RD-100i
OSNA – the new generation of sentinel lymph node analysis in breast cancer
Sentinel node biopsy has rapidly emerged as the surgical procedure of choice for early stage, clinically node-negative breast cancer patients. Conventional intra-operative analysis of the sentinel lymph node (SLN) has, until now, been performed by frozen section or touch imprint with a rapid H&E (haematoxylin and eosin) staining. The sensitivity of these histopathological methods is low because only a small proportion of the lymph node tissue can be investigated in an intra-operative setting. As a result, there is a considerable risk of false-negative results which may only be identified by subsequent post-operative examination.

OSNA (One Step Nucleic Acid Amplification) is a new and widely established diagnostic approach which, for the first time, enables analysis of the whole lymph node in an intra-operative timeframe. A final, fully informed, clinical decision can be taken with confidence, without the requirement for a second surgical procedure or a confirmatory histopathological analysis.
Advanced technology

OSNA is an automated molecular diagnostic assay using a rapid nucleic acid amplification technology (RT-LAMP*) for the detection of Cytokeratin 19 (CK19) mRNA expression. CK19 is an epithelial cell marker and normally not present in lymph node tissue. The amount of CK19 mRNA expression correlates with the size of metastatic foci. The system indicates the extent of the metastatic tumour burden. The basis for the evaluation of the patient result is by comparison to a standard curve with three different calibrators.

Marker selection

During the development of the OSNA method, Sysmex selected 45 candidate mRNA markers from a public human gene expression database. Selection criteria were a high expression level of mRNA in tissues of the mammary gland in combination with minimal or no expression in normal lymph node tissue. The expression ratio of these mRNA markers was evaluated using histopathologically positive and negative lymph nodes (Fig. 1 a+b).

Test of 45 potential marker genes

In a second step the seven most promising candidate markers with high mRNA expression in metastasis positive lymph nodes and minimal expression in negative lymph nodes were further evaluated in a larger number of lymph nodes (Fig. 2).

As a result CK19 mRNA was identified as the most appropriate marker, showing high expression levels in metastatic lymph nodes and low expression levels in non-metastatic lymph nodes, thereby offering the potential for high sensitivity and the capability to discriminate metastatic from non-metastatic lymph nodes.

Fig. 2 Expression of mRNA markers in histopathologically positive and negative lymph nodes

* RT-LAMP = Reverse transcriptase loop-mediated isothermal amplification; licensed under the agreement with Eiken Chemical CO., LTD

Fig. 1a Ratio of mRNA expressions between histopathologically positive and negative lymph nodes

Fig. 1b Expression of each mRNA marker in histopathologically positive lymph nodes

FOXA1 (forkhead box A1), SPDEF (SAM pointed domain containing ETS transcription factor), CEA (carcinoembryonic antigen), TACSTD2 (tumor associated calcium signal transducer 2), MGB1 (mammaglobin), MUC1 (mucin1)
RT-LAMP technology

The innovative amplification technology RT-LAMP is a rapid isothermal procedure offering several advantages in comparison to conventional PCR methods. The amplification reaction takes place within 16 min and no prior RNA purification is necessary. Conditions for sample preparation and the special primer design are tailored to ensure high specificity and to avoid false positive results.

Six different primers are used in RT-LAMP specifically designed to avoid the amplification of CK19 pseudogenes or their transcripts, and furthermore to speed up the reaction of the assay.

LAMP Primer

- Isothermal procedure at 65 °C
- High speed reaction (16 min)
- No RNA purification necessary
- No undesired amplification of pseudogenes and genomic DNA
- High specificity due to 6 primers

Undesired amplification of genomic DNA is avoided by precipitation of DNA at low pH (3.5) during sample preparation and the isothermal reaction temperature at 65 °C.

Blue dyes or radioisotope colloids used for the identification of the SLN do not interfere with the OSNA reaction.
Clinical validation studies and results

The OSNA method has been evaluated in multiple multicentric studies in different countries [1–5]. In all these studies OSNA was compared to an extensive histopathological examination. The lymph nodes were cut with a special cutter device into 4 slices of 1 or 2 mm width. Alternate slices were applied either to the OSNA assay or used for multilevel histology on permanent sections. Sections were taken from 5 levels with skip ribbons of 100, 200 or 250 µm.

In summary, 2313 lymph nodes have been analysed and generated the following performance data:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordance rate</td>
<td>96.5%</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95.6%</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>96.7%</td>
<td></td>
</tr>
</tbody>
</table>

As part of the Japanese study it was demonstrated that the specificity of OSNA in pN0 patients (144 lymph nodes analysed) was 100% (Table 1).

![Table 1 Analysis of 144 lymph nodes from 60 pN0 patients gave a specificity of 100%](image)

Table 1 Analysis of 144 lymph nodes from 60 pN0 patients gave a specificity of 100%

The CK19 mRNA copy number of the negative lymph nodes tested in this specificity study was considerably lower than the cut-off value for the OSNA assay. This strongly indicates that the risk of false positive results can be almost excluded (Fig. 4).

![Fig. 4 Distribution of CK19 mRNA copy number of 144 lymph nodes from 60 pN0 patients](image)

The results of all clinical evaluations show that CK19 mRNA is an excellent molecular marker for the detection of lymph node metastases in breast cancer. In conclusion the OSNA assay can be applied as a diagnostic tool for rapid detection of metastases in sentinel node biopsy samples from breast cancer patients.

It is fully IVD compliant and fully approved for diagnostic use throughout the EU.

References of publications


### RD-100i

**Gene Amplification Detector System RD-100i**

**OSNA (One Step Nucleic Acid Amplification)**

**Parameter**

- CK19 mRNA

**Technology**

- RT-LAMP (Reverse transcriptase loop-mediated isothermal amplification)
- change of transmitted light caused by the precipitation of magnesium pyrophosphate depending on the grade of the reaction

**Displayed parameters**

- CK19 Q (CK19 Qualitative Result)
- CK19 (CK19 Risetime)
- CK19 C (CK19 mRNA-concentration)

**Throughput**

- 4 samples per batch

**Tissue range per sample**

- 50 - 600 mg

**Sample volume**

- 2 µL

**Reagents**

- homogenising reagent LYNORHAG
- amplification reagent LYNOAMP BC
- all reagents are ready for use

**Data storage**

- 2000 samples

**Quality control**

- positive and negative control
- 180 data points per file

**Interfaces**

- host computer connection (RS232, LAN)
- printer connection (USB)

**Dimensions/weight**

- 596 x 548 x 622 / approx. 66

**SNCS optional with Sysmex Service Agent and remote access**

```
CE
```

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

Sysmex Europe GmbH
Bornbarch 1, 22848 Norderstedt, Germany · Phone +49 40 52726 0 · Fax +49 40 52726 100 · www.sysmex-lifescience.com

Sysmex UK Ltd.
Sysmex House, Garamonde Drive, Wymbush, Milton Keynes, MK8 8DF, United Kingdom · Phone +44 870 902 921 0 · Fax +44 870 902 921 · info@sysmex.co.uk · www.sysmex.co.uk