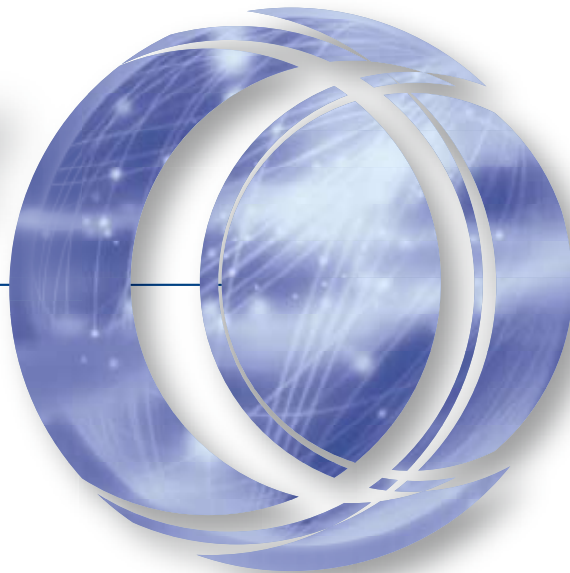


OSNA GLOBAL USER REPORT



OSNA
One Step Nucleic acid Amplification

Vol.6



Sagara Hospital, Kagoshima, Japan

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We shall describe here how OSNA is being used in an institution that is using the assay for diagnosing lymph node metastasis of breast cancer, and the institution's views about it.

I. Background and history of OSNA adoption

At the Sagara Hospital, we have been investing a lot of labor to ensure the accuracy of sentinel lymph node (SLN) biopsy.

The method we had been employing involved cutting of the entire lymph node into 2 mm thick blocks intraoperatively, preparing 3 sets of samples for rapid diagnosis at different levels for each block and observing them under the microscope. After the microscopy, the metastasis data and the size of the metastatic foci (if the result was positive) were reported to the surgeon. The surgeon then decided the extent of dissection required, also taking into account the information gathered preoperatively in cases of micrometastasis. After the surgery, permanent samples were prepared from the rapid diagnosis samples for confirmatory microscopy (Fig. 1).

Method:
 (1) Cutting the entire SLN at 2 mm intervals
 (2) Sectioning: Preparation of 3 serial step sections at 120 μm intervals
 (3) HE staining

Labor:
 1-2 technologists for preparing samples

Rapid diagnosis: Pathologist

Period covered:
 January 2004 to September 2006

Patients biopsied: 762

Report time:
 One SLN: Average 22 minutes
 Concurrent use of staining: Average 40 minutes

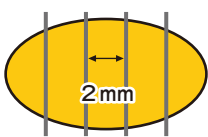


Fig. 1 Intraoperative rapid pathological diagnosis at the Sagara Hospital before induction of OSNA

With cases found to have invasive lobular breast cancer in preoperative tests, intraoperative immunostaining for cytokeratin was also done concurrently (about 40 minutes is required in total) to prevent false negatives, as diagnosis based on histological data alone is difficult in such cases.

The proportion of the work used for intraoperative sentinel lymph node testing, in the diagnostic pathology work, has increased in recent years at the Sagara Hospital also, as both the cases of breast surgery and SLN biopsy cases have been on the increase (Fig.2).

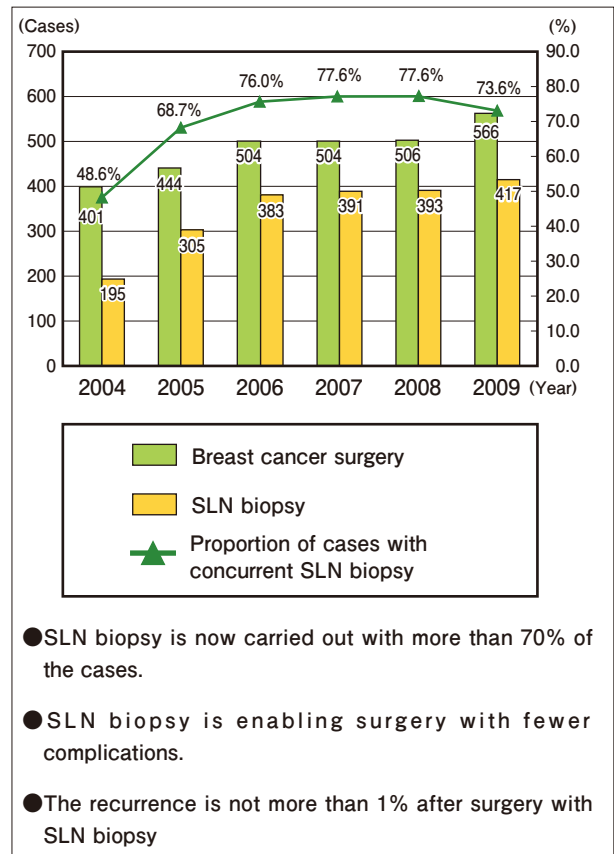


Fig. 2 Changes in the number of cases of sentinel lymph node (SLN) biopsy at Sagara Hospital (combined use of staining and RI)

This has increased the workload of the Pathology Department, and we wanted to somehow increase the work output so that the workload could be managed efficiently without sacrificing accuracy. Since OSNA was covered by the National Health Insurance Scheme the hospital explored the possibility of adopting it.

Prior to the full implementation of OSNA, we examined its accuracy and any likely problems in its clinical application by concurrently using OSNA and intraoperative rapid pathological diagnosis in the period of June to July 2009.

While examining OSNA for possible adoption, we cut the entire lymph node at 2 mm intervals to prepare blocks. When alternate blocks were analyzed, by the two different methods, the results showed 93% concordance. Thus, in our hospital as well, the OSNA assay showed comparable accuracy to intraoperative rapid pathological diagnosis, as had been reported elsewhere (Table 1).

The report time in examining one SLN was 34 minutes with OSNA and 22 minutes for the conventional intraoperative diagnosis (Table 2).

The report time became about 45 minutes when division and search became necessary or more than one SLN was submitted for analysis. This was still within the tolerable range, which made us adopt the OSNA assay for clinical application.

Concordance: 93.4%

53 cases, 61 SLNs		Rapid pathological diagnosis	
		Positive	Negative
OSNA	Positive	9	1
	Negative	3	48

It was decided to adopt OSNA because of the high concordance between the results of the two methods.

Table 1 Combined use of intraoperative rapid pathological diagnosis and OSNA assay (results of diagnosis of metastasis to the sentinel lymph node)

No. of SLN	OSNA (min.)	Intraoperative rapid pathological diagnosis (min.)
1	34	22
2	42	25
3	45	—

With OSNA, the report time did not vary much from case to case, and the procedure could be handled intraoperatively.

Table 2 Combined use of intraoperative rapid pathological diagnosis and OSNA assay (report time)

2. Method of implementing OSNA

The use of OSNA in clinical applications was started at the Sagara Hospital in August 2009.

Four of the 7 pathology technicians had been trained by Sysmex Corporation, and they carry out the OSNA assay. To fully exploit the advantageous features of OSNA, we are now examining the entire SLN with OSNA. Among the SLN submitted for analysis to the pathologists so far (August 2009 to February 2010), an average of 1.2 SLN/patient, and 20.2% (50/248) of the patients were found to be metastasis positive. The report time was about 33 minutes in 56% of the cases, excluding cases with macrometastasis. There have been no accuracy-related issues.

At our hospital, the tests are conducted according to the work flow sequence shown in Fig.3, in response to a request from the Surgery Department at the time of

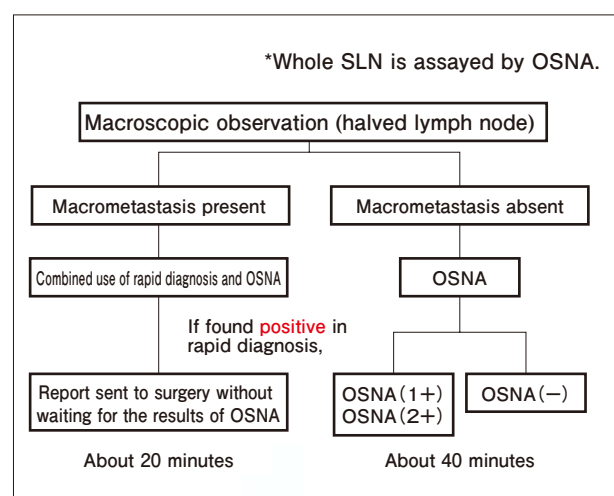


Fig. 3 Work flow of diagnosis of sentinel lymph node (SLN) at the Sagara Hospital (from August 2009).

induction of OSNA for “faster assessment in cases where dissection would be required.” Firstly, the lymph node submitted to the laboratory is cut in half, and observed by a pathologist macroscopically. Then, if the pathologist assesses that it has macrometastasis, intraoperative rapid evaluation samples of the largest cut surface are prepared, and reporting is done in about 20 minutes from the receipt of the sample, as earlier, without waiting for the results of the OSNA assay (Fig.4). On the other hand, if no macrometastasis is found by macroscopic observations, the entire lymph node is assayed by OSNA and the state of metastasis is reported on the basis of the results of OSNA.

Thus, if no report of results is received in about 20 minutes, it means that metastasis is either absent or very small. The surgeons appreciate this method.

As described above, currently, the main purpose of the use of OSNA at our hospital is the accurate detection of micrometastasis and small metastatic foci.

There are some reports that say that there are cytokeratin 19 negative cases, although rare, among breast cancers metastasized into lymph nodes. As this is a rare occurrence, preoperative checking for cytokeratin 19 expression is not routinely done at our hospital. But our testing framework places emphasis on not missing cases of macrometastasis.



Fig. 4 Macroscopic diagnosis by a pathologist in a room adjacent to the operating room

We store the solubilized lymph node preparation left after OSNA at -80°C so that it can be used as a backup sample in case of some intraoperative problem and to meet the possible need for additional tests in the future. To ensure accuracy of the OSNA assay, the reagents and analyzer should of course be properly managed. But we believe that reduction of human error is also equally important. At the Sagara Hospital, we use unique methods for ensuring adherence to the prescribed assay procedure, and for managing the reagents and the test results.

For ensuring strict adherence to the assay procedure laid down, we use an OSNA checklist for each patient (Fig.5). In addition, the basic precautions are posted at visible sites to remind the medical technicians, as a measure to reduce human error (Fig.6).



A view of analysis with RD-100i in the pathology laboratory

OSNA checklist

Date: _____

Patient: _____

Pre-assay preparation and verification

Verify patient information (lymph node size in CT scan, preoperative treatments, age, etc).	
Check whether the numbers on the blue tube and sample tube match.	
Blue tube: Check whether it contains 4 mL of lynorhag.	
Sample tube: Check whether it contains 180 μ L of lynorhag.	
Prepare 1.5 mL tubes.	
Check whether the new calibration curve has been registered.	
Check whether the positive control shows “++” or “+” .	
Register sample order (ID-1).	
Check pipette memory settings (1,000 μ L, 180 μ L, 20 μ L).	

Homogenizing

Check whether any lymph node is remaining in the shaft.	
Check whether any lymph node is remaining in the tube.	

Sample preparation

Check whether the homogenizer operator has changed gloves.	
Take 1 mL from the blue tube and centrifuge (1 minute, 10,000 G).	
Two or more nodes: Note down the number on 1.5 mL tubes.	
Dispense 180 μ L taken from the mid-level into a 1.5 mL tube.	
Dispense 20 μ L into the S tube.	
Stir the S tube and spin down.	
Dispense 20 μ L into the D tube.	
Stir both S and D tubes and spin down.	

Settings of reagents and disposables

Mix the reagent and spin down.	
Mix the enzyme by inverting 5-10 times, and spin down.	
Check whether the reagent has no bubbles.	
Check whether the reagent and samples are set in their proper positions.	
Check whether the cell has been fully pushed in.	
Tip: Check whether at least one rack is fully loaded.	
Confirm that a tip trash bag is set in the Tip Discarding Unit.	
Check for any unregistered parts in the sample order (ID-2, -3 ...).	
On the PC screen, check whether all the cells are marked “G” .	
Check whether the reagent is sufficient for the number of tests to be done.	

After starting the assay

Set the timer.	
Submit the rapid analysis report to Dr Ooi and convey the expected completion time of the assay.	

After completing the assay

Check whether the positive control shows “++” .	
Report the results to Dr Ooi.	
Remove reagent and samples from the analyzer.	
Dispose of used tubes.	
Change the disposal bags.	
Enter the data in the OSNA registry (on the next day).	

Tube No.				
	SN	SN	SN	SN
mg				
Freezing serial No.				
Results				
Report time	~ ()			

Combination use with pathological test:
Yes No.

Waiting time (SLN removal to start of assay):
Yes No. ()min.

TEL:
Before or after the submission of SLN

OSNA assay performed by: _____

Checked by: _____

Verified by: _____

Fig. 5 OSNA checklist



To prevent mix-up of samples and reagents, labels are pasted to indicate their pre-decided positions.



Precautions in the OSNA assay are pasted on the equipment to reduce human error.



Work flow up to result reporting and the basic operating procedure are shown on the side of laboratory equipment.

Fig. 6 Human error prevention measures adopted at the Sagara Hospital

For reagent management, a management table (database) has been created. The date of seal opening, number of times frozen, and number of tests possible with the remaining amount are entered in the table, so that the reagent can be used efficiently without wastage. (Fig. 7)

Similarly, for assay results management, we use a database called “OSNA registry”, where data is entered for each patient. (Fig.8)

Like many specialized hospitals, the Sagara hospital has very smooth coordination between the Pathology Department and the Surgery Department, etc. Probably this is one significant contributing factor for smooth implementation of the OSNA assay.

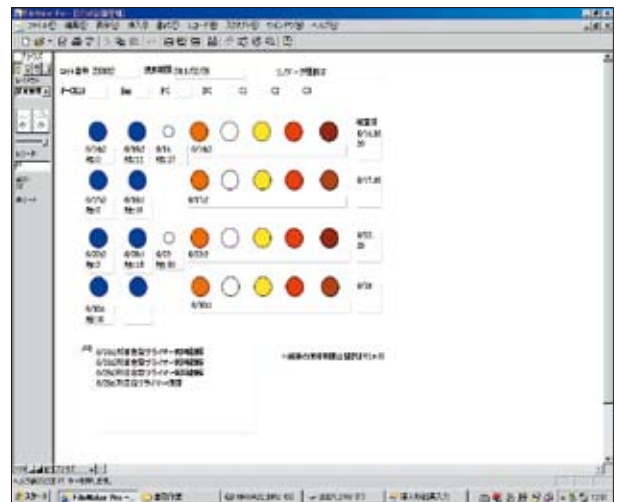


Fig. 7 Reagent management table of the Sagara Hospital



Fig. 8 “OSNA Registry”, the assay results database of the Sagara Hospital

3. Comments after adopting OSNA

Unlike with other conventional pathological tests, the same sample cannot be reviewed in the case of OSNA. This fact was a major concern before deciding on the adoption of the assay. We were not sure whether our laboratory staff, who had little experience with molecular pathology techniques, could handle it. However, the pre-induction training by Sysmex enabled stable application of the assay.

Some errors that our staff encountered with the analyzer have been addressed by prompt and appropriate follow-ups from Sysmex. Thus, it has been functioning smoothly without major downtime that would have affected the diagnosis.

As mentioned in the beginning of this user report, our hospital places great importance on the accuracy of diagnosis. In this regard, after the adoption of OSNA, the detection of positivity for invasive cancer has been found to be comparable to that of the conventional method used earlier, and the Surgery Department places great confidence in the results produced by the Pathology Department. (Table 3)

	OSNA assay	Intraoperative rapid pathological diagnosis
Period	Aug 2009 – Feb 2010	Jan 2004 – Sept 2006
Metastasis positivity	20.2%(50/248)	18.7%(158/762)
Positivity for invasive cancer metastasis	23.0%(50/217)	24.9%(158/633)

Table 3 Detection of positivity for sentinel lymph node metastasis

4. Merits of OSNA

Before the adoption of OSNA, searching for micrometastasis created a heavy workload for the staff of the Pathology Department.

OSNA, however, has relieved them of that work without sacrificing accuracy, and they can use the time saved for other pathological diagnostic work, which is a great advantage.

In addition, as OSNA enables the analysis of the entire SLN for metastasis, it has now become possible to omit dissection of axillary lymph nodes with confidence, based on the results of the OSNA assay. Excessive lymph node dissection is now causing problems like lymphedema. Thus, the assay offers a major benefit to patients also.



5. Prospects for the OSNA assay

The differences between the conventional histopathological diagnosis and the OSNA assay will gradually become clear in the future. We would like to examine how to deal with such differences after accumulating data on a large number of cases, and analyzing the data.

With the OSNA assay, standardized data can be obtained at any medical institution. The approach to axillary lymph node dissection is currently undergoing major changes. Discussions on this issue would be

more objective and accurate if based on data obtained by standardized methods rather than on the results of histopathological observations where the judgment criteria are not so well defined. This, in turn, would contribute to standardization of treatment as well.

This shift in approach towards lymph node dissection is likely to lead to changes in details sought in diagnosis of metastasis to SLN. We hope and expect that objective analysis will advance rapidly with wider application of OSNA.



Dr. Ooi and the staff of the Pathology Department



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