Breast Cancer
Early Detection – the key to effective treatment and better survival

Towards more patient-friendly diagnostics
DiaGenic’s vision is to be a leading provider of molecular diagnostics for the early diagnosis of disease. DiaGenic’s mission is to offer patient-friendly diagnostic tools for the early detection of diseases to improve quality of life and lower costs for society.

*Early detection from just one drop of blood*
Over 1.1 million women are diagnosed with breast cancer annually worldwide. Despite the introduction in most Western countries of mammography screening breast cancer remains the main cause of cancer death amongst women. This suggests that the current method of screening, diagnosis and treatment are not optimal.

5 year survival figures from the American Cancer Society show improved survival from early detection of cancer, with almost 100% five year survival rates for women with stage 0 or stage 1 breast cancer. Mammography is, at present, the main tool for screening and diagnosis (with histopathology/cytology) for women with breast cancer. The sensitivity of mammography is high in symptomatic women but decreases in younger women, women with dense breasts, and for cases of lobular carcinoma. Boyd et al (NEJM 2007) demonstrated that women with 75% or more breast density showed an increased risk of developing breast cancer with an odds ratio of 4.7. In this group, mammography had a sensitivity of 64% across all ages. The rate of cancers missed by mammography in women with extremely dense breasts is 15 times higher than women with low dense breasts. Whilst women of all ages may have high breast density that hinders the quality of mammographic images, it is pre-menopausal women that are more likely to have dense breast tissue since the fat content of the breast increases with age.

Lobular cancer constitutes 5-15% of all breast cancers and is the second largest group after ductal cancers. Due to the architectural structure of lobular breast cancer and the lack of microcalcifications, mammography shows poorer performance for the detection of these lesions.

Tests such as ultrasound and MRI provide additional tools for the diagnosis of breast cancer. MRI has high sensitivity but has poor specificity and is therefore not suitable as a general screening tool. The use of MRI as a screening tool, however, has been recommended by the American Society of Clinical Oncology (ASCO) for high risk women. There are no international guidelines on the performance of MRI, and the high recall rate of MRI leads to an increased consumption of resources and financial demands for health centres.

Why is early diagnosis important?

Early detection of a growing breast tumour is of key importance for patient survival. The 5 year survival rate for early stage breast cancer patients is 100% according to the American Cancer Society.

<table>
<thead>
<tr>
<th>Stage</th>
<th>5-year relative survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>I</td>
<td>100%</td>
</tr>
<tr>
<td>II</td>
<td>86%</td>
</tr>
<tr>
<td>III</td>
<td>57%</td>
</tr>
<tr>
<td>IV</td>
<td>20%</td>
</tr>
</tbody>
</table>

Berg et al (Radiology 2004) demonstrated that whilst mammography had a sensitivity of up to 81% for ductal lesions, lobular lesions were detected at a sensitivity of 34%. Whilst the stage by stage prognosis for ductal and lobular cancers is similar, the mortality of lobular cancers is slightly higher which may in part be due to the difficulty in detecting the lesions by mammography.

The BCtect® test is a simple to use, in-vitro diagnostic test for the quantitative measurement of gene expression in the blood of patients with suspected breast cancer. The intended use for BCtect® is to aid in the diagnosis of breast cancer in adult women. It is intended that the BCtect® test is used together with other clinical evidence to confirm the presence or absence of breast cancer.

The BCtect® gene expression signature consists of a unique set of 96 genes, including quality control genes.

BCtect® is the first blood test to be offered as an adjunctive test to mammography to aid in the detection of early breast cancer.

Table 1: 5-year survival
Early detection is offered by mammographic screening programs; however, the inability to detect all cancers contributes to the continuing high mortality rate for breast cancer. Using the latest in mammographic digital technology, the Digital Mammography Imaging Screening Trial (DMIST) reported only a moderate increase in the overall sensitivity (as defined by a 12-month follow-up period) of digital mammography to 70% compared to 66% sensitivity with film screen technology. The sensitivity of mammography is further reduced in younger women below the age of 50, the age when screening is usually initiated, and in women with high breast density. In a comparative study including data from five prospective studies and including 3571 screened high-risk women with a mean age of 41, the sensitivity of mammography was only 40% Pisano et al (NEJM 2005). Despite these limitations, mammography screening has been shown to help reduce mortality due to breast cancer. However, there is clearly a potential to increase the detection of early cancers and thereby improve the quality of life and survival for breast cancer patients.

Breast cancer
A growing problem in the world

The challenge

Early detection is offered by mammographic screening programs; however, the inability to detect all cancers contributes to the continuing high mortality rate for breast cancer. Using the latest in mammographic digital technology, the Digital Mammography Imaging Screening Trial (DMIST) reported only a moderate increase in the overall sensitivity (as defined by a 12-month follow-up period) of digital mammography to 70% compared to 66% sensitivity with film screen technology. The sensitivity of mammography is further reduced in younger women below the age of 50, the age when screening is usually initiated, and in women with high breast density. In a comparative study including data from five prospective studies and including 3571 screened high-risk women with a mean age of 41, the sensitivity of mammography was only 40% Pisano et al (NEJM 2005). Despite these limitations, mammography screening has been shown to help reduce mortality due to breast cancer. However, there is clearly a potential to increase the detection of early cancers and thereby improve the quality of life and survival for breast cancer patients.

RNA - The ideal biomarker for complex diseases

The uniform chemical nature of RNA makes this analyte very well suited for biomarker studies and several signatures using tumour tissue have been developed. Several of them are now commercially available and used as prognostic or prediction markers. However, markers based on gene expression in malignant tissue are not useful for diagnostic purposes.

Peripheral blood is highly suitable for diagnostic use as it is readily obtainable and provides a large biosensor pool in the form of gene transcripts. Peripheral blood has the potential to reflect responses to changes in the immediate and distant environments in the form of detectable alterations in the levels of selected RNA transcripts. Gene expression profiling is affected by technical variables such as collection, transportation, storage of blood samples, RNA extraction and choice of microarray platform. These challenges can be met by close attention to study design to remove bias in study populations, and by implementation of quality control procedures to ensure consistency in technical variables.

Our approach using the expression pattern from multiple informative genes is more suited to the multifactorial nature of breast cancer than existing singleplex blood based biomarkers (tumour markers such as CA 15-3) thus providing a higher accuracy.
Development of BCtect®

BCtect® has been developed through an extensive and rigorous clinical program involving many hundreds of patient samples from hospitals in Scandinavia.

Proof of concept
Using a macroarray platform with 1536 randomly selected cDNA probes, DiaGenic were able to demonstrate for the first time that peripheral blood gene expression provides information that can distinguish breast cancer patients from healthy age matched controls with high accuracy (Breast Cancer Research 2005).

Discovery
Several whole genome studies using alternative microarray platforms were carried out to select the most informative genes from more than 30,000 available gene probes. This approach ensured that genes with no known function could also be included in our selection. A cohort of 140 subjects containing both control subjects (benign lesions and mammographically clear controls) and subjects with breast cancer (histology/cytology confirmed breast cancer) was recruited. More than 700 informative gene transcripts (RNAs) were identified which clearly distinguished breast cancer patients from age matched controls with a high accuracy of 81% (sensitivity 82% and specificity 79%).

Prototype development
To identify the optimal gene signature, 384 of the most informative gene transcripts were selected and validated using a real-time PCR platform. A new cohort of 123 patients was selected and a gene expression signature of 96 genes including several control genes was identified.

BCtect® validation
To ensure a robust and reliable clinical test, the BCtect® test was developed and validated using a multi-centre study design. More than 550 individual blood samples were recruited from 5 recruitment sites in Scandinavia and the US were collected. The majority were used in calibration of the test (223 samples), an additional set 109 samples was used for validation of the BCtect®. In total, 332 patient samples from 5 hospitals in Sweden, Norway and US were used in the final calibration and validation of the BCtect®.

Clinical validation of BCtect®

Of the total 332 patients, a large set 223 patients was selected to develop a robust calibration model for BCtect®. The remaining 109 patients from 5 different hospitals of which 2 had not been used in calibration was used for validation of BCtect®.

The accuracy of the BCtect® test in the combined calibration and validation set (N=332) is shown in the Table entitled “Overall Performance Characteristics”. BCtect® correctly predicted the class of 240/332 samples (including 126/176 BC samples and 114/156 control samples composed of women with benign lesions and women without mammographic findings).

The performance of BCtect® with the validation cohort alone was similar to the combined data with a sensitivity of 69% and specificity of 74%.

The ROC curve and area under the curve (AUC) from the validation study (N=109) and the combined calibration and validation study (N=332) are shown below.
Clinical validation of BCtect®

Ensuring uniformity of results

Table 2: Demographic and background data

<table>
<thead>
<tr>
<th>Demographic information</th>
<th>Validation cohort (N=109)</th>
<th>Combined cohort (N=332)</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>Breast cancer (N=55)</td>
<td>Non-breast cancer (N=54)</td>
</tr>
<tr>
<td>Mean</td>
<td>56</td>
<td>50</td>
</tr>
<tr>
<td>Min</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>Max</td>
<td>87</td>
<td>70</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>Pre-menopausal</td>
<td>Post Menopausal</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>69</td>
</tr>
</tbody>
</table>

Table 3: Overall performance characteristics

<table>
<thead>
<tr>
<th>Validation cohort (N=109)</th>
<th>Combined cohort (N=332)</th>
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<tbody>
<tr>
<td>Accuracy</td>
<td>72% (8.5%)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>69% (12.2)</td>
</tr>
<tr>
<td>Specificity</td>
<td>74% (11.7)</td>
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</table>

Table 4: Menopausal status

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<tr>
<td>Accuracy</td>
<td>72%</td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>73%</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>70%</td>
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Table 5: Performance by stage

<table>
<thead>
<tr>
<th>Validation (N=55)</th>
<th>Combined cohort (N=176)</th>
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<tbody>
<tr>
<td>Early stage (0-I)</td>
<td>74%</td>
</tr>
<tr>
<td>Late stage (II+)</td>
<td>66%</td>
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</table>

Lesion size
In the clinical studies BCtect® detected lesions as small as 4mm in diameter. No statistical relationship between lesion size and test score has been noted and therefore the minimum detectable lesion size has not been determined.

Receptor status
The performance characteristics of BCtect® are independent of tumour receptor status (ie: oestrogen, progesterone and HER2). In particular, the performance of BCtect® with triple negative tumours is the same as with receptor positive tumours.

Medications
Medications were taken by 47% of the validation cohort and 50% of the calibration cohort. Medications were most commonly taken for hypothyroidism, hypercholesterolaemia, cardiac conditions, diabetes, asthma and depression. No effect of medications on test score or performance characteristics has been noted in the BCtect® development studies.

Hormonal supplements
Hormonal supplements containing oestrogen and/or progesterone in the form of contraceptives or hormone replacement therapy were taken by 76 subjects in the combined cohort. BCtect® performed with 79% accuracy indicating that hormonal supplements do not influence the performance of BCtect®.

Menstrual cycle
Studies performed with BCtect® with repeated weekly sampling show no relationship between the test performance and menstrual cycle.

Breast cancer type
Lobular cancer is often difficult to image with mammography due to the architectural structure of the lesion and the lack of tell-tale calcifications. BCtect® performs equally well with lobular cancer as for ductal cancer. The sensitivity of the test for detection of lobular cancer is 74% in the validation set and 75% in the combined study population.

Limitations of BCtect®
- • BCtect® is an in-vitro test to aid in the diagnosis of breast cancer. The results should be used together with other clinical evidence to confirm the presence or absence of breast cancer. If the BCtect® result is inconsistent with other clinical evidence, further investigation is necessary to confirm the diagnosis.
- • BCtect® is intended for use in adult women.
- • BCtect® is not for use with pregnant women.
- • BCtect® has not been documented to discriminate between breast cancer and other types of cancer.
- • BCtect® may show reduced test specificity with acute inflammatory conditions.

Clinical validation of BCtect®

Cancer stage
BCtect® has an equal sensitivity for early stage (Stage 0, 1) and late stage (Stage 2+) breast cancer (Table 5). It has been well documented that earlier detection of breast cancer has benefits for the patient’s quality of life and survival. BCtect® with early stage breast cancer showed a sensitivity of 74% for the validation population and 71% for the combined study population.

Table 5: Performance by stage

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Convenient and easy to use

The BCtect® test has been designed for routine use, with the focus on patient acceptability and strict quality control to ensure confidence in the results.

- A simple blood sample collected in a PAXgene™ tube (CE marked and FDA cleared) is all that is required to perform BCtect®.
- Following overnight storage at room temperature the sample is shipped to our reference laboratory, DNAVision, in Belgium.

- RNA is extracted and converted to cDNA using an FDA approved protocol. Extensive quality control is carried out to ensure high sample quality.
- Gene expression measurements are performed using BCtect® microfluidic cards and BCtect® software.

DiaGenic offers a service with centralized testing to ensure high and consistent quality. Key to this concept is the cooperation we have established with the accredited laboratory DNAVision in Belgium. They undertake all the analytical work and ensure a uniform and rigorous quality for all laboratory processes.

TEST DESCRIPTION

BCtect® is a blood based gene expression test using real time RT-PCR to measure the quantity of 96 specific RNA transcripts in blood. BCtect® software analyzes the overall pattern of gene expression to derive a test score. Values above zero are classified as positive for the presence of breast cancer. Values below zero are classified as negative (absence of breast cancer).

QUALITY CONTROL

The blood sample passed the quality checks for the test.

Samples have undergone RNA and gene expression quality controls to ensure test consistency and accuracy.

TEST RESULT

The BCtect® result is positive. The result is consistent with the gene expression pattern seen in patients with breast cancer.

The BCtect® should not be used as a stand alone test. It is recommended that it is used together with other screening/diagnostic tests to confirm the presence or absence of breast cancer.

For enquiries see www.diagenic.com or contact customerservice@diagenic.com
What is the DiaGenic Breast Cancer Test (BCtect®)?
The DiaGenic BCtect® is a blood based test for early detection of breast cancer.

How does the test work?
The DiaGenic BCtect® works by detecting unusual patterns of gene activity in a persons blood which indicates the presence of breast cancer. If the test is positive further investigation is required to establish the final diagnosis.

Who might benefit from the test?
• BCtect® has been developed for the early detection of breast cancer. An investigational study conducted shows that the test is as effective in detecting breast cancer in pre-menopausal women as in post-menopausal women.
• Many reports document that mammography is less effective in pre-menopausal women because breast tissue is denser. BCtect® is useful in pre-menopausal women and women with high breast density.
• The investigational study also shows that BCtect® effectively detects lobular carcinomas, which often lack micro-califications and can be more difficult to detect by conventional mammography.
• BCtect® could be used alongside mammography in women who have normal mammograms but are particularly anxious about having breast cancer.

What are the benefits of BCtect® to the patient?
• BCtect® has been developed to detect early stage breast cancer (stage 0 and I). Early detection improves overall survival.
• Early detection may increase the options available for treatment.
• BCtect® can detect lobular cancer, which is sometimes missed by mammography.
• Only a simple blood sample is required from the patient.
• The blood sample can be taken by a phlebotomist at Collection Centres, Medical Centres etc.

How was the BCtect® developed?
• The genes used for BCtect® were selected following several clinical studies. These studies used blood samples from European and US subjects, and demonstrated that an overall accuracy between 75% to 85% could be achieved for both pre- and post-menopausal women.
• BCtect® is able to detect the early stages of breast cancer, stage 0 and stage I, as well as late stage cancer in ductal cancer as well as lobular cancer which are often missed by mammography.
• BCtect® has been developed in accordance with the European Union’s regulatory demands (In-vitro Diagnostic Directive) allowing the test to be CE marked. The CE mark ensures compliance with the directive and ensures the user that the test is reliable, repeatable, safe and efficacious.

How are the results of BCtect® reported?
The results of the BCtect® test are reported as either normal/ negative or abnormal/positive.

Is a normal/ negative BCtect® a guarantee that my patient does not have breast cancer?
• BCtect® is validated in a large multicentre study. As with all other tests, false negative results can occur. BCtect® must always be used in conjunction with the patient’s clinical data and results from other tests (mammography) in order to establish a final diagnosis.
• It is recommended that patients with a negative/normal BCtect® clinical examination and mammography are followed up according to local programs and recommendations, taking into consideration the patient’s risk profile.

How do I follow up an abnormal/positive BCtect® result?
A positive test result should be followed up by an appropriate imaging modality to localize the lesion and if necessary a biopsy or fine needle aspiration to obtain a definitive diagnosis.

What do I do if an abnormal/positive BCtect® result is not confirmed by diagnostic mammography or ultrasound?
• It is possible that BCtect® may detect cancers prior to their detection by imaging tests like mammography. This may occur because the cancer is too small for detection by mammography, or the cancer is hidden by dense breast tissue. It is recommended that further clinical examination and a high quality MRI scan are performed in order to establish a final diagnosis.
• As with any medical test, BCtect® may provide a false positive result and the final diagnosis must be based upon an overall clinical evaluation of the patient.
• If BCtect® is positive but concurrent mammography, clinical examination and high quality MRI are all negative, the patient should be reassured that they are free of cancer and regular follow up arranged.

Where is the test performed and how can I order it?
The blood sample can be taken by a phlebotomist at Collection Centres, Medical Centres etc. The BCtect® test will be performed at a centralised certified laboratory in Belgium (DNAvision).

How should the blood sample be collected?
The sample must be collected in a special blood collection tube called PAXgene. Quality checks are carried out on the blood sample to ensure that the conditions for the test are complied with. If a blood sample does not pass the quality checks, the patient and their doctor are informed and asked to supply a further sample.

What is the turnaround time for the BCtect® results?
The results of BCtect® will typically be available within 7 days from shipment of the sample to the central laboratory.