraoperative techniques, adjacent and more thorough permanent section histology is likely to confirm metastases seen with frozen section or touch imprint cytology. The specificity of frozen section diagnosis is commonly reported as near 100%, perhaps due in part to the ability to re-examine the exact tissue used to make original frozen section diagnoses. Frozen section re-examination was not part of the current study, since an analogous histologic re-examination of the exact tissue for the GeneSearch™ BLN Assay is not possible.

A direct comparison of the sensitivities of the proposed assay and frozen section histology results with each other is shown in the following table:

GeneSearch result	Frozen section result		Total
	positive	negative	
Positive	75	11	86
Negative	2	2	4
Total	77	13	90

The sensitivity of the proposed assay in this comparison was 95.6% while the sensitivity of frozen section histology was 85.6%. The difference in sensitivity (10.0%, 95% confidence interval of the difference 17.6% to 2.4%) was statistically significant (p = 0.01). Therefore, in a direct comparison of the GeneSearch test kit and frozen section histology the GeneSearch kit has a statistically higher sensitivity than frozen section histology. The ratio of true positive subjects using the GeneSearch test kit to the true positive subjects in frozen section histology is 1.12 (95% confidence interval 1.04 to 1.19).

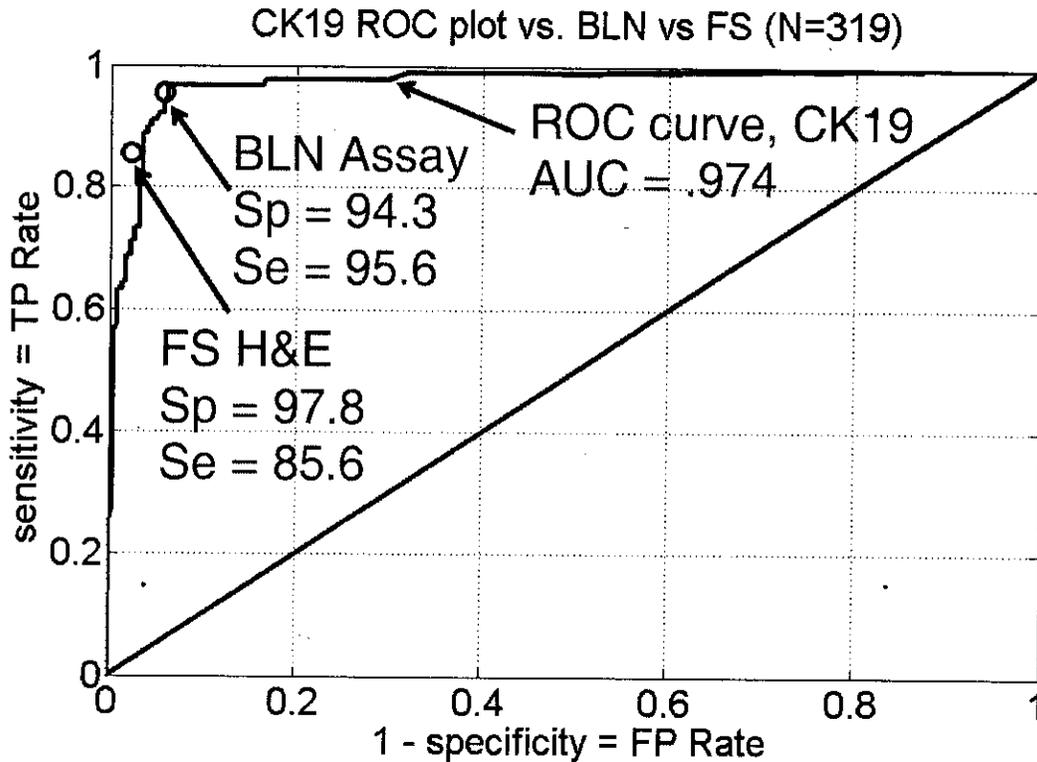
A direct comparison of the specificities of the GeneSearch test kit and frozen section histology results with each other is shown in the following table:

GeneSearch result	Frozen section result		total
	positive	negative	
Positive	0	14	14
Negative	5	210	215
Total	5	224	229

The specificity of the GeneSearch test kit in this comparison was 93.9% while the specificity of frozen section histology was 97.8%. The difference in specificity (-3.9%, 95% confidence interval of the difference -7.6% to -0.2%) was marginally significant statistically ($p = 0.037$). Therefore, in a direct comparison of the GeneSearch test kit and frozen section histology, the GeneSearch test kit has a marginally lower specificity than frozen section histology. Of note also from this data, the false positive rate of the GeneSearch test kit is 2.8-fold higher (95% confidence interval 2.44 to 3.16) than the false positive rate of frozen section histology (14 of 229 for the proposed assay compared with 5 of 229 for frozen section histology). In this regard, the true positive rate for the proposed assay is 1.12-fold higher than frozen section histology. Due to the small number of false positive subjects in this comparison, it is not clear if the ratio of false positive rates is clinically meaningful, though marginally and statistically different.

The operating characteristics of the assay (sensitivity, specificity, PPV, false positive rate) are specific to the cutoffs selected for the internal control PBGD and the MG and CK19 markers. If the cut-offs are changed, different operating characteristics are obtained. By varying the cut-off for a marker over its range of values, all possible pairs of sensitivity and specificity are obtained for that marker. A receiver operating characteristic curve plots all possible pairs of sensitivity and 1 – specificity. The ROC plot for the CK-19 marker is given in Figure 2. The specific sensitivity and 1-specificity pair for the BLN test kit and frozen section are superimposed on the plot. The Figure shows that the GeneSearch BLN test kit and frozen section are at different positions in relation to the curve for CK-19. To obtain operating characteristics for the GeneSearch BLN test kit more similar to frozen section, the cut-offs for the BLN assay would need to adjust, which is not possible as designed. For example, if the cut-offs for the MG and CK19 markers were adjusted from ≤ 31 Ct and ≤ 30 Ct, respectively, to ≤ 24.7 Ct and ≤ 25.5 Ct the GeneSearch BLN test kit would then have specificity 97.8% (222/229), the same as frozen section, but would have sensitivity 74.4% (67/90), lower than frozen section.

Figure 2. Receiver-Operator Characteristics curve for CK 19 marker as a backdrop against which to compare operating characteristics of BLN assay and FS.



The performance of the GeneSearch™ BLN Assay in combination with other intra-operative histological techniques has not been examined.

9. Pooling of data by site

The rate by site of positive lymph nodes per patient ranged from 9.1% to 44.4% when using site pathologist's categorization of node results. The mean overall rate of positive histology by site using site pathologist's H&E histology categorization was 26.0%. For the hypothesis that the mean rate of 26% was equivalent to the pooled rate of positive histology by site, the probability was 0.045 (Chi square value 18.65 for 10 degrees of freedom). The rate of positive node status by site appears to be statistically significant, though marginally so. It is not clear if the differences in rate of positive node status by site are significant clinically. In addition to the difference by site in rate of positive nodal histology, the mean age of subjects by site also was significantly different. The overall mean age was 60 years. The mean age by site ranged from 52 years to 67 years and was statistically significant ($p < 0.001$ by analysis of variance; F-value 5.02 for 10 and 410 degrees of freedom). The rate of positivity of estrogen receptor status of the primary tumor (expressed as positive or negative) for all subjects was 79.3%. The rate of positivity of estrogen receptor status by site was statistically significant ($p = 0.002$; F-value 2.83 for 10 and 386 degrees of freedom), ranging from 43% to 100%. The rate of positivity of HER/2 receptor by site (expressed as positive or negative) for all subjects was 26% and was not significantly different by site ($p > 0.05$; F-value 1.26 for 10 and 371 degrees of freedom). The size of the primary tumor for all subjects was 1.8 cm. The mean size of the primary tumor by site was not significantly different by site ($p > 0.05$; F-value 0.95 for 10 and 410 degrees of freedom). Of 5 patient characteristics, nodal status, age, and estrogen receptor status were significantly different by site while HER/2 receptor status

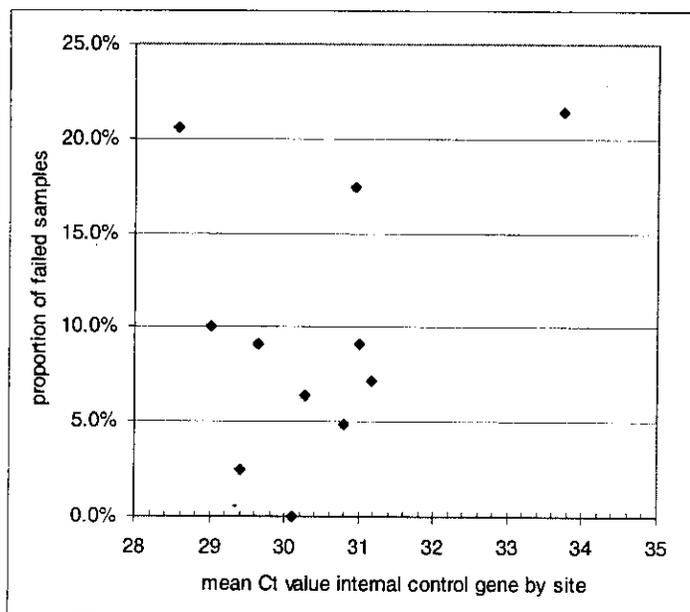
and tumor size were not significantly different by site. It is unclear if the differences by site in the clinical parameters of age, estrogen receptor status, and node status are meaningful.

Among three sites with at least 63 subjects, the worst sensitivity estimate was 77.8% and the worst specificity estimate was 92.2%. A Breslow-Day statistical test was made for significant heterogeneity over sites in the odds ratio (sensitivity / 1 – sensitivity times 1 – specificity / specificity) by site. The test indicates that heterogeneity among sites was not quite statistically significant ($p = 0.066$). The mean odds for all sites of a positive assay result when positive by H&E histology was 85.4 times greater than the odds of a negative assay result when positive by H&E histology (95% confidence interval 35.2 to 206.8). The odds ratio when all sites are pooled was 146.2. The mean odds ratio by site was not statistically different from the pooled odds ratio, indicating sufficient homogeneity of assay performance to suggest pooling of all subjects across all sites.

The proportion of subjects with positive GeneSearch assay results by site ranged from 14% to 48% (mean rate = 30% \pm 2.2%). The proportion of subjects with positive assay results by site includes subjects with invalid assay results. The rate of assay positive subjects by site was not statistically different by site from the pooled rate of positive assay results ($p > 0.05$; Chi square value 14.099 for 10 degrees of freedom). Exclusion of subjects with invalid assay results did not significantly alter the rate of assay positive subjects by site, nor did exclusion give significant differences by site compared with the pooled rate of assay positive subjects.

An assessment of operators using the proposed assay was performed by analyzing the rate of invalid assays per site. The overall rate of subjects with invalid assays was 8.1% (34 of 421 subjects). The rate by site ranged from 0% to 21.4%. For the hypothesis that the rate of subjects with invalid assays by site was 8.1%, the probability was 0.0114. This analysis indicates that the rate of subjects with invalid assays by site was not equivalent with the overall rate of 8.1%, suggesting differences in operator use of the assay by site. It is possible that operator differences by site could reflect differences in the tissue assessed by the sites, as suggested in the marginally significant difference in rate of lymph node status (positive or negative) by site. One possible measure of potential differences in tissue analyzed by site could be differences in the mean cycle threshold value (Ct) of the internal control gene. One would hypothesize that the expression of the internal control gene would be similar in all tissue when used by equally experienced operators at all sites. To this end, analysis of variance of the mean cycle threshold values of the internal control gene was performed by site. The overall mean Ct value of the internal control gene for all subjects with valid or invalid assay results was 30.8 Ct \pm 0.17 Ct (\pm standard error of the mean). The mean Ct value of the internal control gene ranged from 29.6 to 34.9 Ct. The probability of equivalent Ct values for the internal control gene by site was < 0.001 . The difference in mean Ct value of the internal control gene by site remained even when subjects with invalid assay results were excluded. There was no obvious relationship between the rate of subjects with invalid assays by site and the mean Ct value of the internal control gene by site, as shown in the following graph:

Figure 3. Proportion of subjects with in valid assay results by clinical site



As shown in the graph, the three sites with the highest rate of subjects with invalid assays had mean Ct values of the internal control gene below, above and, at the overall mean Ct (30.8) value for the internal control gene. Other sites with lower rates of subjects with invalid assays generally had mean Ct values within 1.5 Ct of the overall mean Ct (i.e. from 29.3 Ct to 32.3 Ct). Based on this information, it is not entirely possible to distinguish if operators at certain sites were more subject to higher rates of invalid assays or the tissue from subjects at those sites were more subject to higher rates of invalid assays.

10. Histological results of clinical study

a) *Central Pathologist Agreement of histology classification*

The overall agreement of two primary central pathologist's review of slides on a subject level was 98.3%. Ninety-two (92) subjects were evaluated with macro-metastases by one or both central pathologists (91% agreement). In 7 of 92 subjects (7.6%) a diagnosis of macro-metastases was classified as micro-metastases by the other pathologists. Nineteen subjects evaluated by one or both central pathologists were classified as micro-metastases. In 6 of these 19 cases, the other pathologist classified the subject as negative. Recognizing the difficulty of evaluating H&E slides, it is unclear if the 98% agreement is significantly different from perfect agreement.

b) *Site Pathologist Agreement of histology classification with Central Pathology Classification*

Agreement was compared between the site pathologist's H&E evaluation versus the central pathologist's H&E evaluation, though the slides read by each pathologist may not have been exactly the same slide as read by the other due to the sectioning scheme of the study. The observed agreement in all diagnoses was 92.2% (95% confidence interval 89.1% to 94.6%), as indicated in the following table:

Site pathology	> 2 mm	0.2-2 mm	<0.2 mm	negative	total
>2 mm	79	3	0	4	86
0.2-2 mm	5	9	0	9	23
<0.2 mm	0	2	0	4	6
Negative	3	1	1	288	293
#N/A	2	3	0	8	13
Total	89	18	1	313	421

Note that 13 subjects had no histology categorization (#N/A) by site pathology. These subjects were not included in the agreement calculations since no categorization is available for comparison with central pathology.

The agreement of a positive diagnosis, i.e. macro- and micro-metastases of tumor to lymph node tissue, was 83.5%. The agreement of a negative diagnosis, i.e. no metastases or metastases consisting of isolated tumor cells and tumor clusters, was 93.9%. Of 107 subjects classified by Central pathologists as positive, 89.7% of subjects were categorized with a positive histology by the site pathology categorization. Of 306 subjects with central pathology categorization of negative histology results, 95.8% (293) of subjects were categorized as negative histology by the site pathology categorization.

As part of the histopathological evaluation in cases where there were disagreements in diagnosis of metastases, the central pathologists re-evaluated the slides actually examined by the site pathologist to confirm or not confirm the site pathological evaluation. Even if the tissue was mis-sampled between site pathology and central pathology there was the opportunity for central pathology to re-evaluate slides. If, after central pathology review, by 2 different pathologists, there remained a discrepancy in diagnosis, the disagreement could also be interpreted as difference between pathologists as well as differences in examination of differently sampled tissue.

c) Comparison of the number of positive nodes per patient by the GeneSearch Test Kit compared with standard histology

The number of cancer positive nodes in a subject when positive by the proposed assay was compared with the number of nodes positive by Overall Histology. The tabulation by subject and is as follows:

# nodes histology positive	# nodes assay positive					total
	0	1	2	3	≥4	
0	277	16	1	1	0	295
1	13	59	7	1	1	81
2	0	4	25	0	1	30
3	0	0	1	3	1	5
≥4	0	0	0	1	4	5
total	290	79	34	6	7	416

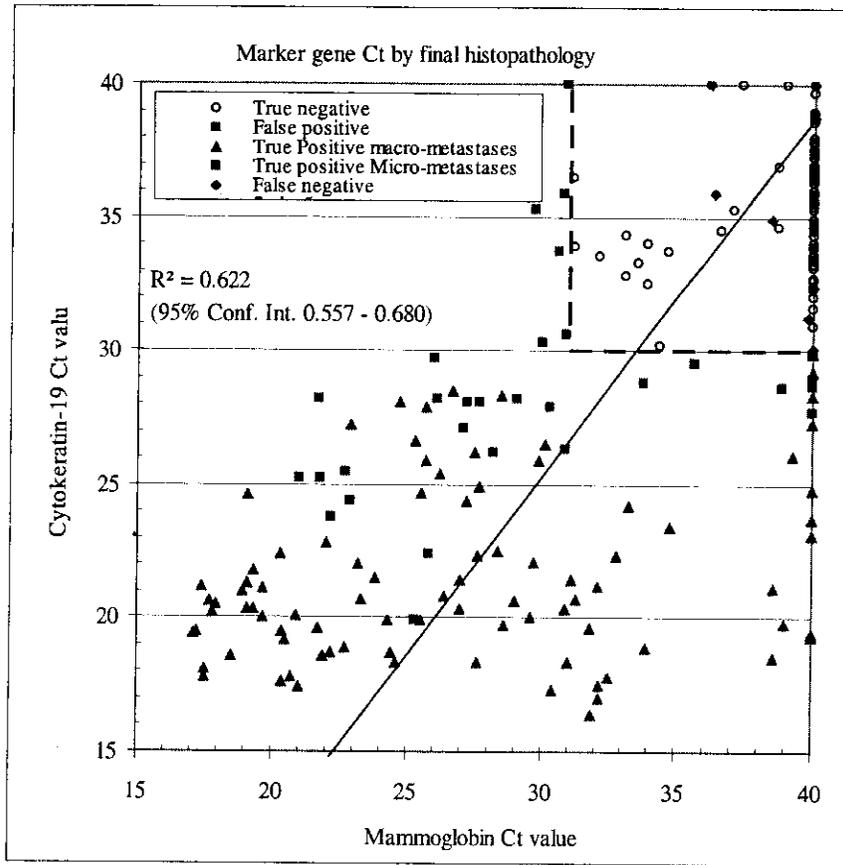
In 18 subjects the assay classified the node as falsely positive (assay positive in 1 or more nodes) but histology was negative. Of 295 subjects who were classified as negative by

Overall Histology, these 18 subjects (6.1%) were categorized as pN1 by the assay while histology would have classified the subjects as pN0. Of 121 subjects who were categorized as positive by Overall Histology, 13 subjects (10.7%) were classified as pN0 by the assay while histology classified the subjects as pN1 or higher. Three subjects were classified as pN2 by the assay but were pN1 by histology, while 4 of 5 subjects classified as pN2 by histology were classified as pN1 by the assay. The assay categorized 35 patients (8.4%) differently (over-staged or under-staged) than did Overall Histology. Concordance between the assay and histology for the number of positive lymph nodes per patient between histology and assay is imperfect. The reasons for divergence are not established. Staging is a histological exercise and the assay is not intended for staging purposes. The 2002 AJCC revised staging system for breast cancer includes mention of molecular techniques such as RT-PCR used to detect nodal metastases. According to that system, and absent histologic findings of sentinel lymph node metastases, positive results from a molecular test are consistent with a staging status of pN0(mol+). Test results are not otherwise intended for use in assigning tumor stage.

d) Marker Ct value correlation with histological category and size of metastasis

The Spearman rank correlation coefficient was calculated using Ct value for mammoglobin and cytokeratin-19 stratified by final histology results in 6 categories (P(MA), P(MI), P, N(CL), N(ITC), and N). The correlation coefficient for mammoglobin vs. the 6 histology categories was 0.77 and was 0.74 for cytokeratin-19. The correlation coefficients for each marker indicate a correlation with final histology category. For 87% of subjects with macrometastases, Ct values for mammoglobin were less than or equal to 26 and cytokeratin-19 Ct values were less than or equal to 25. Further the Spearman rank correlation coefficient of mammoglobin Ct values vs. cytokeratin-19 Ct values was 0.74. The following graph illustrates the correlation of Ct value against each other and vs. the test outcomes, true and false positive as well as true and false negative:

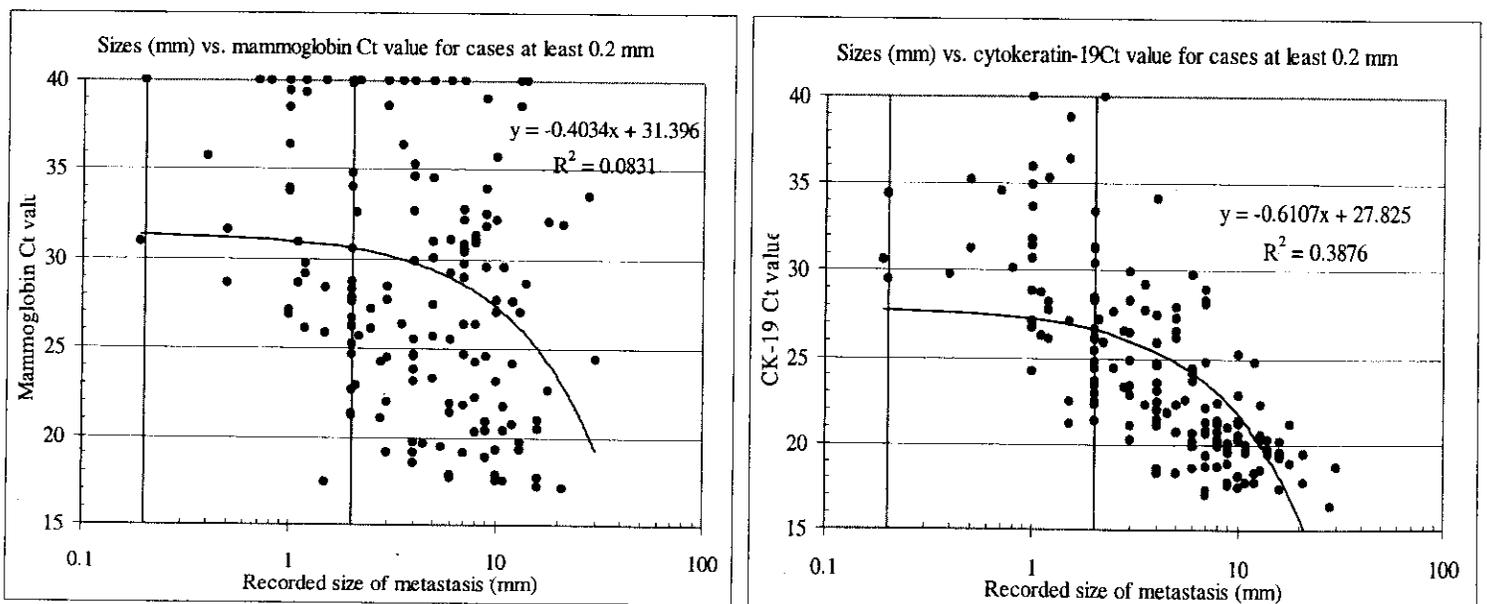
Figure 4. Correlation of Ct value against each other and vs. the test outcomes



Note that the box indicated in the upper right corner shows the cutoff values for each marker.

The FDA requested of the sponsor the pathologist measured size of metastases for each subject, where available, for comparison of metastasis size with marker Ct value. Metastasis size was linearly correlated with Ct value for mammoglobin and cytokeratin-19. The following graphs indicate the relationship.

Figure 5. Relationship of recorded metastasis size and Ct value



Note on the graphs that the metastasis sizes are shown on a logarithmic scale. The line of correlation is linear but appears curved on a logarithmic plot. The graphs illustrate the modest correlation between Ct values of mammoglobin and cytokeratin-19 with measured size of H&E visualized metastases. The correlation for cytokeratin-19 with metastasis size ($r^2 = 0.39$, $p < 0.001$) is more evident than the correlation of metastasis size with mammoglobin ($r^2 = 0.08$, $p < 0.001$). Also indicated on the graphs are the vertical lines indicating the metastasis sizes of 0.2 mm and 2.0 mm. The graphs illustrate some scatter in Ct values with metastasis size but generally metastases greater than 2.0 mm have lower Ct values. There are relatively few measured metastases between 0.2 mm and 2.0 mm. The assay uses a combination of both markers each with separate Ct values correlated with size-ordered histological categories in order to find appropriate Ct cutoff values. The graphs imply a modest or weak correlation of metastasis size with the size-ordered histological categories typical of the AJCC staging manual.

XI. Special Specimen Collection and Preparation Recommendations

The GeneSearch™ BLN test kit has been designed to detect specific breast tissue RNA markers in lymph nodes. For accurate test results, care must be taken to properly collect and prepare lymph node samples. The following guidelines will help to ensure that node samples are of the highest quality for processing:

- It is suggested that the sentinel lymph node (SLN) excision be performed prior to removal of the primary tumor. This will allow the evaluation of the SLN's to occur while the surgeon is removing the primary breast tumor. The SLN removal is expected to be performed through a dedicated axillary incision and prior to incision for the breast surgery (e. g. lumpectomy).
- Avoid contaminating lymph nodes with breast tissue or the primary tumor tissue. Testing breast tissue or the primary tumor with the GeneSearch™ BLN test kit can yield false-positive assay results. Clean surgical instruments and surgical trays must be used.
- False-positive results were observed with most lymph nodes from patients diagnosed with lymphoma. Patients diagnosed with any type of cancer other than breast cancer may not be good candidates for the GeneSearch™ BLN test kit.
- Lymph node tissue should be placed in a fresh transport container after excision, labeled appropriately and immediately transported to the pathology cut-in area.
- On arrival to the pathology area and before preparation of the lymph node, clean the cutting board and spread a fresh disposable surface on the cutting board. Put a fresh blade on the scalpel and change into a fresh pair of gloves. Change gloves, scalpel blades, forceps and cutting surface between lymph nodes. This is essential to minimize sample cross-contamination.

- Clean the lymph node of any fibroadipose tissue (fat) following standard procedures for the histopathology area. Fat is a known interfering substance in the GeneSearch™ BLN test kit. Check for and remove any non-lymph node material.
- The lymph node should be prepared as soon as possible to minimize RNA degradation. If tissue must be held for any period of time before processing (e.g., while waiting for additional lymph nodes), keep the tissue on the weighing paper until processing begins. DO NOT place the tissue in the homogenization buffer and allow it to remain for any period of time. Each lymph node should be processed as a separate specimen. Do not use any tissue fixatives on the lymph node prior to preparation. Tissue is stable for 45 minutes at room temperature after removal from the patient. If tissue will not be homogenized within 45 minutes, it should be flash frozen in liquid nitrogen and placed in the freezer at -65°C or below until testing will commence.
- Remove the lymph node from its container with gloved hands and clean forceps (do not use forceps that have been in contact with other tissue) and place onto the fresh disposable surface.

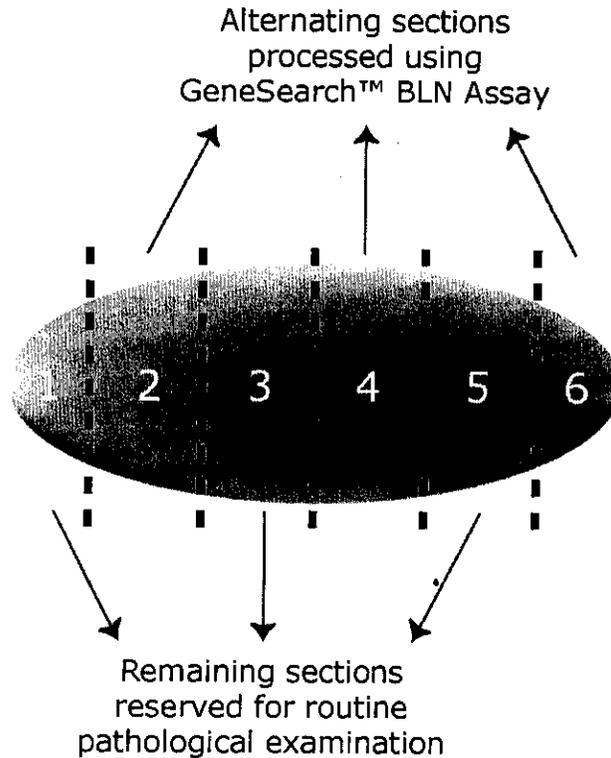
Histological determination of permanent sections of the tissue specimen using the Veridex clinical study lymph node cutting scheme is required. Therefore a portion of the lymph node sample must be retained for standard histological determination. In the clinical study, 50% of the lymph node sample was retained for standard histological determination.

All sentinel lymph nodes were bisected along the short axis. Lymph nodes 6.0 mm or less in length were bisected to produce two (2) lymph node tissue portions. Larger lymph nodes were cut along the short axis into an even number of lymph node tissue portions of approximately the same thickness, as indicated in the table below. This procedure assured that all lymph node portions were between 1.5 to 3.0 mm in thickness, and that there was an equal number of tissue portions for histology and the GeneSearch™ BLN Assay. The following figure shows an example of lymph node sharing between the GeneSearch™ BLN Assay and histology for a lymph node that is approximately 12 mm in length.

Table 13. Sentinel Lymph Node Cut-in (based on node size) and Sharing Between the GeneSearch™ BLN Assay and Histology as done in the Clinical Studies.

Node size (longest dimension in mm)	Total number of node tissue portions
≤ 6	2
> 6 and ≤ 10	4
> 10 and ≤ 15	6
> 15 and ≤ 20	8
> 20	10 or more (each portion ≤ 3 mm thick)

Figure 6. Example of lymph node sharing between the assay and histology for a lymph node that is approximately 12 mm in length.



After any desired touch imprint cytology slides had been taken, alternating lymph node tissue portions from the same lymph node are combined, processed and tested following the Sample Preparation, RNA Purification, and RT-PCR sections of the Instructions For Use.

Slide sections are recommended to be 4 to 6 μm thick, with three (3) sections taken from each 1.5 mm to 3.0 mm fixed lymph node portion. The three (3) sections are taken from levels approximately 150 μm apart.

XII. CONCLUSIONS DRAWN FROM THE STUDIES

A. Safety

The Instructions for use contain the following information in a black-outlined box:

The GeneSearch™ Breast Lymph Node (BLN) test kit may be used in conjunction with sentinel lymph node biopsy for a patient who has been counseled on use of this test and has been informed of its performance. False positive results may be associated with increased morbidity. False negative and inconclusive test results may be associated with delayed axillary node dissection. Clinical studies so far are inconclusive about a benefit from treatment based on findings of breast cancer micrometastases in sentinel lymph nodes.

Refer to the patients' and physicians' brochures, which are available on the Veridex Web Site or by contacting Veridex Customer Technical Services.

When a patient is scheduled to undergo sentinel lymph node biopsy and the intraoperative use of the assay is intended to contribute to a decision to proceed, or not proceed, to further lymph node dissection, the patient will not have the opportunity to directly participate in the

intraoperative decision. Therefore, prior to surgery, the patient should be made aware of the risks and benefits in the use of the assay in addition to the information provided regarding sentinel lymph node biopsy. The provision of information need not imply specific informed consent for the assay or for lymph node biopsy procedures. The above information can contribute to the information given to the patient.

The performance of the GeneSearch™ BLN Assay for patients receiving neoadjuvant treatment has not been established.

B. Effectiveness

The agreement between the GeneSearch™ BLN Assay and thorough permanent section histology (Overall Histology being site and central slides) with review by at least two independent pathologists (355 of 383 subjects with valid assay results) was 92.7%; 95% confidence interval 89.6% to 95.1%. (Table-10) This is similar to the agreement between Site Pathology review versus Central Pathology review of different H&E sections from the same lymph nodes (389 of 408 = 95.3%; 95% confidence interval 90.8% to 97.2%). In the Pivotal Study, subject invalid result rates were 4.2% when at least 40 assay runs had been completed.

The likelihood of an assay positive result when Overall Histology is positive (> 0.2 mm) is 87.6% (see table x), at worst 80.4% (lower 95% confidence interval), at best 92.9% (upper 95% confidence interval). An assay result used intraoperatively that was false negative compared to later positive permanent section histology results would mean that any necessary additional axillary lymph node dissection would be performed in a second surgery rather than during the surgery for sentinel lymph node biopsy. In histologically positive patients, the likelihood of an assay false negative result and the failure to be able to perform the additional axillary node dissection during the same surgery is 12.4%; at worst 19.6%, at best 7.1%.

The likelihood of an assay negative result when Overall Histology is negative is 94.2%, at worst 90.9%, at best 96.6%. An assay result used intraoperatively that was false positive compared to later negative permanent section histology results would mean that a complete axillary dissection performed on the basis of the positive assay result during the same surgery as the sentinel lymph node biopsy procedure may have been unnecessary based on histological evidence alone. In histologically negative patients, the likelihood of an assay false positive result and the possibility of an unnecessary complete axillary node dissection is 5.8%, at worst 9.1%, at best 3.4%.

The total rate of disagreement that can be expected between the assay and permanent section histology as performed in the Pivotal Study is 7.7% (at worst 10.7, at best 5.3). This represents 32 subjects of 416 in the Pivotal Study with disagreement between the assay and histology results -- 15 assay false negatives and 17 assay false positives.

When a patient tests assay negative, there is a 94.9% likelihood (at worst 91.7, at best 97.1) that later histology will also be negative. When a patient tests assay negative and histologically detectable metastatic cancer is found later, that patient will typically require a second surgical procedure for complete axillary node dissection. In assay negative patients, the likelihood of histologically detectable metastatic cancer is 5.1%; at worst 8.3%, at best 2.9%.

When a patient tests assay positive, the chance histologically detectable cancer also will be found is 86.2% (see table 9), at worst 78.8%, at best 91.7%. When a patient tests assay positive and histologically detectable metastatic cancer is not found later, a complete axillary dissection may have been performed unnecessarily on the basis of the assay positive result. In assay positive patients, the likelihood that histologically detectable metastatic cancer will not be found is 13.8%, at worst 21.2%, at best 8.3%.

In addition to the potential morbidity from the axillary lymph node dissection, there is a potential for additional morbidity due to over-treatment with adjuvant therapy. If any axillary nodes show histologically detectable cancer, then the axillary dissection was likely warranted despite the sentinel nodes testing histologically negative. The likelihood of the presence of histologically detectable metastatic cancer in axillary nodes when sentinel nodes are histologically negative is approximately 5-10%¹²⁻¹⁴

Patients with false negative assay results may not receive a needed axillary node dissection and potentially would fail to receive needed adjuvant therapy or would incur a delay in such therapy. Failure to adequately treat metastatic cancer can result in earlier distant metastases and increased risk of shorter long-term survival¹⁵⁻¹⁷. A falsely negative assay result may be recognized when the residual node portions are evaluated within days by permanent section histology. Such patients could then have an appropriate axillary node dissection in a second surgery and likely would receive the appropriate adjuvant therapy. Permanent section histology itself conducted following current guidelines¹⁸ is reported to have false negative rates of 9-12.7%^{19,20}.

In a matched data set, the GeneSearch™ BLN Assay sensitivity was 95.6% compared to permanent section H&E and intraoperative frozen section sensitivity was 85.6% evaluation (86 of 90 for the assay *versus* 77 of 90 for frozen section evaluation). When permanent section histology was negative, the GeneSearch™ BLN assay identified 3.5% more patients as positive than did the frozen section evaluation (13 of 229 for the assay *versus* 5 of 229 for frozen section evaluation).

C. Risk Benefit Analysis

Several risks and benefits must be considered when counseling patients about the use and analyzing lymph node tissue using the GeneSearch™ BLN Assay. The most significant risk involves a false-positive result, in which otherwise cancer-free axillary lymph nodes are removed based on a falsely positive intra-operative result from the GeneSearch™ BLN Assay in the absence of confirming histopathology results. The greatest benefit is the opportunity to forego axillary node dissection and its associated complications based on an accurate negative GeneSearch™ BLN test kit result.

1. Risks

False-positive, false-negative, and invalid assay results are potential risks of the GeneSearch™ BLN Assay. A false-positive result would occur if the patient's SLNs are negative, but the assay returns a positive result (metastatic cancer would not be present but the assay result falsely indicates the presence of cancer) until confirmed or not by permanent section histology. A false-negative result would occur if the patient's SLNs are positive, but the assay returns a negative result (metastatic cancer would be present but the assay result

falsely indicates the absence of cancer) until confirmed or not by permanent section histology. An invalid assay result delays a decision to remove ALNs since an assay result is not returned intraoperatively. The risk of a false-negative result is mitigated by subsequent histological assessment since retention of node tissue occurs and additional tissue testing (or re-examination of current tissue slides) is performed. The results may reflect the true status of the SLN tissues evaluated by BLN and H&E since both could be correct on their particular tissue assessed or may be the result of imperfections in either test evaluations. However, it is possible that both the GeneSearch™ BLN Assay and the histological assessment could have incorrect results.

Sample contamination from breast tissue, primary tumor tissue or breast lymph node tissue from another patient may cause erroneous results. False-positive results may occur if the lymph nodes were obtained from patients diagnosed with any type of cancer other than breast cancer. Issues known to affect sample quality include excessive fat tissue, total sample weight less than 50 mg, and fixed (rather than fresh) tissue. The results of the GeneSearch™ BLN Assay may not be informative if the specimen quality or quantity is inadequate.

In the case of a false-positive result, the ALNs of the patient are removed intraoperatively. Post-surgery, additional histological testing may show that the H&E result is negative. This may be because the 'cancer' was not present in the node sample evaluated by the additional testing and was only present in the assay sample, or it may be because the assay sample truly was a false-positive. The removal of the remaining ALNs may have negative consequences, such as arm swelling, pain and limited motion of the arm.

The long-term overall survival or disease-free survival outcomes for a patient of a positive GeneSearch™ BLN Assay result and a negative histological result has not been assessed. The long-term outcomes of permanent section alone have not yet been fully examined, particularly with regard to micrometastatic cancer in axillary lymph nodes.

From the primary overall analysis, when the assay is used intra-operatively, a positive assay result would indicate an 86% risk of metastatic breast cancer in the subject. When the assay result is negative, the risk of no metastatic breast cancer would be 95% (no worse than 91% based upon the lower 95% confidence interval of the negative predictive value). Therefore when a negative assay result occurs intra-operatively there is a high degree of confidence (at least 95%) that the subject is absent loco-regional breast cancer metastases and there is little need for complete axillary lymph node dissection to occur during the sentinel lymph node biopsy surgery. This high degree of confidence when assay negative provides a reasonable clinical rationale for concluding that the subject does not yet have breast cancer metastatic by lymphogenous spread to the local axillary region. It further supports the conclusion that there is sufficiently little risk of metastatic cancer (at most 8% risk of metastatic cancer though assay negative) to not proceed to level I axillary node dissection and the associated morbidity from that operation. Thus the patient could be reasonably spared that surgical intervention without serious risk of metastatic breast cancer from lymphogenous spread of the disease.

The risk of metastatic breast cancer when assay positive is 86%; at least 79% and as much as 92%. The risk of lymphogenous spread of breast cancer is sufficiently high that there could be a justifiable clinical rationale for level I axillary node dissection and histopathological evaluation of other lymph nodes than sentinel lymph nodes. Balancing that risk is the absence of metastatic breast cancer though the assay is positive. In this study the rate of non-

metastatic breast cancer though assay positive is estimated to be 14% and as much as 21% (1- lower 95% confidence limit of positive predictive value). This fact suggests that when assay positive that 21% of subjects at most would be subjected to an unnecessary level I axillary node dissection even though absent metastatic spread of breast cancer to the regional lymph nodes. The fact that at most 5% of breast cancer patients with metastatic disease would fail to have a needed surgical intervention because of a negative GeneSearch assay must also be clinically weighed with the fact that at most 21% of subjects would undergo an unnecessary surgical intervention because of a positive GeneSearch assay.

When test positive there is a 13.8% chance of sending a subject to an unnecessary lymph node dissection due to falsely detecting the presence of cancer metastases in lymph nodes. In addition to the potential morbidity from the axillary lymph node dissection, there is also the potential for additional morbidity due to over-treatment with chemotherapy, if such therapy is applied. A falsely positive test result could be recognized when undergoing an axillary node dissection and thus lower the potential for over-treatment with chemotherapy. Likewise, the chance of the presence of histologically detectable metastatic cancer when test negative is 5.1%. These subjects would fail to receive a needed axillary node dissection and would potentially fail to receive needed chemotherapy in the short term or incur a delay in needed chemotherapy when cancer metastases are subsequently noted because metastatic cancer is present. A falsely negative test results could likely be recognized when permanent section histology is thoroughly evaluated after the sentinel lymph node biopsy procedure. Such patients could then have an appropriate axillary node dissection and reduce the potential for failure to begin appropriate chemotherapy.

The sponsor chose to classify subjects with invalid assay results as assay negative. If the assay is invalid because the external controls or internal control were outside their specification limits, then the assay result would be classified as no test result. Since some of the invalid assay results could include histologically positive or negative results for the subject node status, a clinical decision would likely be made based on the final histology result when other intra-operative histology procedures have not been performed. In addition, some of the invalid assays could be assay positive as well as assay negative on subsequent re-testing of a clinical sample and so it appears appropriate for the laboratory to classify the assay results as a no test result. In some cases, there may be sufficient RNA homogenate to re-isolate RNA for re-testing of the specimen. If an invalid assay result would occur intra-operatively then it makes clinical sense to make no node determination based upon the proposed assay result in the absence of other intra-operative histology results but defer a clinical decision until a final H&E histology determination can be made. Therefore, it would not be unreasonable clinically to lack an assay result intra-operatively. It would clinically result in a deferred decision on the node status of the patient in the absence of other intra-operative histology results.

Based upon the clinical study, the rate of invalid assays was 8.1%. The rate of invalid testing occurred after laboratory personnel had been trained in the appropriate performance of the assay. The rate of invalid assay results may be higher if lab personnel are insufficiently trained. The invalid rate declined to less than 10% after approximately 30 assay runs based upon data from the clinical study, and to 4.2% after approximately 40 assay runs. An invalid assay result delays a decision to remove ALNs since an assay result is not returned intra-

operatively. This could result in the need for a second surgery depending upon the histopathology determination.

The 8.1% of invalid test results does not consider the situation where a valid result wasn't used because it took too long to obtain to be used intraoperatively. The clinical study was not a study of BLN assay timing in conjunction with the surgery, as BLN assay results were not used to make surgical decisions on subjects.

2. Benefits

The benefit of the GeneSearch™ BLN Assay is the potential to forego ALND and its associated complications based on a negative GeneSearch™ BLN Assay result. Because the SLNs act as a doorway to the ALNs, negative SLN samples indicate that cancer has not spread to the ALNs. In turn, the surgery to remove the ALNs would not need be performed, sparing the patient the extra time in the operating room and the significant complications that could arise from ALND.

The GeneSearch™ BLN Assay is a tool used as support for surgeons. The assay has been designed to be performed by a technologist. The pathologist reviews all assay results, in conjunction with all available clinical information, before making a diagnosis.

Another advantage of the GeneSearch™ BLN Assay is that the tissue being tested is sampled thoroughly via homogenization. The sample being tested is a representation of the entire tissue being evaluated, unlike histology which assesses only a small sampling of the tissue.

Table 14. A summary of potential consequences of true and false test results

True Positive	False Positive
<ul style="list-style-type: none"> • ALND performed without need for second operation • Histologically verified as positive by permanent section from SLNB • Potential clinical benefit from excising known micrometastases 	<ul style="list-style-type: none"> • ALND performed; Potential recognition of false test result when histology from ALND evaluated • No histological verification, pending ALND result • Over-staging potentially resulting in over-treatment and increased morbidity • Little or no clinical benefit from excising unseen micrometastases
True Negative	False Negative
<ul style="list-style-type: none"> • No ALND performed • Histologically verified as negative by permanent section from SLNB 	<ul style="list-style-type: none"> • Under-staging, ALND delayed, patient recalled for second operation • Permanent section detects tumor several days after breast resection; Potential recognition when thorough histology performed from permanent section SLNB

XIII. PANEL RECOMMENDATION

At an advisory meeting held on November 16, 2006, the Immunology Devices Panel recommended that the PMA for the GeneSearch™ BLN Assay be approved subject to submission to and approval by, the Center for Devices and Radiological Health (CDRH) of 6 conditions.

XIV. CDRH DECISION

CDRH concurs with the Panel recommendation of November 2006, and issued a letter to the sponsor on March 15, 2007, advising that the PMA was approvable subject to several conditions.

The applicant's manufacturing facility was found to be in compliance with the device Quality System regulations (21 CFR part 820) on May 8, 2007.

XV. APPROVAL SPECIFICATIONS

Directions for use: See the labeling.

Hazards to Health from Use of the Device: See Patient brochure, Physician brochure, and Warnings and Precautions in the Instructions for Use.

Post approval requirements: See Approval order.

XVI. REFERENCES

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4. Backus J, Green GA, Xu M, et al. Intra-operative Molecular Analysis of Sentinel Lymph Nodes for the Management of Breast Cancer Surgery. *Clin Cancer Res*; in press.