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Horizon Scanning Technology Prioritising Summary Update

GeneSearch™ Breast Lymph Node Assay

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PRIORITISING SUMMARY UPDATE 2010

REGISTER ID:	**000295**
NAME OF TECHNOLOGY:	**GENESEARCH™ BREAST LYMPH NODE (BLN) ASSAY**
PURPOSE AND TARGET GROUP:	**WOMEN UNDERGOING SENTINEL LYMPH NODE BIOPSY FOR BREAST CANCER**

2010 SAFETY AND EFFECTIVENESS ISSUES:

The performance of the GeneSearch™ BLN Assay against routine permanent-section histology was investigated in a US study with 12 participating centres (level III-1 diagnostic evidence). The study design incorporated two phases. Firstly, a beta trial (n=304) was conducted to establish thresholds of mammaglobin and cytokeratin 19 expression correlating with metastasis greater than 0.2mm.[1] Subsequently, a validation trial (n=416) was undertaken to confirm threshold cut-offs. Patients aged at least 18 years with diagnosed invasive adenocarcinoma of the breast and scheduled for sentinel lymph node biopsy (SLNB) were eligible for the study. Males were not excluded.[2] Trained site personnel performed the BLN Assay and results were blinded to all personnel involved in any testing (Julian et al 2008).

Sentinel lymph nodes were identified and dissected following the standard procedures of each centre. Sectioning of each node along the short axis was performed, yielding multiple tissue samples 1.5 to 3mm thick. Alternating tissue samples were designated for histology or the BLN assay. The samples reserved for histology were prepared for post-operative permanent-section slides stained with haemotoxylin and eosin (HE). Tissue samples were also prepared for intra-operative cytopathology and frozen section, routinely performed at some sites. The HE, cytopathology and frozen section samples were designated as site slides and provided the basis for patient management. Additional sections from three nodal levels 150µm apart were prepared for HE slides and evaluated by the central study pathologists (central slides). This extensive sampling was undertaken to optimise histological assessment. Since all histological methods are limited by the examination of tissue samples, methods that exhaust more of the lymph node in question are less subject to sampling bias. Sections were also stained using immunohistochemistry (IHC)[3], either on site or by an independent

[1] The accepted size for nodal metastases of clinical relevance among the majority centres performing sentinel lymph node biopsy, in accordance with American Joint Committee on Cancer (AJCC) criteria.
[2] Since male breast cancer is an especially rare condition, only five males participated in each of the two trials. Females represented 299 and 411 of the participants in the beta and validation trials, respectively.
[3] IHC exploits antigen-antibody interactions for histological purposes. Slide mounted tissue sections are incubated with antibodies specific to targeted antigens (proteins), producing a primary reaction signal. The signal is amplified using a second antibody complexed with an enzyme and, in the presence of a substrate and chromagen, coloured deposits form at the sites of antigen-antibody binding. The coloured deposits can then be viewed by standard microscopy.

laboratory. These slides were evaluated by the site pathologist or the central pathologists. When a cancer greater than 0.2mm on any slide was independently confirmed by two pathologists, the node in question was regarded as positive for metastasis. Two or three central pathologists independently evaluated each HE and IHC central slide, while at least one central pathologist was required to confirm positive site slides. If either of the final site or final central result, or both reported metastases, the overall histology result (OHR) for the node was positive.

Alternating sections of nodal tissue not designated for histology were combined for each node and processed according to the BLN Assay manufacturer's instructions. The resulting homogenate formed the basis for the reverse-transcription PCR to determine expression levels of mammaglobin (MG) and cytokeratin 19 (CK-19) mRNA corresponding to metastases larger than 0.2mm. An internal control marker was included in the reaction.[4] Cut-off values were established from the beta study data set and raw cycle threshold (C_t) data for these markers were sent to the study sponsor, Veridex LLC, for analysis.[5] Cut-offs for MG and CK-19 were examined to find all combinations that gave assay specificity of at least 95 per cent, then sensitivity was maximised.

Using the predetermined cut-off values, 123 out of 416 (29.6%) validation study patients had a positive BLN Assay result, 260 (62.5%) had a negative result, and 33 (7.9%) had an invalid result. Many invalid results were due to a degree of inexperience among assay operators. However, a review of the errors enabled successful revision of the assay training program, as seen in one clinical site which entered the study later. Personnel at this site were trained with the improved program and of the 82 patients tested, two (2.4%) had invalid results. Invalid results were not included in the final analysis. In regard to histology, 121 (29.1%) out of 416 validation study patients had a positive OHR and 295 (70.9%) were negative. The overall performance of the BLN Assay was compared at the patient level with permanent-section histology in adjacent node tissue with an observed sensitivity of 87.6 per cent (95% CI [80.4%, 92.8%]) and specificity of 94.2 per cent (95% CI [90.9%, 96.6%]). These results validated the cut-off values selected from the beta study, and assay performance in both trials was high by comparison with OHR (Table 1). To reiterate, this provides only a relative estimate of the assay's true performance since different tissue is tested by the two methods with inherent sampling limitations.

[4] The gene encoding porphobilinogen deaminase is often referred to as a 'housekeeping' gene and is constitutively expressed (always turned on) in all cells. The properties of this internal control marker were used to establish test functionality. If the test is working correctly, a signal for the control marker should be observed for all samples.

[5] The sponsorship and data management role of Veridex LLC in this study constitutes a conflict of interest issue.

Table 1 GeneSearch™ BLN Assay overall performance against overall histology results (OHR)

Study	No. of Patients	Sensitivity % [95 % C]	Specificity % [95 % CI]	PPV* %	NPV† %	Agreement‡ %
Validation	416	87.6 [80.4, 92.9]	94.2 [90.9, 96.6]	86.2	94.9	92.3
Beta	304	82.4 [72.6, 89.8]	96.3 [92.9, 98.4]	89.7	93.4	92.4

*PPV = positive predictive value

† NPV = negative predictive value.

‡High agreement for the number of SLNs positive in a given patient indicate that assay cut-off values were suitably selected to detect only metastases of clinical relevance.

Results of the BLN Assay compared to routine histological intra-operative tests are summarised in Table 2. In the validation study, BLN Assay sensitivity was 10 per cent higher than observed for frozen section (95.6% versus 85.6%; p=0.04). Specificity was not statistically different (p=0.09).

Table 2 GeneSearch™ BLN Assay performance versus site intra-operative histological tests using OHR as the comparator test

Study	Test	No. of Patients	Sensitivity % [95% CI]	Specificity % [95% CI]	PPV %	NPV %
Validation	BLN Assay	319	95.6* [89.0, 98.8]	94.3 [90.5, 97.0]	86.9	98.2
	Frozen section	319	85.6 [76.6, 92.1]	97.8 [95.0, 99.3]	93.9	94.5
Beta	BLN Assay	171	83.6 [71.2, 92.2]	94.8 [89.1, 98.1]	88.5	92.4
	Frozen section	171	76.4 [63.0, 86.8]	96.6 [91.4, 99.1]	91.3	89.6
Validation	BLN Assay	29	63.6 [30.8, 89.1]	100 [81.5, 100]	100	81.8
	Cytology	29	45.5 [16.8, 76.6]	100 [81.5, 100]	100	75.0
Beta	BLN Assay	58	84.2 [60.4, 96.6]	92.3 [79.1, 98.4]	84.2	92.3
	Cytology	58	57.9 [35.5, 79.8]	100 [91.0, 100]	100	83.0

*p-values (< 0.05) were reported only for sensitivity and specificity of the BLN Assay versus frozen section in the validation study. Large differences in the remaining point estimates comparing the methods are clearly evident, however where wide confidence intervals are involved, the observed differences are unlikely to be significant.

It is important to note that the BLN Assay performance is dependent on the size of metastases. Observed values for sensitivity differed on the basis of whether a node contained micrometastases or macrometastases.[6] In the validation study, for OHR-positive patients with macrometastases (n=94), the sensitivity of the BLN Assay was 97.9 per cent (95% CI [92.5%, 99.7%]). For patients with micrometastases (n=23),

[6] Micrometastases are defined by the AJCC as greater than 0.2mm and up to 2.0mm, while macrometastases are greater than 2.0mm.

sensitivity was 56.5 per cent (95% CI [34.5%, 76.8%]). Discrepancies between test results that occur when different parts of a node are examined are likely to be greater when micrometastases are present because these are less distributed throughout a node than macrometastases. Disagreement was also seen in the detection of micrometastases when comparing different histological sections from the same node pieces. Site pathologists found metastases on site slides in only 75 per cent (95% CI [47.6, 92.7]) of patients with micrometastases identified by central pathologists on central slides using nearby tissue. The majority (9/15) of the BLN Assay "false negative"[7] results were seen among patients with only one OHR-positive node, all such nodes containing micrometastases. Eight of the same 15 patients had nodes confirmed positive on only one set of slides (site or central) as opposed to both. Similar results were seen for the majority (15/17) of BLN Assay-positive/OHR-negative patients who were BLN Assay-positive for a single node.

Lymph nodes that gave differing results between OHR and the BLN Assay for the validation study were subjected to further, independent molecular testing. This provided an estimate for the percentage of disagreement likely to have resulted from the assay and histology evaluating different parts of a given node. An RNA extract from residual homogenate was used to establish cut-offs for four markers[8] that would yield 100 per cent specificity with a lower confidence limit no less than 95 per cent. As a control, residual assay samples of eleven BLN Assay-negative and OHR-negative nodes from 11 patients underwent independent molecular testing. All tested negative indicating high sensitivity for both tests. Of the 15 nodes that were BLN Assay-positive and OHR-negative, 11 (73%) had a positive independent molecular test result. In addition, it would be expected nodes that were BLN Assay-positive and OHR-positive would all test 100 per cent positive with molecular testing. However, molecular testing was only positive in 15 out 23 (65%) of these samples. These last two results indicate that differential sampling occurs when the BLN Assay and histology is conducted on sections of lymph nodes. That is, metastases may not occur uniformly in the lymph node giving rise to what appears to be "false positives" and "false negatives" with the different techniques. Results of the test were for confirmation purposes only and not included for calculation of BLN Assay performance. Technicians involved in this additional testing were blinded to previous test results.

Further sub-analysis found a high correlation between the size of metastases and the BLN Assay's C_t (cycle threshold) values for MG and CK-19. Since these values are a

[7] "False negatives" and "false positives" from either test may in fact be true results for a given tissue sample. The differential sampling means that comparison could be made between two portions of tissue for which one contains metastatic cells and the other is free from metastasis. The results of the two tests can therefore be different and yet both correct.

[8] PIP, PDEF, B305D and B726. Understanding the complex functions of these markers is not necessary within this context. The importance is in the role of the additional test's ability to confirm the BLN Assay results.

measure of the time taken to reach a positive result, the lower C_t values represent a higher measure of positivity, corresponding to larger or more extensive metastases which express markers at a higher level. Figure 1 shows data for nodes from 383 patients in the validation study with conclusive OHR and valid BLN Assay results.

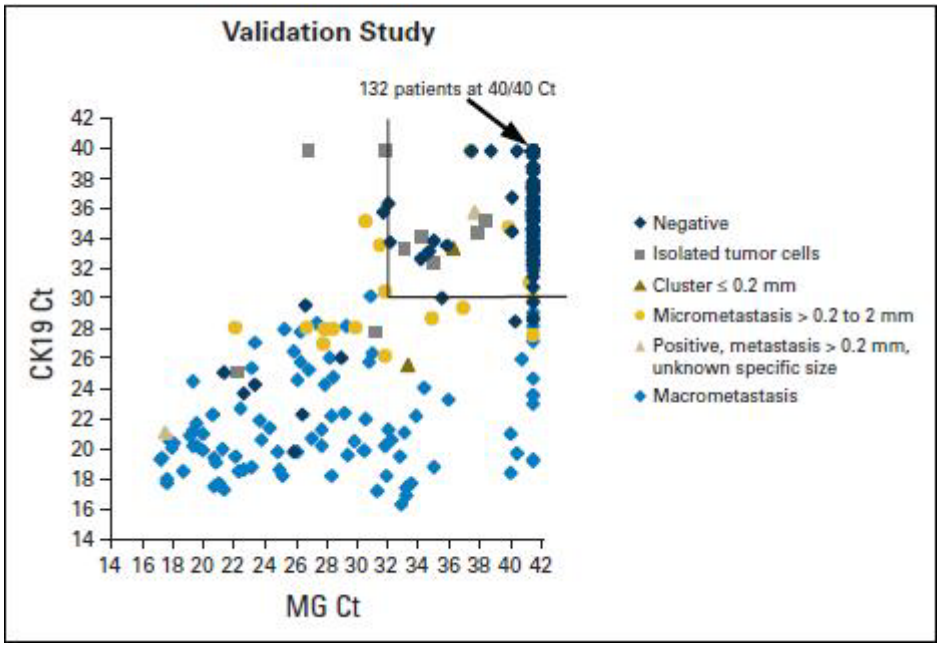

Figure 1 Correlation between GeneSearch™ BLN Assay C_t values and metastasis determined by histology for the validation study. The lowest C_t values found on any node for a given patient are plotted. BLN Assay results falling within the boxed section to the upper right were regarded as negative. Cut-offs are represented by the boundaries of this region. 132 overlapping data points are shown at the 40/40 point indicating no signal was detected for either MG or CK-19 (Julian et al 2008).

Data were collected on the time required to conduct the BLN Assay. Results for a patient were available within 36 to 46 minutes for one to three nodes when the operator was experienced with the assay.[9] Time at the lower end of this range was attainable if the testing laboratory was in the vicinity of the surgery suite and if there were a maximum of two sentinel nodes to be tested. Another four to six minutes were required for each additional node. Final results were automated and available on a computer screen or by print out.

Overall, this study showed high agreement between the BLN Assay and moderately extensive permanent-section histology on adjacent node samples. The assay gave higher sensitivity than intra-operative histology. However, reporting on specificity was ambiguous and wide confidence intervals were associated with all differences between specificity for the assay and intra-operative techniques against post-operative HE histology. Even so, there is evidence that the performance of the BLN Assay is actually higher than observed. Apparent 'false positives' (BLN Assay-positive/OHR-negative results) may in fact be correct as shown by independent molecular testing.

[9] An experienced operator will have tested a minimum of 30 patients.

For most (73%) of BLN Assay-positive/OHR-negative samples, the independent test confirmed positivity, indicating that the portions of node tested did contain metastases, but that these were confined to that particular sample and absent in the tissue assessed by histology. This shows the importance of more extensive tissue sampling to detect metastases. Application of the BLN Assay could minimise the effect of sampling bias because a larger portion (50%) of each node is analysed (Julian et al 2008).

The multicentre study discussed to date formed the basis for several single site studies in Belgium, the UK and Italy. Studies in these countries all applied the threshold cut-off values for mammaglobin and CK-19 determined in the original beta trial involving 12 sites. The study conducted in Belgium recruited 78 breast cancer patients scheduled for lumpectomy and compared the performance of the BLN Assay with routine histology (Martin Martinez 2009). Sentinel lymph nodes were identified using the standard procedure of the institute and excised prior to lumpectomy to avoid possible contamination of lymph nodes during tumour manipulation. A total of 123 SLNs were evaluated by histology[10] and the BLN Assay.

SLNs were divided along the short axis to produce fresh samples of 1.5 to 2.0mm thickness and alternatively processed by the BLN Assay and post-operative histology, using a protocol similar to Julian et al 2008. For clarity, a diagram of the process is shown in Figure 2. BLN Assay results were not used for patient management and nodes were only considered positive for metastases greater than 0.2mm as defined by the AJCC. Patients and pathologists were blinded to BLN Assay results, while operators of the assay were blind to histological results.

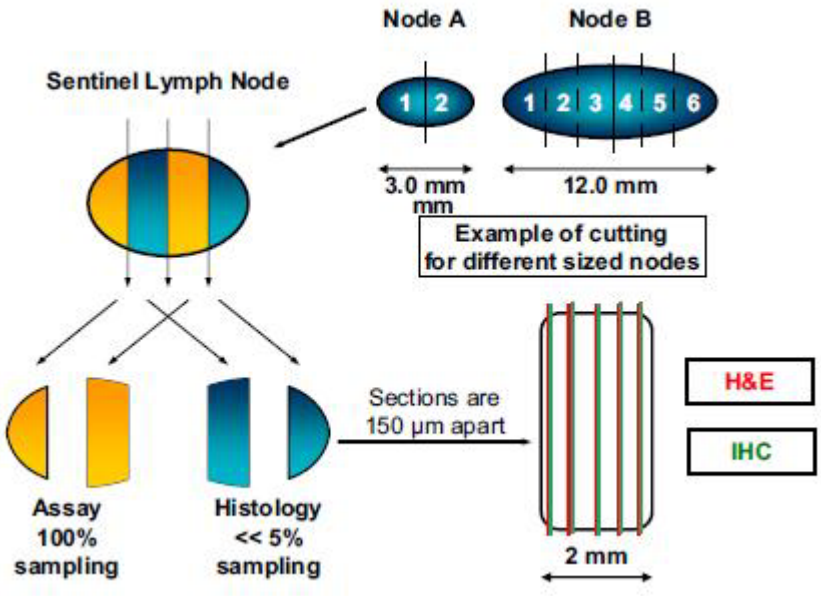

Figure 2 Sentinel node sampling procedure (Martin Martinez et al 2009).

[10] HE and IHC.

Results from the assay were available within 35 to 45 minutes, with 14 out of 78 patients testing SLN metastasis positive. Histological analysis found 13 out of the 78 patients were positive for metastases greater than 0.2mm. Agreement between the techniques was 96 per cent (75 out of 78 patients). Two of the 14 BLN Assay-positive cases had no metastases identified when examined by HE and IHC, while one BLN Assay-negative case had a micrometastasis of 0.25mm when assessed by IHC. For almost all histologically node negative patients, expression levels of the cancer markers mammaglobin and CK-19 did not reach levels for positivity and 21 patients had no signal at all detected by the RT-PCR. Three cases had histologically identified sub-micrometastases, considered by the institution as clinically irrelevant (less than 0.2mm). Two of these were isolated tumour cells and all three were BLN-Assay negative. Only one case, a micrometastasis detected by IHC alone, had a 'false negative' assay result. In this instance, both mammaglobin and CK-19 were expressed at levels near the cut-off values. Compared to HE and IHC sections, the BLN assay had a sensitivity of 92 per cent (12/13) and specificity of 97 per cent (63/65). Positive predictive value was 86 per cent (12/14) and negative predictive value was 98 per cent (63/64). Statistically significant correlations were found between the size of metastases identified by histology and each of the markers, mammaglobin (ρ=0.76) and CK-19 (ρ=0.71) ($p < 0.0001$).

The financial and operational involvement of Veridex LLC in this study was substantial. Veridex supplied assay equipment free of charge, funded acquisition of specimens and labour costs associated with testing, and provided training for assay operators (Martin Martinez et al 2009) (level III-1 diagnostic evidence).

Another study of 253 patients (Veys et al 2009a) was conducted at the same institute on the basis of the work by Martin Martinez (2009). This study was later extended to include a further 114 patients (Veys et al 2009b). For the purposes of this update, only the results of the latter publication are reported. Selection criteria were in accordance with Julian et al 2008 and Martin Martinez et al 2009. However, the main difference in the work led by Veys was the adoption of the BLN Assay as an intraoperative test for patient management[11]. The BLN Assay was positive in 19.6 per cent (72/367) of patients with a sensitivity of 89 per cent and positive predictive value of 76.5 per cent, compared with histology. Specificity of the assay was 94.5 per cent and negative predictive value was 97.5 per cent. Significant correlations between size of metastasis and markers, mammaglobin (ρ=0.62) and CK-19 (ρ=0.64) were observed ($p < 0.0001$). These results lend further support for the BLN Assay as a complementary tool to conventional histology for decisions about axillary lymph node dissection. Not only may the risk of additional disease in non-SLNs be predicted, but also, cycle threshold values for mammaglobin and CK-19 could provide valuable information regarding the size and extent of metastases (level III-1 diagnostic evidence).

[11] The BLN Assay was CE marked in August 2006. Having investigated extensively, Jules Bordet Institute now performs axillary lymph node dissection on the basis of assay results.

A study conducted in the UK (Mansel et al 2009) recruited 82 breast cancer patients to investigate the BLN Assay, employing procedures and eligibility criteria in line with Julian et al 2008. The performance of the assay, comparative to histology, was similar to that observed in the previously described studies (level III-1 diagnostic evidence). The results of an Italian trial (n=293) also obtained similar results with a similar research protocol (level III-1 diagnostic evidence). However, unlike the preceding studies, sensitivity was favoured over specificity due to more extensive sampling of the node achieved by sectioning and sampling at closer intervals (Figure 3) (Viale et al 2008).

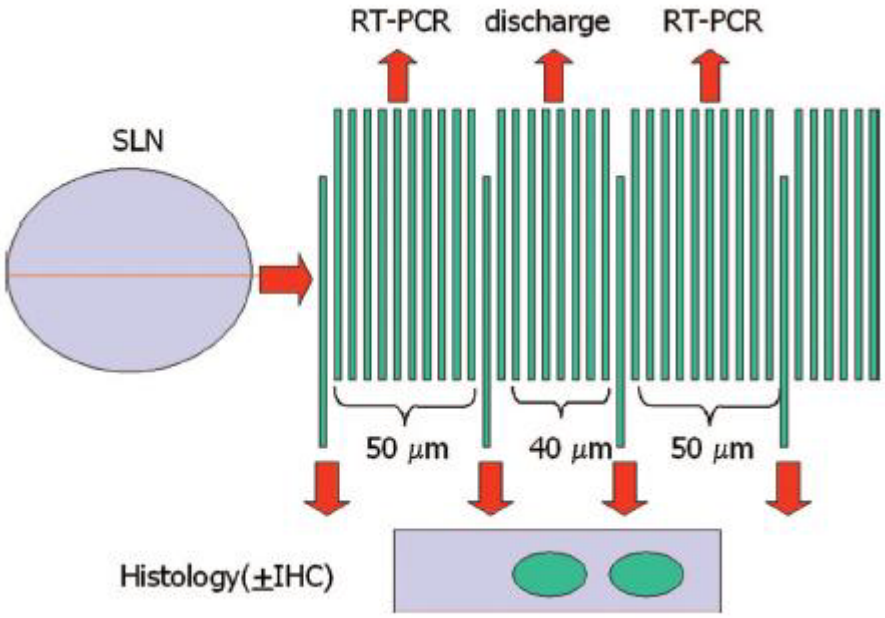

Figure 3 Node sampling and sharing procedure for the assay and histology. This pattern was continued until the node was exhausted (Viale et al 2008).

OTHER ISSUES

The work by Veys et al (2009b) brought special attention to the choice of markers for the detection of lymph node metastases using molecular methods. Whereas the BLN Assay uses mammaglobin and CK-19, other assays such as OSNA[12] (Schem et al 2009; Tamaki et al 2009; Visser et al 2008) use only CK-19. The rationale in support of OSNA is its ability to detect CK-19 (widely expressed in human cancers) with high sensitivity. Conversely, RT-PCR used in the BLN Assay can sometimes be unreliable in detection of CK-19 due to the presence of pseudogenes[13] and contamination of benign epithelial cells. OSNA can quantitatively detect CK-19 without interference from pseudogenes, and differentiate contamination of a few epithelial cells and the existence of isolated tumour cells from metastasis of clinical relevance using an

[12] One-step nucleic acid amplification.
[13] Genomic DNA similar to normal genes in sequence, but lacking components essential for protein expression.

established cut-off value (Tamaki et al 2009). In support of the BLN Assay over OSNA, Veys et al (2009b) found that among 71 BLN Assay-positive patients[14], 48 were positive for both mammaglobin and CK-19, 15 were positive for CK-19 only and eight (11%) were positive for mammaglobin only. Notably, two of the eight cases positive for mammaglobin only had clinically relevant metastases detected by histology. Consequently, even if OSNA is highly sensitive and specific, it potentially misses some positive cases detectable by the BLN Assay.

2010 SUMMARY OF FINDINGS:

Evidence from the studies examined shows that specificity and sensitivity of the BLN Assay are at least as high as conventional post-operative histology. While metastasis to the sentinel lymph nodes may be simultaneously ruled in by one test and ruled out by the other, this does not necessarily imply an erroneous result in either diagnostic method. This is a reflection of differential sampling, which may be used to advantage by undertaking the BLN Assay in tandem with post-operative histology. There is an obvious advantage in determining metastasis intra-operatively, then progressing to axillary lymph node clearance during the course of one surgical procedure rather than two. Complications and additional morbidity are avoided for the patient, while demand on health system resources is reduced. Even so, the BLN Assay has the advantage of testing more lymph node tissue on a hypothetical post-operative basis. Patients diagnosed by both histology and the BLN Assay, whether intra-operatively or post-operatively, are more likely to have an accurate result than patients examined by histology alone. Finally, an additional advantage is that the assay can be performed by trained technicians, making it more accessible than histological techniques where expert pathology is limited or under significant pressure.

2010 HEALTHPACT ASSESSMENT:

The BLN Assay offers a clear benefit in the role of detecting lymph node metastasis, thereby informing decisions on whether or not axillary lymph node clearance should be performed. The assay has high potential for complementing conventional histology for improved diagnosis on the basis of more extensive examination of lymph node tissue. Furthermore, the ability to avoid additional costs and morbidity associated with axillary lymph node dissection during a second procedure add to the advantages seen in the performance of this technology. However, during the preparation of this update for publishing, it was discovered that the manufacturer has withdrawn the BLN Assay. Therefore, no further review of this technology by HealthPACT is warranted.

2010 LIST OF STUDIES INCLUDED:

Total number of studies	5
Level III-1 diagnostic evidence	5

[14] One case with missing C_t data.

2010 REFERENCES:

Julian, T. B., Blumencranz, P. et al (2008). 'Novel intraoperative molecular test for sentinel lymph node metastases in patients with early-stage breast cancer', *J Clin Oncol,* 26 (20), 3338-3345.

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Schem, C., Maass, N. et al (2009). 'One-step nucleic acid amplification-a molecular method for the detection of lymph node metastases in breast cancer patients; results of the German study group', *Virchows Arch,* 454 (2), 203-210.

Tamaki, Y., Akiyama, F. et al (2009). 'Molecular detection of lymph node metastases in breast cancer patients: results of a multicenter trial using the one-step nucleic acid amplification assay', *Clin Cancer Res,* 15 (8), 2879-2884.

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Veys, I., Majjaj, S. et al (2009b). 'Evaluation of the histological size of the sentinel lymph node metastases using RT-PCR assay: a rapid tool to estimate the risk of non-sentinel lymph node invasion in patients with breast cancer', *Breast Cancer Res Treat*.

Viale, G., Dell'Orto, P. et al (2008). 'Comparative evaluation of an extensive histopathologic examination and a real-time reverse-transcription-polymerase chain reaction assay for mammaglobin and cytokeratin 19 on axillary sentinel lymph nodes of breast carcinoma patients', *Ann Surg,* 247 (1), 136-142.

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PRIORITISING SUMMARY

REGISTER ID: **000295**

NAME OF TECHNOLOGY: **GENESEARCH™ BREAST LYMPH NODE (BLN) ASSAY**

PURPOSE AND TARGET GROUP: **WOMEN UNDERGOING SENTINEL LYMPH NODE BIOPSY FOR BREAST CANCER**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

- ☒ Yet to emerge
- ☐ Experimental
- ☐ Investigational
- ☐ Nearly established
- ☐ Established
- ☐ Established *but* changed indication or modification of technique
- ☐ Should be taken out of use

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

- ☐ Yes — ARTG number
- ☒ No
- ☐ Not applicable

INTERNATIONAL UTILISATION:

COUNTRY	LEVEL OF USE		
	Trials Underway or Completed	Limited Use	Widely Diffused
United States of America	✓		
Belgium		✓	
United Kingdom	✓		
France	✓		

IMPACT SUMMARY:

Veridex, LLC provides Genesearch BLN (Breast Lymph Node) with the aim of detecting the spread of breast cancer to lymph nodes within the breast and arm. In combination with permanent section Haematoxylin and Eosin (H&E) staining analysis of the lymph node, the data obtained from the GeneSearch™ BLN Assay can assist in staging of the patient. (FDA 2006) The technology would only be available through tertiary hospitals due to the requirement for a molecular biology diagnostic laboratory. The assay is for patients undergoing sentinel lymph node biopsy during breast cancer surgery. Currently, this technology is not in use in Australia.

BACKGROUND

Sentinel lymph node biopsy (SNB) for breast cancer is an emerging diagnostic technique that is undergoing clinical trials in Australia (e.g. SNAC trial). SNB is

based on the idea that cancer spread occurs in a non-random fashion and is hence more likely to spread first to the lymph nodes to which the tumour drains (Giuliano et al 1994). The testing of "sentinel" lymph nodes is an indicator of how far the cancer has spread within the patient. If the sentinel nodes are found to be cancer positive the patient may undergo axillary clearance (AC). AC is the removal of all of the lymph nodes from the armpit, and is associated with significant morbidity such as wound infection; numbness of the arm, shoulder, armpit and chest; seroma; lymphoedema; and shoulder stiffness (Schijven et al 2003). If the sentinel nodes are negative for cancer the patient is prevented from undergoing potentially more damaging AC. Recently, the SNAC trial reported a positive outcome of SNB versus AC (Wetzig 2005). In Australia, 74% of breast cancer patients present with T1 stage (<20 mm) or ductal carcinoma *in situ* and the majority of these patients have metastasis negative lymph nodes (Malycha 2003). SNB is of most benefit to patients with negative nodes as they are prevented from undergoing AC.

The GeneSearch™ BLN Assay is *dependent* on the adoption of SNB in preference to immediate AC, as the assay is designed to be used intra-operatively to screen sentinel lymph nodes for clinically relevant (>0.2mm) breast cancer metastases. Specifically, the GeneSearch™ BLN Assay aims to improve the accuracy of metastasis detection during intra-operative sentinel lymph node biopsy. The GeneSearch™ BLN Assay consists of 2 parts; a RNA purification kit and a real time reverse-transcription polymerase chain reaction (RT-PCR) kit. The real time RT-PCR assay qualitatively detects the expression of two genes, Mammaglobin (MG) and Cytokeratin 19 (CK19). Mammaglobin and Cytokeratin are markers specific for breast cancer cells and are not expressed at high levels in normal lymph node tissue (Bernstein et al 2005; Schoenfeld et al 1997; Zehentner & Carter 2004). The assay involves the sectioning of the lymph node with alternative sections homogenised, the RNA extracted and real time RT-PCR performed on this RNA. If external RT-PCR controls are valid then the samples are compared against pre-determined thresholds for each gene and internal RT-PCR controls. The samples are then designated positive, negative or invalid, depending which markers are above or below these thresholds (Veridex 2006). The real time RT-PCR is performed on a Cepheid Smart Cycler® Diagnostic System. The alternate, spare lymph node sections can be embedded and used for post-operative permanent H&E staining to verify and supplement the information provided by the GeneSearch™ BLN Assay.

CLINICAL NEED AND BURDEN OF DISEASE

In Australia, the number of females diagnosed with breast cancer was estimated to be 13,261 in 2006 and is predicted to rise to 14,800 in 2011. The age-standardised incidence of breast cancer in females was 117 per 100,000 in 2002, 80% above the 1983 level. In 2004 the age-standardised rate of death from breast cancer was 23.4 per 100,000 females, decreasing from 31.0 deaths per 100,000 in 1990. There were 2,641 female deaths due to breast cancer in 2004, with an average of 601 additional cases

per year from 2000–2004 in which breast cancer was an associated cause but not the underlying cause of death (AIHW & NBCC 2006).

In 2003-04 there were 23,598 hospital separations where the primary cause of hospitalisation was breast cancer and on average each separation was 3.9 days (AIHW & NBCC 2006).

The GeneSearch™ BLN Assay is not applicable to all patients undergoing breast cancer surgery. Only the population undergoing SNB are candidates for testing with GeneSearch™ BLN Assay. The initial findings of the SNAC trial indicate that only 46% of women initially included into the trial passed the exclusion criteria, (54% were excluded due to tumour size greater then 3 cm or axillary involvement), and were hence suitable for SNB (Gill 2004).

DIFFUSION

The Veridex GeneSearch™ BLN Assay has pre-market approval in the USA and is CE marked as an *in vitro* diagnostic device in Europe (Veridex, Personal communication). The GeneSearch™ BLN Assay is used routinely at the site of the European clinical trial, Jules Bordet Institute, Brussels, Belgium (Veridex, Personal communication). It has not yet been marketed as an *in vitro* diagnostic device in the USA, Canada, Japan or Australia. The product has been in clinical trials as an investigational use only (IUO) device in the United States.

COMPARATORS

Following AC, histological analysis, using H&E staining, is conducted post-operatively on formalin-fixed and paraffin-embedded slices of sentinel nodes. This is the gold standard with regard to diagnosis of breast cancer metastasis (MSAC 2005). Intra-operative comparators include gross inspection of the target lymph node by the surgeon, or either frozen section or imprint cytology of the sentinel lymph nodes. Frozen section or imprint cytology require the availability of a pathologist to assess the test samples. The GeneSearch™ BLN Assay is aimed to replace intra-operative tests for breast cancer metastasis in sentinel lymph nodes.

Table 3 GeneSearch™ BLN Assay compared to Intra-operative histology

	GeneSearch™ BLN Assay	Histology based tests (Frozen section (FS), imprint cytology (IC))
Advantages	Objective	Can determine metastasis size directly (FS only)
	Samples more of lymph node (less sampling error)	Can determine location of tumour within lymph node (FS only)
	Only requires verification of results by Pathologist	Can distinguish true from iatrogenic positives (FS only)
	Standardised Test	Slightly shorter to perform (10 - 40 minutes)
	Lower operator to operator variability	Greater Specificity (97.8 % vs. 94.3 %) (FS only)
	Greater sensitivity (95.6 % vs. 85.6 %)	
Disadvantages	Tissue used in assay cannot be subsequently used for histology	Subjective
	Less information for patient staging (no metastasis size measurements or location of tumour)	Requires more time from an expert Pathologist
	Take slightly longer to perform (35-50 minutes)	Higher operator to operator variability
		Not standardised, each lab has own methods
		Samples less of lymph node (greater sampling error)

Adapted from (Cserni et al 2003)

EFFECTIVENESS AND SAFETY ISSUES

There are no known contraindications for the GeneSearch™ BLN Assay. The GeneSearch™ BLN Assay is an *in vitro* test and therefore can have no direct negative impact on the patient. Histological information about the lymph node is completely destroyed during its processing within the assay. Due to the destruction of useful information the FDA has stated, in its pre-market approval, that the test may only be conducted as a *complementary* test to the post-operative gold standard, H&E staining of permanent lymph node sections (FDA 2006). H&E staining and the GeneSearch™ BLN Assay result in qualitatively different information and conflicts may arise between these two testing methods. The PPV of the GeneSearch™ BLN Assay was calculated to be 86% compared to H&E staining (FDA 2006). Although this would imply that 14% of patients testing positive with the GeneSearch™ BLN Assay would later test negative with H&E staining, evidence was presented at the FDA pre-market approval meeting indicating that the majority of these "false positives" are in fact true positives and that H&E staining itself misses some positives due to its limited sampling of the lymph node (FDA 2006).

Staging of the breast cancer patient within their disease course, which is critical for post-surgical treatment, is currently based on histological data obtained during SNB or AC. The information from the GeneSearch™ BLN Assay is qualitatively different to that obtained from the current histological methods e.g. the size of metastasis and its location with in the lymph node. Hence, some controversy exists about how best to stage patients solely tested with the GeneSearch™ BLN Assay (FDA 2006). Due to this problem the FDA ruled that the GeneSearch™ BLN Assay must be conducted alongside post-operative H&E staining.

The GeneSearch™ BLN Assay is only useful when a SNB is a surgical option for a patient, as a patient undergoing AC can be staged using the established H&E staining post-operatively. In Australia, as reported by the RACS SNAC trial, half of the patients presenting were ineligible for SNB as they either had large (> 3 cm) or multicentric tumours, and therefore had AC immediately (Wetzig et al 2005). The GeneSearch™ BLN Assay is not applicable in the diagnosis of these patients as they are not candidates for SNB. No long-term morbidity/mortality data exist for patients undergoing SNB, and therefore, by extension, for patients being tested with the GeneSearch™ BLN Assay.

In the clinical trial conducted by Veridex, which was submitted to the FDA, the sensitivity of the GeneSearch™ BLN Assay was reported to be 87.6 % (95% CI [80.4, 92.9]) and the specificity was 94.2 % (95% CI [90.9 to 96.6]) (FDA 2006) (level III-2 diagnostic evidence).

COST IMPACT

A capital investment of $US 40000 is required for establishment of the GeneSearch™ BLN Assay at a particular site. The kit would cost $US 2250 for 30 tests (Veridex, personal communication). Each patient requires one test per lymph node and two controls (positive and negative). The test itself would cost about $US 300 per patient, assuming two lymph node tests and two controls are performed per patient, i.e. four tests per patient (Veridex, personal communication).

Through the reduced burden on pathologists and an increased sensitivity, and therefore reduction in second surgery costs, the GeneSearch™ BLN Assay is predicted to be more cost effective than current comparators (Veridex, personal communication), although this is not supported by evidence at this early stage in the product's development.

ETHICAL, CULTURAL OR RELIGIOUS CONSIDERATIONS

A false positive test result from the GeneSearch™ BLN Assay could lead to the patient receiving AC rather than SNB alone. AC has been associated with significant morbidity versus SNB.

A false negative test result from the GeneSearch™ BLN Assay means that an existing metastasis/metastases were not detected and the incorrect surgical procedures and/or post-surgical treatment may be administered to the patient e.g. failure to perform AC. Although the patient may be monitored over the course of their disease course, and thus the false negative result may be later amended to the correctly positive result, there is a significant risk, potentially fatal, to the patient receiving a false negative result.

OTHER ISSUES

All research on the GeneSearch™ BLN Assay was conducted by the Veridex or affiliates. The FDA gave pre-market approval for the GeneSearch™ BLN Assay with several conditions.

CONCLUSION:

As recently reported in the initial findings of the RACS SNAC trial (Wetzig 2005), SNB alone reduced the hospital stay to 1.8 days from 2.8 days for SNB plus AC. The Genesearch test is designed to increase the sensitivity of intra-operative diagnosis of the spread of metastatic breast cancer cells to sentinel lymph nodes of patients undergoing lumpectomy. Hence it may play a role in the uptake/improvement of SNB and therefore indirectly facilitate a reduction in breast cancer patient morbidity.

If the GeneSearch™ BLN Assay is to play a role in reducing the mortality of breast cancer patients it will be through more accurate diagnosis of breast cancer metastasis during SNB. As yet there is no data to indicate whether SNB itself lowers the mortality rate among breast cancer patients. Hence, it is unclear whether the GeneSearch™ BLN Assay would have any indirect effect on breast cancer mortality until further investigation into SNB concludes.

HEALTHPACT ACTION:

The GeneSearch™ assay may have benefits in terms of a standardised approach to the determination of nodal status. However, the widespread usefulness of this assay may depend on whether or not the assay, or the genes that are targeted in the assay, are patented. HealthPACT have therefore recommended that this technology be monitored in 12 months time.

SOURCES OF FURTHER INFORMATION:

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LIST OF STUDIES INCLUDED

Total number of studies

Level III-2 Diagnostic evidence 1

SEARCH CRITERIA TO BE USED:

Breast Neoplasms/*pathology/surgery

Lymphatic Metastasis

*Sentinel Lymph Node Biopsy

Axilla

Lymph Node Excision/*adverse effects

Sensitivity and Specificity

Tumor Markers, Biological