OSNA
Breast Cancer Management
Be one step ahead in sentinel node analysis
**Breast cancer – new diagnostic tools**

Breast cancer is the most common malignant disease in women, killing more than 400,000 worldwide every year. The status of the axillary lymph nodes is the most important prognostic factor. SYMEX has developed a new intra-operative method for the analysis of sentinel lymph nodes called OSNA (One Step Nucleic Acid Amplification).

Clinical studies in Europe and Japan indicate that the sensitivity and specificity of the OSNA test is comparable to those of very extensive histopathological examination. As results can be available within 30 minutes, the test can be performed intra-operatively and therefore provides the capability to avoid second surgeries.

**Conventional intra-operative lymph node analysis**

Sentinel node biopsy is emerging as the surgical procedure of choice in early stage, clinically node-negative breast cancer patients. When intra-operative diagnosis of the SLN (sentinel lymph node) is performed, metastases are detected by frozen section or touch imprint with rapid haematoxylin and eosin (H&E) staining. However with these methods only a small portion of the lymph node tissue is analysed in an intra-operative setting, impacting on the accuracy of the result. Many investigators have reported that the intra-operative H&E-based histopathological examination has a false negative rate up to 52%.

**OSNA – intra-operative lymph node analysis**

The newly established OSNA method amplifies cytokeratin19 (CK19) mRNA by a specific and sensitive isothermal procedure called RT-LAMP* (Reverse Transcription Loop-Mediated Isothermal Amplification). The progress of the amplification is monitored in real-time. Blue dyes or radioisotope colloids used for the identification of the SLN do not interfere with the OSNA reaction.

In the OSNA assay, no purification of RNA is required. Accordingly, the total process of the OSNA assay can be completed in about 30 minutes when RD-100i, an automated instrument for amplification and detection of CK19 mRNA is used. The OSNA assay quantitatively measures the amount of CK19 mRNA which is directly related to the size of metastatic foci. In addition, the OSNA assay has the capability to analyse the whole lymph node. Therefore, the OSNA assay can analyse the real size of metastatic foci in a lymph node, meaning that it can discriminate both macrometastases from micrometastases and micrometastases from non-metastases. Results are displayed as (++), (+) for macrometastases, (-) for micrometastases and (-) for negative.

**Characteristics of OSNA**

- Short amplification time allows intra-operative detection of sentinel lymph node metastases.
- Sensitivity and accuracy in combination with the capability of analysing the whole lymph node leads to discrimination of both macrometastases from micrometastases and micrometastases from non-metastasis.
- Availability of reliable results during the operative procedure helps to avoid second surgeries.
- High degree of automation and an easy-to-use procedure enables users without experience in molecular biology to perform the analysis.

* RT-LAMP = Reverse Transcription Loop-Mediated Isothermal Amplification; licensed under the agreement with Eiken Chemical Co., Ltd.
Marker selection

During the development of the OSNA method, SYSMEX selected 45 candidate mRNA markers, from the public EST database. The expression ratio of these mRNA markers was evaluated using histopathologically positive and negative lymph nodes (Fig. 2).

Test of 45 potential marker genes and β-actin as housekeeping genes

7 markers were selected and further evaluated in individual lymph nodes (Fig. 3). CK19 mRNA was identified as the best marker, showing high expression levels in metastatic lymph nodes and low levels in non-metastatic lymph nodes, thereby offering both the potential for high sensitivity and the capability to discriminate metastatic from non-metastatic lymph nodes.

Primer design and avoidance of CK19 pseudogenes

Primers act as specific starter molecules for the amplification of the target of interest. Conventional molecular approaches use only 2 primers. The OSNA assay uses 6 different primers which have been specifically designed to avoid the amplification of CK19 pseudogenes or their transcripts which can lead to false positive results.

Furthermore, undesired amplification of genomic DNA is avoided due to precipitation of DNA at low pH during sample preparation and the isothermal reaction temperature of 65°C.

Marker selection

During the development of the OSNA method, SYSMEX selected 45 candidate mRNA markers, from the public EST database. The expression ratio of these mRNA markers was evaluated using histopathologically positive and negative lymph nodes (Fig. 2).

Test of 45 potential marker genes and β-actin as housekeeping genes

7 markers were selected and further evaluated in individual lymph nodes (Fig. 3). CK19 mRNA was identified as the best marker, showing high expression levels in metastatic lymph nodes and low levels in non-metastatic lymph nodes, thereby offering both the potential for high sensitivity and the capability to discriminate metastatic from non-metastatic lymph nodes.

Primer design and avoidance of CK19 pseudogenes

Primers act as specific starter molecules for the amplification of the target of interest. Conventional molecular approaches use only 2 primers. The OSNA assay uses 6 different primers which have been specifically designed to avoid the amplification of CK19 pseudogenes or their transcripts which can lead to false positive results.

Furthermore, undesired amplification of genomic DNA is avoided due to precipitation of DNA at low pH during sample preparation and the isothermal reaction temperature of 65°C.
Clinical studies and data

The OSNA method has been evaluated in several multicentric studies in different countries. In all these studies OSNA was compared to a very extensive histopathological examination. The lymph nodes were cut into 4 slices of 1 or 2 mm width, with 2 alternate slices being used for the OSNA assay and 2 alternate slices being used for multilevel histochemical investigation.

In a multicentric study in Japan, performed in 6 centres, 325 lymph nodes from 101 patients were analysed. 43 lymph nodes were positive with both methods, 276 lymph nodes were negative in OSNA and histopathology. These data resulted in a concordance rate of 98.2% (Table 1).

Table 1: Result table of an intra-operative, multicentric study in Japan*: OSNA results are displayed as (+++) for macrometastases, (+) for micrometastases and (–) for negative lymph nodes

<table>
<thead>
<tr>
<th></th>
<th>Pathological Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>macro</td>
<td>(+)</td>
</tr>
<tr>
<td>OSNA</td>
<td>34</td>
</tr>
<tr>
<td>(++)</td>
<td>0</td>
</tr>
<tr>
<td>(+)</td>
<td>0</td>
</tr>
<tr>
<td>(–)</td>
<td>2</td>
</tr>
</tbody>
</table>

Concordance rate: 98.2% (95% C.I.: 0.919 – 0.958)

The specificity in pN0 patients (144 lymph nodes analysed) of the Japanese intra-operative study was 100% (Table 2).

Table 2: Analysis of 144 lymph nodes from 60 pN0 patients gave a specificity of 100%; OSNA results are displayed as (+++) for macrometastases, (+) for micrometastases and (–) for negative lymph nodes

<table>
<thead>
<tr>
<th></th>
<th>Pathological Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>macro</td>
<td>(+)</td>
</tr>
<tr>
<td>OSNA</td>
<td>0</td>
</tr>
<tr>
<td>(++)</td>
<td>0</td>
</tr>
<tr>
<td>(+)</td>
<td>0</td>
</tr>
<tr>
<td>(–)</td>
<td>0</td>
</tr>
</tbody>
</table>

Specificity: 100% (95% C.I.: 0.935 – 0.993)

In summary at the time of writing about 1500 lymph nodes have been analysed with a specificity of 96.5 – 100% and a concordance rate of 96.1 – 99.1%.

The CK19 mRNA copy number of these negative lymph nodes was considerably lower than the cut-off value for the OSNA assay (Fig. 4). The probability that the copy number of a histopathologically negative lymph node exceeds the cut-off value of the OSNA assay is thus extremely low (less than 0.5%). The results strongly indicate that the OSNA assay does not give a false positive result.

Design and specifications may be subject to change due to further product development.