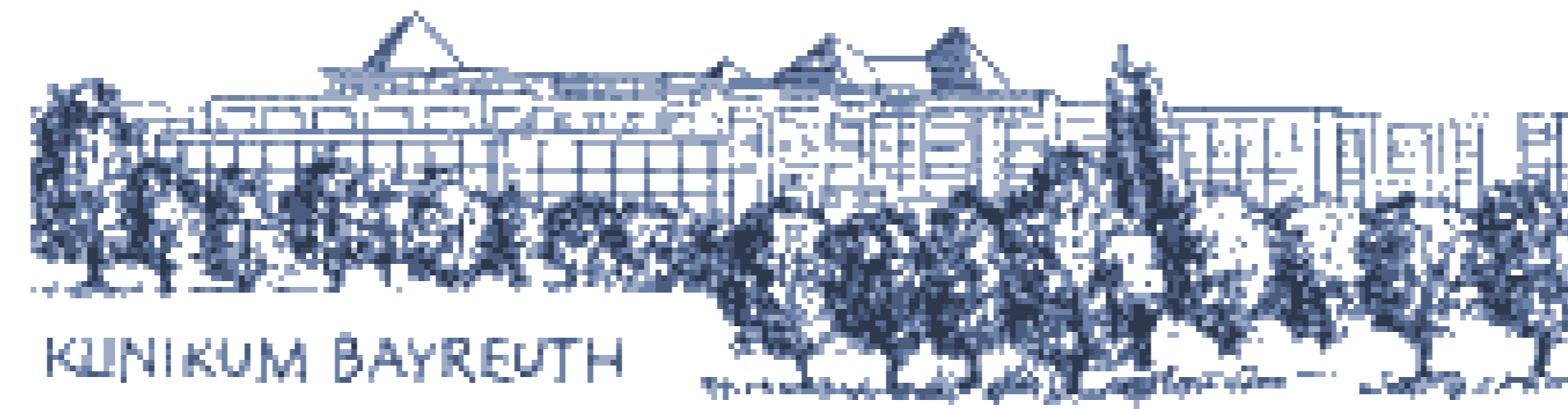


INTRA-OPERATIVE DETECTION OF SENTINEL LYMPH NODE METASTASIS IN BREAST CANCER BY OSNA (ONE STEP NUCLEIC ACID AMPLIFICATION)

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Introduction

Despite recommendations from international and national breast cancer guidelines there is no standardised histopathological procedure for intra-operative and post-operative analysis of the sentinel lymph node (SLN). In this study we used the molecular diagnostic OSNA assay for intra-operative SLN analysis in breast cancer patients.

OSNA is based on CK19 mRNA amplification and has shown to be as accurate as intensive post-operative histology in several studies (see literature below).

Material and Methods

The semi-automated OSNA assay follows a short sample preparation step and subsequent rapid amplification of CK19 mRNA without prior isolation of mRNA (figure 1).

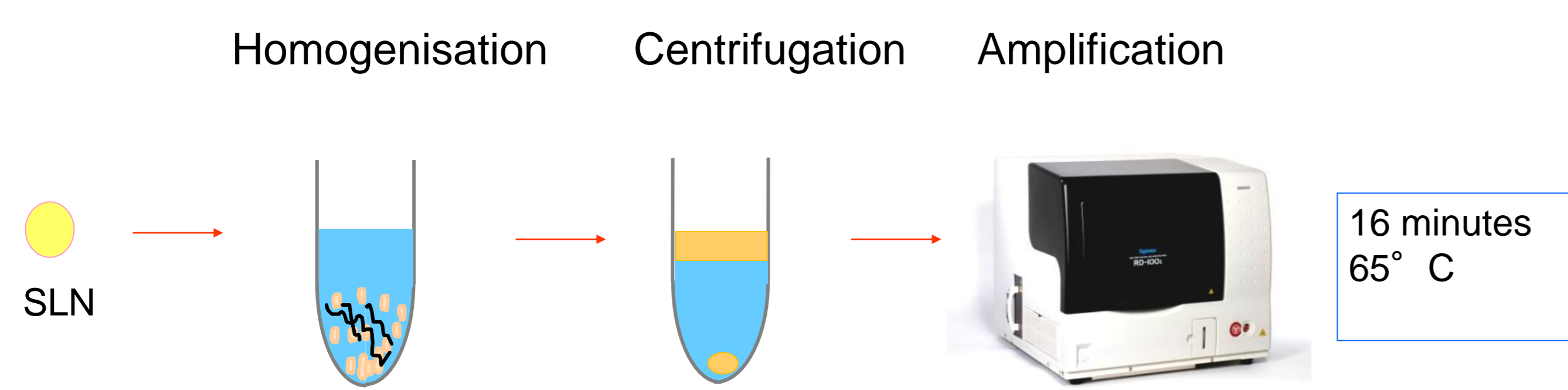


Figure 1: The OSNA assay.

Eighty SLNs from 47 breast cancer patients were included in this study so far. A 1 mm middle slice was reserved for intra-operative frozen section staining with Haematoxylin&Eosin (H&E)(figure 2). The rest of the SLN was homogenised and analysed with the automated OSNA system RD-100i (Sysmex, Kobe, Japan). Results were displayed as (++) equivalent to a macrometastasis, (+) for a micrometastasis, (-) for negative, and led to direct axillary dissection if positive.

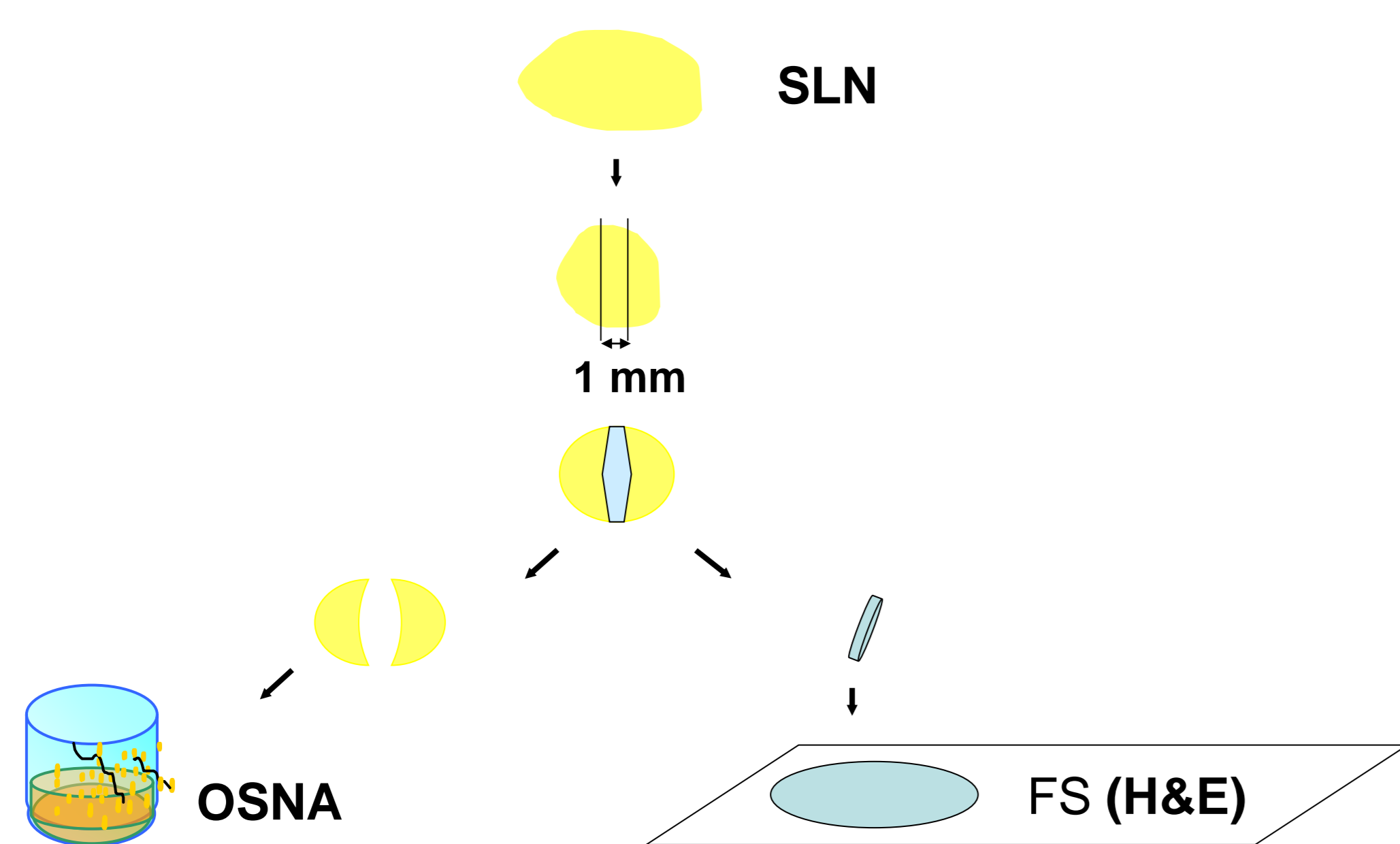


Figure 2: The study design.

Literature:

Molecular diagnosis of sentinel lymph nodes for breast cancer: one step ahead for standardization. Laia BV, Marcos MB, Refael CM et al. *Diagn Mol Pathol* 2011(1): 18-21.

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Molecular detection of lymph node metastases in breast cancer patients: results of a multicenter trial using the one-step nucleic acid amplification assay. Tamaki Y, Akiyama F, Iwase T, et al. *Clin Cancer Res*. 2009 Apr 15;15(8):2879-84.

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. Schem C, Maass N, Bauerschlag DO et al. *Virchows Arch*. 2009 Feb;454(2):203-10.

Intra-operative rapid diagnostic method based on CK19 mRNA expression for the detection of lymph node metastases in breast cancer. Visser M, Jiwa M, Horstman A et al. *Int J Cancer*. 2008 Jun 1;122(11):2562-7.

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Results

In 20 patients OSNA gave a positive result (22 SLNs with ++, 12 SLNs with +), resulting in a positivity rate of 42.6%. In 27 patients OSNA was negative, with one patient having a very small micrometastasis in the 1 mm middle slice. 6 patients were OSNA positive/histology negative, thereby avoiding a second surgical intervention as axillary dissection was performed intra-operatively.

Table 1: Results of SLN analysis with OSNA and histology.

N=47 patients		Pathological Examination		
		Positive		Negative
		Macrometastasis	Micrometastasis	
OSNA	++	14		
	+			6
	-		1	26

In 14 patients 1 SLN was analysed, in 19 patients 2 SLNs, in 11 patients 3 SLNs, in 3 patients 4 SLNs with the mean analysis time of 29.5, 37, 40, and 51 minutes, respectively (table 2).

Table 2: Time span for SLN transport, preparation and OSNA.

		1 OSNA sample	2 OSNA samples	3 OSNA samples	4 OSNA samples
Transport and SLN isolation [min]	Range	3 - 10	3 - 25	4 - 20	10
	Mean	6.6	8.9	10.5	14.0
Homogenisation and amplification [min]	Range	25 - 37	31 - 40	27 - 48	50
	Mean	29.5	37.0	40.0	51
Total intra-operative Time [min]	Range	30 - 42	35 - 60	37 - 65	60
	Mean	36.1	45.8	50.5	65

Conclusions

OSNA is a standardised technique for intra-operative SLN investigation which could replace both intra-operative and post-operative histology as most or all of the tissue can be analysed during the primary surgery.