



SUMMARY OF SAFETY AND EFFECTIVENESS

1.1 General Information

Device Trade Name:	GeneSearch™ Breast Lymph Node (BLN) Test Kit, GeneSearch™ RNA Sample Preparation Kit, and GeneSearch™ BLN Assay Protocol Software Compact Disk
Device Generic Name:	GeneSearch™ BLN Assay
Applicant's Name and Address:	Veridex, LLC 33 Technology Drive Warren, NJ 07059 USA
Contact Name:	Debra Rasmussen
Establishment Registration Number:	3004582358
Pre-market Approval (PMA) Application Number:	P060017

1.2 Intended Use Statement

For *in vitro* diagnostic use only.

The GeneSearch™ Breast Lymph Node (BLN) Assay is a qualitative *in vitro* test for the rapid detection of clinically relevant (> 0.2 mm) metastases in lymph node tissue removed from breast cancer patients. Results from the assay can be used to guide the decision to excise additional lymph nodes and to aid in patient staging.

1.3 Device Description

The GeneSearch™ BLN Assay is a real time Reverse Transcriptase-Polymerase Chain Reaction (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). The GeneSearch™ BLN Assay is composed of:

- GeneSearch™ BLN Test Kit: contains all the reagents required for performing RT-PCR (reverse transcription and amplification) and fluorescent detection of amplicon.
- GeneSearch™ RNA Sample Preparation Kit: contains reagents and spin columns for rapid lymph node tissue homogenization and RNA purification.
- GeneSearch™ BLN Assay Protocol Software compact disk (CD): includes parameters for performing and analyzing the GeneSearch™ BLN Assay

The device functions with the Cepheid SmartCycler® Instrument, an integrated nucleic acid amplification and detection instrument system based on the Cepheid proprietary micro processor-controlled I-CORE™ (Intelligent Cooling/Heating Optical Reaction) module.

The presence of metastases in axillary lymph nodes is the most important prognostic indicator in breast cancer and provides important staging information.¹⁻³ The status of the sentinel lymph nodes (SLNs) has been shown to accurately reflect the presence of metastases in the axillary lymph nodes (ALNs) in patients with breast cancer.⁴⁻⁹ As summarized by Yared, et al.,¹⁰ multiple studies show that SLN examination has a sensitivity of 83.4 to 100% for the detection of axillary nodal metastases using paraffin embedded Hematoxylin and Eosin (H&E) histology.

When SLN dissection (SLND) is conducted, typically the patient undergoes complete ALN dissection (ALND) only when one or more SLNs test positive for the presence of metastases. Patients with negative SLNs are spared the significant 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.¹¹ Rapid assessment of the cancer status of SLNs 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 the same information intra-operatively that is provided by post-operative formalin-fixed paraffin-embedded Hematoxylin and Eosin (H&E) (permanent section) histology and immunohistochemistry, i.e., the absence or presence of clinically actionable lymph node metastases in patients that have been diagnosed with breast cancer.¹² The test has been designed to detect the presence of metastases >0.2mm in lymph nodes.^{13,14} The amplification, detection of fluorescence and the interpretation of the signals is done automatically using the Cepheid SmartCycler® Instrument.

Assay Components

Table 1: GeneSearch™ Breast Lymph Node (BLN) Assay		
Component	Volume	Composition
Master Mix	0.30 mL	1 vial, contains Tris buffer, 0.05% Bovine Serum Albumin, 0.08% ProClin 300, primers and probes
Enzyme Mix	0.30 mL	1 vial, contains Tris buffer, 0.08% ProClin 300, DNA (Deoxyribonucleic Acid) polymerase (enzyme) and a proprietary stabilizing agent
Negative Control	0.05 mL	1 vial, contains Tris buffer, plasmid DNA, and 0.08% ProClin 300
Positive Control	0.05 mL	1 vial, contains Tris buffer, plasmid DNA, and 0.08% ProClin 300

Table 2: GeneSearch™ RNA Sample Preparation Kit		
Component	Volume	Composition
Homogenization Buffer	100 mL	Contains ≥ 25% guanidine thiocyanate
Wash Buffer 1	8 mL	Contains < 10% guanidine thiocyanate, 10% ethanol
Wash Buffer 2	2 mL	Contains a proprietary compound mixture, sodium azide (0.09%), pH 7.5
RNase-free Water	1.9 mL	RNase-free Water
RNA Spin Columns	10 each	Spin Column

1.4 Principles of Operation

The GeneSearch™ BLN Assay is a real time Reverse Transcriptase-Polymerase Chain Reaction (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 quantitative 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 have been demonstrated to correlate with detection of metastasis by the current method of paraffin-embedded (permanent section) H&E histology with the additional use of immunohistochemistry (IHC) on H&E negative samples.

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 background levels in normal lymph node tissue.

The SLNs are the first lymph nodes into which lymphatic fluid drains. As a result, these nodes are more likely to contain cancer cells if metastasis has occurred. SLN biopsy permits the intraoperative identification and evaluation of the nodes in the lymphatic basin, which are at highest risk for metastasis. In order to maximize the uniformity of sampling, lymph nodes are divided into sections and alternating sections are combined and processed using the GeneSearch™ BLN assay. The remaining sections may be used for routine pathology 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.

Using the GeneSearch™ BLN Assay, the real-time RT-PCR reaction is performed in a homogeneous, one-step, fully contained reaction. Three gene markers, (mammaglobin, CK19, and an internal control gene), 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 mixture, (a buffer containing marker-specific DNA primers, deoxyribonucleoside triphosphates (dNTPs), DNA Polymerase, and marker-specific DNA probes) 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 that are detected utilizing sequence-specific DNA probes.

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 three 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.

Interpretation of Results

The software will generate results in the form of a report. The patient result is displayed in the run report and will be "Positive", "Negative", or "Invalid", based on the results of the gene markers and the results of the external controls (positive and negative) and of the internal control.

The GeneSearch™ BLN Assay software will analyze real-time PCR fluorescence data against pre-determined assay cut-offs to derive a qualitative assay result. Test data will be analyzed on a per patient basis and will include the results of internal and external controls run concurrently with the patient sample(s). In patient assays for which all necessary assay controls give valid results, the patient result will be either "Positive" or "Negative". If mRNA is detected above the threshold fluorescence levels for either MG or CK19, the specimen is considered positive. If mRNA from the breast cell markers is not detected, and the internal control confirms correct specimen preparation and product performance, the specimen is considered negative. External controls are included in each run to assure that primers and reaction mixtures are functioning. In patient assays for which one or more of the necessary controls gives an invalid result, the patient result will be "Invalid." This strict interpretation mitigates a host of user errors that could lead to false results. An overview of the integrated process follows:

Sentinel Lymph Node
Excised by Surgeon
(Patient Sample)



Veridex Sample
Preparation Kit (RNA
Extraction)



Veridex BLN Kit for
Reagent Preparation
(RT-PCR Amplification
including Markers)



Cepheid Smart Cycler II
for Thermal Cycling
(RT-PCR
Amplification)



Cepheid Smart Cycler II
Software for Data
Analysis, Storage and
Veridex Algorithmic
Interpretation



Patient Report



Quality Control Features

Several quality control features have been included with the GeneSearch™ BLN Assay. Along with MG and CK19, the RT-PCR step also amplifies an internal control gene RNA (PBGD, porphobilinogen deaminase). The PBGD RNA is constitutively expressed in SLN tissue and is expected to be amplified in all negative samples. The RNA internal control is effectively a control against falsely negative results and is not required to be positive if the sample is positive for either assay marker. The amplification of the internal control RNA indicates that the GeneSearch™ BLN Assay procedure in a negative patient sample was performed successfully. Furthermore, positive and negative controls are included to monitor reagent quality and instrument performance, as they relate to the performance of the GeneSearch™ BLN Assay. Finally, to minimize the possibility of contamination, the entire RT-PCR amplification and detection procedure is performed in a single closed tube that is not opened after the reagents have been added.

Quality Control - Internal Control

The internal control consists of detection of mRNA from a constitutively expressed gene in lymph node tissue as a control against false negative results. The results of this control are obtained as one of the multiplexed gene markers in the specimen reaction tube. This control monitors the sample quality, sample preparation, and assembly of the RT-PCR reaction in the specimen reaction. It is possible that extremely high expression of the breast cell-specific markers will inhibit detection of the internal control. As a result, assays in which one or both cancer markers are positive in at least one node are considered valid, regardless of the result observed for the internal control.

Quality Control - External Controls

External controls are provided for both markers MG and CK19 (within the Positive Control) and the internal control PBGD (within the Negative Control). These controls must be included with each run. External controls consist of linearized plasmids containing sequences capable of being amplified and detected by the primers and probes used in the GeneSearch™ product. The controls monitor reagent quality and instrument performance as they relate to assay performance.

Quality Control - Contamination Controls

The Positive Control and Negative Control also serve as contamination controls. The Positive Control does not contain the target sequence for the internal control and serves as its contamination control. The Negative Control does not contain the cancer marker target sequences, and serves as their contamination controls. The contamination control system protects against environmental contamination or nonspecific products that could result in incorrect assay results.

Table 3: Expected Control Results for the GeneSearch™ BLN Assay			
	MG	CK-19	PBGD
Positive Control (External)	+	+	-
Negative Control (External)	-	-	+
Internal Control	-	-	+

1.5 Contraindications

There are no known contraindications for the GeneSearch™ Breast Lymph Node Test Kit, GeneSearch™ RNA Sample Preparation Kit and GeneSearch™ BLN Assay Protocol Software CD.

See labeling for warnings and precautions.

1.6 Warnings and Precautions

For *in vitro* diagnostic use only.

The warnings and precautions can be found in the GeneSearch™ BLN Assay labeling.

1.7 Alternative Practices and 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 clinically actionable lymph node metastases in patients that have been diagnosed with breast cancer.

Intraoperative techniques would eliminate the need for a secondary surgery. Frozen sections (FS) and imprint cytology (IC) are the most common intraoperative procedures currently used to determine the pathology of SLNs. 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%.

Recently, Veronesi et al,⁷ have described a combination of intraoperative step FS with rapid immunohistochemistry to arrive at an intraoperative final diagnosis. However, this procedure is very labor-intensive and will lead to a significant increase in the operating time.¹⁵ Ultra rapid immunohistochemistry protocols have recently been described.¹⁸ In an initial evaluation involving 33 SLNs, this technique was highly specific (100% specificity) with a sensitivity of 88.2%. These authors concluded that serial sections with H&E staining are essential for correct

lymph node classification, and IHC cannot be a substitute for a careful examination by means of multiple step sections of lymph node specimens.

Although it would be of great advantage to patients and surgeons if the regional lymph node dissection, when necessary, could be performed in the same operating session as the SLN procedure and excision of the primary tumor, the sensitivity of current methods is not suitable for intraoperative assessment, particularly when highly skilled pathologists are not available.

1.8 Potential Adverse Effects of the Device on Health

The GeneSearch™ Breast Lymph Node Test Kit, GeneSearch™ RNA Sample Preparation Kit and GeneSearch™ BLN Assay Protocol Software CD are for in vitro diagnostic use, thus there is no direct adverse effect on the patient. Failure of the product to perform as intended, or errors in the use of the product, may lead to a false result.

A false positive result using the GeneSearch™ BLN Assay is not considered a significant patient or public health concern. If this were to occur, the patient may undergo an unnecessary axillary lymph node dissection. It is conceivable that misdiagnosis may lead to the patient having to undergo unnecessary treatment.

A false negative result using the GeneSearch™ BLN Assay would mean that nodal metastases (> 0.2 mm) may go undetected. In this case, there is a potential safety concern for the patient. However, a negative result, whether true or false, in a clinical lab would result in clinical follow-up for metastases or re-occurrence of the primary cancer.

The external and internal controls of the GeneSearch™ BLN Assay are designed to minimize the rate of false positive and false negative results.

1.9 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 [REDACTED] 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 [REDACTED] and has not been withdrawn for any safety issues.

The GeneSearch™ BLN Assay is a novel, qualitative, *in vitro* product. It functions as a complete system of reagents and instrumentation for the rapid detection of breast cancer cells in lymph node tissue. The product has been in use as an investigational device on patient samples for clinical trials in the United States since July 2004, but was not used for patient management or treatment decisions. It was recently shipped to [REDACTED] where a field study began in the second quarter of 2005. Investigational Use Only kits were also recently sent to [REDACTED] where an internal study has been completed, and a subsequent study is set to begin later in 2006.

The Cepheid SmartCycler® instrument system and core software have been in use in the [REDACTED] [REDACTED] as a device for use with IVD assays since Nov. 18, 2002 and the safety profile (or post-market experience) is good. Cepheid conducted a recent field correction of the software (letter July 22, 2005). The software anomaly is described as presenting itself when the software is handled in a particular manner. This software anomaly did not effect the GeneSearch™ BLN Assay IUO software and has been corrected by Cepheid prior to the approval of this PMA.

1.10 Summary of Studies

Two prospective [REDACTED] clinical trials were conducted to gather the data from which to determine the proper Ct cutoffs for the GeneSearch™ BLN Assay (training set, Cutoff Study, 12 sites, n = 306) and to validate the chosen cutoffs in an independent subject set (test set, Pivotal Study, 11 sites, n = 423). Both studies had identical methods (described below).

Methods: During scheduled sentinel lymph node (SLN) removal procedures, clinical site personnel performed the GeneSearch™ BLN Assay testing on SLNs freshly removed from female or male patients at least 18 years of age diagnosed with invasive breast cancer. The GeneSearch™ BLN Assay results were compared to rigorous permanent section H&E and IHC sectioning (described below) evaluated by pathologists who were blinded to BLN assay results. Two independent pathologists evaluating a node as having a metastatic focus >0.2 mm was required for a node (or subject) to be categorized as “positive.”

Sentinel Lymph Node Cut-In and Sharing: 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. This procedure assured that all node slabs were between 1.5 - 3.0 mm in thickness, and that 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 intraoperative touch preparation slides had been taken (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. 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.

Test Comparator - Histology Interpretation: The combination of permanent section H&E and IHC was used as the comparator test method in these studies to determine the performance of the GeneSearch™ BLN Assay. The study data set includes the results for both the standard Site Slides (used by the sites for patient management) and for the slides taken specifically for the study (Central Slides). Permanent section H&E results for all nodes and IHC results for H&E negative results were collected. Central Slides were evaluated only by Central Pathologists. Two Central Pathologists independently read all of the slides. A third pathologist evaluated the slides when the diagnoses of the first two Central Pathologists disagreed. For positive Site Slides, the slides were sent to a Central Pathologist to further confirm positivity. Thus, all histology positive samples in the study data set were confirmed by two independent pathologists. The Pathologists were blind 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, as described here. Two Central Pathologists independently read Central Slides. If the results from the two pathologists were discordant, a third Central Pathologist independently read the same slides. The final Central Slide result was determined by majority rule. For Site Slide results, positive site pathologist evaluations had to be confirmed by Central Pathologists either on the Central Slides or by evaluation of the Site Slides themselves. 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. A node was considered P(MA) only if two Central Pathologists agreed on that level of metastasis.

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.

Overall Histology Results Interpretation for a Subject: It was the subject's Overall Histology result that was used for performance calculations for the GeneSearch™ BLN Assay. The subject's Overall Histology result was negative if the Overall Histology result of all nodes was negative, and positive if at least one node was positive. The subject's Overall Histology result was considered UND if one node was evaluated as UND and there were no other nodes, or all other nodes were either UND or negative. The subject's Overall Histology result was equal to the most positive result seen in any of their nodes, e.g., if one node was P(MA), one P(MI), and one negative, the patient result was P(MA).

Intraoperative Histological Evaluations: 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 measured against permanent section histology (Overall Histology as described above). 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.

Cutoff Study Results: 274 subjects with valid GeneSearch™ BLN Assay results and defined Overall Histology results were used to determine cutoffs for the assay internal control (PBGD), MG, and CK19 markers. Data from an additional 30 subjects with invalid GeneSearch™ BLN Assay results and two with Overall Histology results of UND were excluded from the MG and CK19 cutoff determinations, as these subjects would not provide any numerical contribution to

cutoff calculations. Cutoffs for PBGD, MG, and CK19 markers were determined as < 36 Ct, ≤ 31 Ct, and ≤ 30 Ct, respectively. The performance of the GeneSearch™ BLN Assay with these cutoffs in the 274-subject data set was 91.1% sensitivity (95% confidence interval: 82.5% - 96.4%) and 95.9% specificity (95% confidence interval: 92.1% - 98.2%).

Pivotal Study Results: There were 423 subjects (418 females and 5 males) with SLNs removed who met all protocol inclusion criteria. 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%).

SLN Disposition: 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.

Overall Histology Results: Five of the 421 subjects with study histology results had Overall Histology results of UND and were not included in assay performance calculations. The prevalence by Overall Histology was 29.1% (121 positive subjects of 416), and ranged from 14.3 – 45.5% across all sites. H&E was positive in 120 subjects, and there was one positive subject identified as P(MI) by IHC alone. Positives were most often P(MA) (77.7%), with 19.0% being P(MI), and 3.3% of undetermined size > 0.2 mm (P). Most negatives (93.2%) were completely histologically negative with no evidence of tumor cells (N), 4.7% N(ITC) and 2.0% N(CL).

Central Pathologist Agreement Evaluating the Same Slides: The overall positive/negative agreement on a subject level between the two primary central pathologists evaluating the same slides was 98.3%. There were 92 subjects evaluated with a macrometastasis by one or both central pathologists. Only one of these 92 subjects (1.1%) was found negative by the other pathologist. In seven of the 92 subjects (7.6%), the other pathologist evaluated the subject as having micrometastases. There were 19 subjects evaluated by one or both Central Pathologists as having a micrometastasis (and not evaluated with macrometastases). In 31.6% of these cases (6/19), the other pathologist evaluated the subject as negative. The non-uniform distribution of micrometastases in nodes lowers the probability of correctly evaluating metastases at earlier stages of breast cancer.^{19,20} These results illustrate the difficulty of distinguishing between positivity and negativity for clinically relevant metastases.^{20,21}

Agreement between Site Pathologist's Results and Final Central Pathologists' Results from Different H&E Slides: Comparisons were made between the site pathologists' H&E results used for patient management *versus* the central pathologists' final H&E results on Central Slides. In seven cases one evaluation determined that the subject had macrometastases while the other evaluation found the subject negative. In 12 cases one evaluation determined that the subject had

micrometastases while the other found the subject negative. These 19 subjects represent 4.7% of the 408 subjects with both site and central pathology results. These findings illustrate the inadequacy of current sampling techniques used for histological evaluation of lymph nodes, since significant metastases can be missed in nearby tissue left uncut on the block.^{19,22}

Confirmation of Site Slide Positivity: There were 113 subjects reported positive by the Site Pathologists. Central Slides were also positive in 82.1% of those cases. Of the 21 subjects reported positive by the site and found negative on Central Slides, central pathologist(s) confirmed positivity on the Site Slides in 61.9% (13/21). In four of the 21 (19.0%) subjects, both central pathologists found the Site Slides negative. In four additional site-positive subjects, missing central pathologist data precluded confirmation of Site Slide positivity -- they remain UND. These findings illustrate the subjective nature of histological slide evaluation.²²

GeneSearch™ BLN Assay Performance Calculations: Of the 421 subjects with GeneSearch™ BLN Assay results, the assay result was invalid for 34 (8.1%), whether due to external control (13) or subject sample (21) failures. Subject invalid result rates declined to 4.2% with increased operator assay experience (when at least 40 assay runs had been completed). For the purposes of performance calculations, these invalid results were not excluded but were treated as assay “negative,” since these results do not provide the clinician with evidence of nodal metastases. Performance calculations were based on the 416 subjects with defined Overall Histology results (the five subjects with Overall Histology results of UND were not included). The GeneSearch™ BLN Assay overall performance (with 95% confidence intervals, 95% C.I.) is shown below compared to Overall Histology. Also shown is assay performance from the Cutoff Study evaluated by the same methods (assay invalids are treated as “negative” and subjects with incomplete histology are excluded) and the combined performance from both studies.

Table 4: GeneSearch™ BLN Assay Clinical Performance					
STUDY	N	Sensitivity (95% C.I.)	Specificity (95% C.I.)	Positive Predictive Value	Negative Predictive Value
Pivotal	416	87.6 (80.4-92.9)	94.2 (90.9-96.6)	86.2	94.9
Cutoff	304	82.4 (72.6-89.8)	96.3 (92.9-98.4)	89.7	93.4
Combined	720	85.4 (79.9-90.0)	95.1 (92.9-96.8)	87.6	94.2

Fourteen of 15 subjects with assay false negative (FN) results had only one positive node by Overall Histology. Of the 15 FN subjects, two were due to external control failures being interpreted as “negative” for the purposes of assay performance calculations. In these two cases, subject sample Ct values were actually positive for both CK19 and MG. Of the 13 valid assay

negative subjects, most had small metastases with nine being P(MI), three P, and only one P(MA) by Overall Histology. Eight of the 13 had positivity found only on Central Slides or Site Slides, but not on both.

There were a total of 17 subjects with assay false positive (FP) results. Fifteen of the 17 were positive by the assay on only one node.

It is probable that differences in results between the GeneSearch™ BLN Assay and Overall Histology were due predominantly to tissue sampling since the assay evaluated different portions of the node than did histology. This conclusion is supported by differences seen between the evaluations of the two sets of H&E slides collected for the Pivotal Study discussed above. The effect of tissue sampling is evident when comparing Site pathologists' results on Site H&E Slides to the central pathologists' results on Central H&E Slides. This is a comparison of H&E evaluations of different sections from the same portions of the node. Site Pathology had the following "performance" *versus* Central Pathology:

N	Sensitivity (95% C.I.)	Specificity (95% C.I.)	Positive Predictive Value	Negative Predictive Value
408	94.1 (87.6-97.8)	95.8 (92.8-97.7)	88.1	98.0

The lack of perfect agreement is due predominantly to different sections (samplings) from the node tissue being taken for Site Pathology evaluation *versus* Central Pathology evaluation.

Sensitivity of the GeneSearch™ BLN Assay for the 94 subjects with P(MA) was 97.9% (C.I.: 92.5-99.7%). For the 23 subjects with P(MI), assay sensitivity was 56.5% (C.I.: 34.5-76.8%). The sensitivity for micrometastases for Site Pathology H&E compared to Central Pathology H&E on different but nearby sections of the node was 80% (C.I.: 51.9-95.7%). These results again show the difficulty of detecting small and infrequently occurring metastases with limited sampling, and the importance of evaluating more than a small proportion of the node.

There were 31 subjects who had breast surgery conducted immediately prior to the SLND. There was 100% agreement between the Overall Histology result and the GeneSearch™ BLN Assay result in these subjects, indicating that conducting breast surgery prior to the SLND did not cause false positive assay results due to contamination of the lymph nodes with breast tissue.

For the five male subjects, assay results were in 100% agreement with Overall Histology. Three were negative (N) and two were P(MA).

There were 10 subjects who were receiving cancer treatment of chemotherapy (9) or radiation (1) at the time of the SLND. The assay results agreed with Overall Histology (7 negative and 2 positive) in all but one of these subjects. That subject was receiving chemotherapy and was

negative by the assay and P(MI) only by IHC. The GeneSearch™ BLN Assay, Site Slides H&E and Central Slides H&E were negative for this subject.

Subanalyses indicated that GeneSearch™ BLN Assay performance was similar in subjects with varied tumor histology (invasive ductal, invasive lobular, or other invasive breast cancer), tumor sizes, and tumor stages.

The number of axillary lymph nodes that are found with metastases is an important prognostic indicator and is used to make treatment decisions.¹⁻³ The number of nodes found positive in a subject by the GeneSearch™ BLN Assay compared to the number found positive in the same subject by Overall Histology is shown in the table below. The Kappa value of agreement between the two tests was 0.75 (95% confidence interval 0.68 to 0.81). Kappa values above 0.61 are considered indicative of substantial agreement.²³

Table 6: Number of Nodes found Positive by the GeneSearch™ BLN Assay versus Histology				
Histology (No. Positive Nodes)	GeneSearch™ BLN Assay (No. Positive Nodes)			
	0 (NEG)	1	2	>= 3
0 (NEG)	278	15	1	1 ^a
1	14	57	8	1
2	1	5	23	1
≥ 3	0	0	1	10

^aThe subject with three assay-positive nodes had isolated tumor cells reported by Site IHC in four of the five SLNs.

Of the 17 cases where the assay was interpreted as FP, 15 (88.2%) subjects were identified as assay positive in only one node. Similarly, of the 15 cases where the assay was interpreted as false negative, 14 (93.3%) subjects were histology positive in only one node. These results are expected when the differences are due to sampling of different node tissue in subjects with less metastatic spread of disease.

Spearman nonparametric correlation coefficient analysis found that there was a high correlation between assay cancer analyte Ct values and level of metastases reported by Overall Histology (n=383, conclusive histology and valid BLN results). The correlation coefficient was 0.77 for MG and 0.74 for CK19 Ct values *versus* the six histology categories of P(MA), P, P(MI), N(CL), N(ITC) and N. The following graph shows that relationship. There are 128 overlapping subjects with negative histology at the 40/40 point in the graph. Those subjects with CK19 Ct values less than 25 or MG Ct values less than 26 were highly likely to have macrometastases (90.9%, 80 of 88).

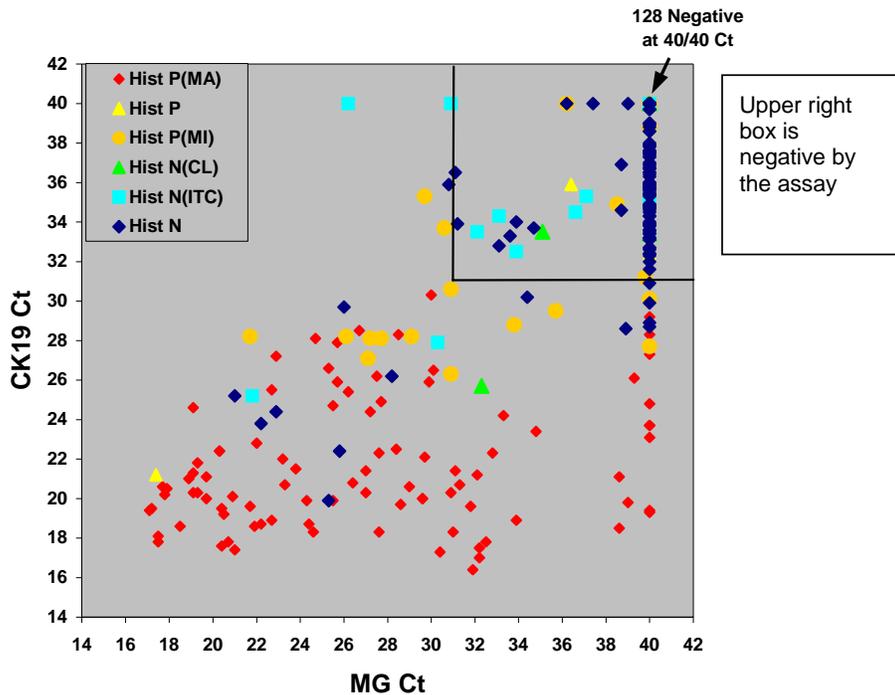


Figure 1. Distribution of GeneSearch™ BLN Assay Analyte Ct Values by Size of Metastases

Additional molecular testing of assay false negative and false positive samples: Sampling different portions of the node can lead to disagreements between the GeneSearch™ BLN Assay result and the Overall Histology result. Testing the residual assay sample for the expression of other gene markers associated with metastases can provide further information on the node portions tested only by the assay. The Sponsor developed a molecular test (Confirmatory Molecular Test) designed to have very high specificity. This test was a modification of previously published methods.¹² The test had four molecular markers that were not used in the GeneSearch™ BLN Assay.

One node from each of 11 subjects who tested negative in both the GeneSearch™ BLN Assay and overall Histology (true negative, TN) were tested in the Confirmatory Molecular Test. All 11 nodes tested negative.

One Overall Histology-positive node was tested from each of 10 GeneSearch™ BLN Assay FN subjects. Residual sample was unavailable in five FN subjects. All 10 nodes tested negative in the Confirmatory Molecular Test, suggesting that the node portions tested in the GeneSearch™ BLN Assay did not contain metastases.

Of the 23 true positive (TP) nodes from 22 TP subjects tested in the Confirmatory Molecular Assay, 15 nodes (65.2%) from 15 subjects (68.2%) confirmed as positive, 7 (30.4%) were negative, and one (4.3%) invalid. Lack of 100% agreement was expected since the Confirmatory Molecular Assay was designed to maximize specificity as opposed to sensitivity.

Of the 17 subjects with assay FP results, 13 had residual assay sample available. A total of 15 FP node samples were tested with the Confirmatory Molecular Test. Eleven of the 15 (73.3%) FP nodes and nine of the 13 (69.2%) FP subjects tested positive in the Confirmatory Molecular Test. The proportion of confirmed positive nodes was similar in TP (65.2%) and FP (73.3%) nodes, suggesting that metastases were present in the majority of assay “false” positive samples. In the subject with the three nodes that tested FP in the GeneSearch™ BLN Assay, the Confirmatory Molecular Test confirmed positivity in all three nodes.

Comparison of the GeneSearch™ BLN Assay Performance to Current Intraoperative Tests: The table below compares the performance of the GeneSearch™ BLN Assay to that of current intraoperative methods that were in use at the Pivotal Study sites. For GeneSearch™ BLN Assay performance calculations, the data set was limited to those 324 subjects who had intraoperative frozen section (FS) results. In all cases, the comparator test was permanent section histology (Overall Histology).

Sensitivity of the GeneSearch™ BLN Assay was 95.6% compared to 85.6% for FS and 45.5% for intraoperative touch preparations (ITP). Sensitivity of the GeneSearch™ BLN Assay for subjects with P(MA) was 100% (76/76) compared to 90.8% (69/76) for FS and 57.1% (4/7) for ITP. Sensitivity of the GeneSearch™ BLN Assay for subjects with P(MI) was 81.8% (9/11), and was 54.5% (6/11) for FS and 25.0% (1/4) for ITP.

Specificity for both current intraoperative methods was greater than 97%. Due to the limited sampling involved in current intraoperative techniques, adjacent and more thorough permanent section histology is likely to confirm any metastases seen with FS or ITP.

These data show that the GeneSearch™ BLN Assay detected more metastases than did current intraoperative histological techniques, despite the fact that the comparator test (Overall Histology) was conducted on different portions of the node than those on which the assay was conducted. FS results were, in contrast, generated on the same portions of the node as Overall Histology.

Table 7. Performance of the GeneSearch™ BLN Assay versus Current Intraoperative Tests					
Test	N	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV	NPV
BLN Assay	319	95.6 (89.0-98.8)	94.3 (90.5-96.9)	86.9	98.2
FS	319	85.6 (76.6-92.1)	97.8 (95.0-99.3)	93.9	94.5
I TP	29	45.5 (16.7-76.6)	100 (81.5-100)	100	75.0

GeneSearch™ BLN Assay Reproducibility: 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 CK19. There were a total of four panel members with one being negative for both MG and CK19. Starting from the RNA isolation step, each operator tested panel samples in duplicate in each run using three different lots of the GeneSearch™ Breast Lymph Node (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 Assay qualitative results were in 100% agreement with the known presence or absence of target for all individual markers (PBGD, MG, and CK19). Percent Coefficients of Variation (CV) for all marker Ct values were ≤ 6.82% for intra-run, inter-run, inter-site, inter-operator, and inter-lot analyses. Standard deviations were ≤ 1.88 in all cases.

1.11 Conclusions Drawn from the Studies

The agreement between the GeneSearch™ BLN Assay and thorough permanent section histology with review by at least two pathologists is similar to the agreement between Site Pathology review versus Central Pathology review of different H&E sections from the same nodes. In addition, the absolute number of positive nodes identified by the GeneSearch™ BLN Assay is similar to the number identified by permanent section histological evaluation. In a matched data set, the GeneSearch™ BLN Assay identified more clinically relevant metastases (> 0.2 mm) than did intraoperative histological techniques.

The Reproducibility Study data show that GeneSearch™ BLN Assay results are highly reproducible on both a quantitative and qualitative level across sites, operators, lots, days, and within runs.

These data support the safety and effectiveness of the GeneSearch™ BLN Assay for the rapid detection of clinically relevant (>0.2 mm) metastases in lymph node tissue removed from breast

cancer patients. The data also indicate that GeneSearch™ BLN Assay results can safely and effectively be used to guide the decision to excise additional lymph nodes and to aid patient staging.

1.12 Benefit Analysis (Safety and Effectiveness)

As a diagnostic test, the GeneSearch™ BLN Assay involves partial or full use of the excised sentinel lymph node(s) from a patient for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is already undergoing a sentinel lymph node dissection for current pathology diagnostic evaluations. The benefits to a breast cancer patient of rapid, objective detection of clinically relevant (>0.2 mm) metastases in the lymph node tissue outweigh any potential adverse event or risk to the patient or user due to assay malfunction or operator error.

The potential risks encountered with *in vitro* diagnostic tests are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for these devices. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

1.13 Panel Recommendation and FDA Decision

Following review of this PMA by the Agency, (placeholder only), statement will be included in the finalized Summary of Safety and Effectiveness

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