

'One-Step Nucleic Acid Amplification' for rapid molecular analysis of breast cancer lymph nodes: *the way towards one stop sentinel node surgery?*



Kylie L Snook¹, Mark W Kissin (PI)¹, Graham T Layer¹, Peter Jackson¹, Corinne S de Vries², Sami Shousha³, H Dudley Sinnett³, Ezra Nigar⁴, Hemant Singhal⁴, Yoon Chia⁵, Giles Cunnick⁵
OSNA UK Investigator Group

¹Guildford Breast Unit, Royal Surrey County Hospital and ²The University of Surrey, Guildford; ³Charing Cross Hospital & Imperial College, London; ⁴Northwick Park Hospital, Harrow; ⁵Wycombe General Hospital, High Wycombe, United Kingdom

Background

The OSNA® (One Step Nucleic Acid Amplification) system (Sysmex) has been developed to rapidly amplify CK19 mRNA from tissue lysates of lymph nodes (LNs), allowing detection of metastatic tumour deposits >0.2mm in around 30 minutes for a single node. A qualitative result ("++" macrometastasis, "+" micrometastasis or "-" no metastasis) and a quantitative result (copy number of CK19mRNA) is given for each LN sample analysed. A positive node is one that is "++" or "+". Concordance is reported as 98% with multilevel histopathology¹.

Objectives

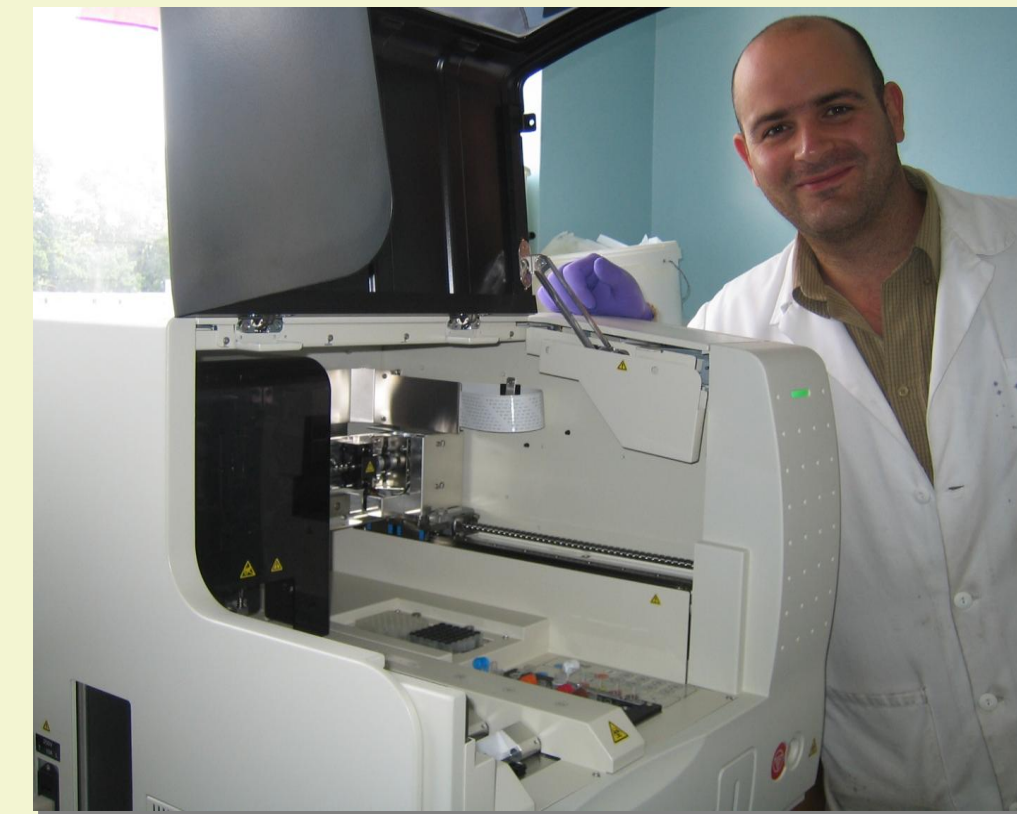
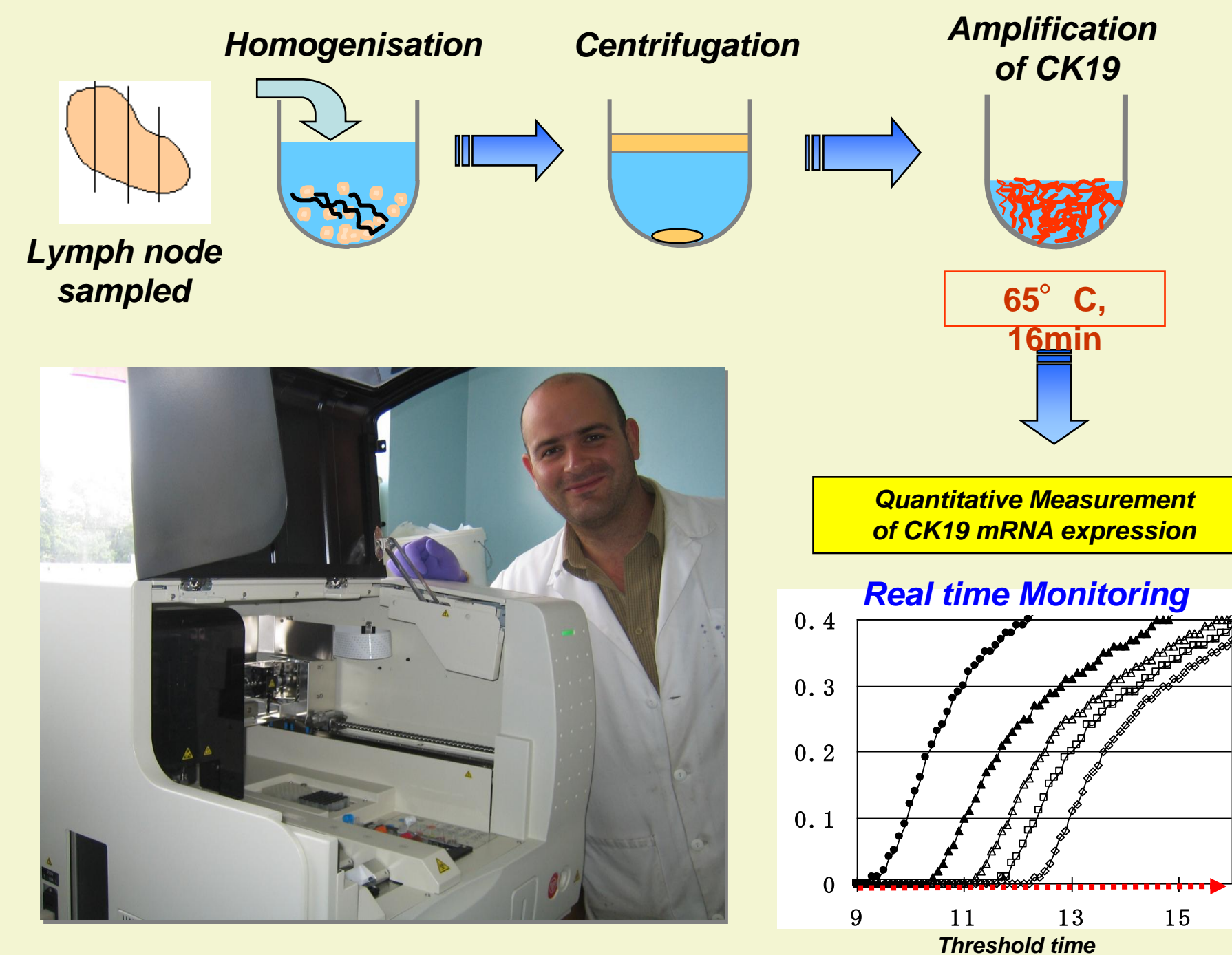
We are conducting a multicentre prospective study in 4 busy hospital breast cancer units (>300 new cancers/year) to evaluate the feasibility of OSNA for intraoperative diagnosis of sentinel lymph nodes (SLNs) in our UK hospital settings, and to determine concordance of OSNA analysis with multilevel haematoxylin & eosin (H&E) and immunohistochemical (IHC) examination. We undertook a 'technical phase' to familiarise ourselves with the technique using both sentinel and non sentinel lymph nodes. Our aim was to achieve a minimum of 80% concordance for at least 40 lymph nodes. These results are reported along with early results of the 'intraoperative' phase of our study examining only SLNs immediately on removal from patients.

Conclusions

- OSNA is a promising technique for intraoperative examination of LNs and is more informative than histopathology as 50% volume of the node is examined
- Concordance of OSNA with histopathology can never be 100% due to tissue allocation bias
- There is a learning curve associated with the procedure
- A UK quality assurance programme for new users of molecular SLN analysis would ensure units become proficient with the technique prior to 'live' use

Methods

OSNA Technique



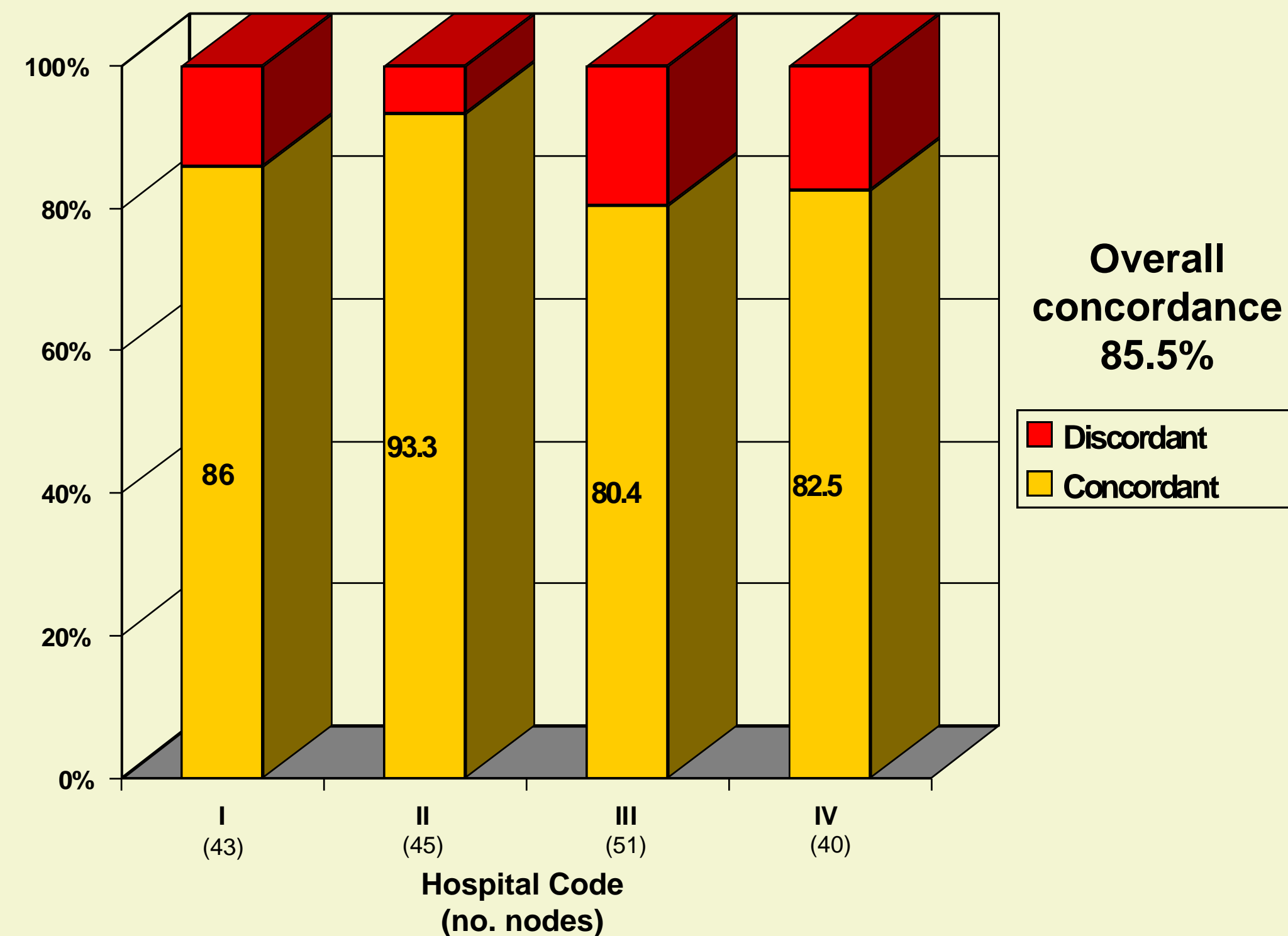
Lymph nodes were removed using standard surgical techniques, defatted and cut into 4 x 1 or 2mm slices depending on size and weight. Alternate slices were snap frozen at -80 C for subsequent OSNA analysis; remaining slices underwent H&E and IHC (CK19 and AE1/AE3) examination (5 levels, 0.25mm multistep sections). Tissue lysates were prepared according to standard procedure. OSNA was performed and results correlated with histopathology findings. Investigators were blinded to OSNA results when reporting histology. Discordant cases were reanalysed with OSNA x 3 times, PCR for CK19mRNA and Western Blot for CK19 protein.

Reference

1. Tsujimoto et al. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients. *Clinical Cancer Research* 2007; 13 (16): 4087 – 4816.

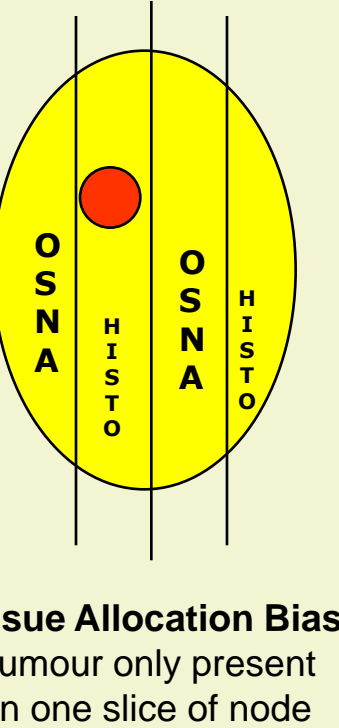
Results

OSNA Concordance by Hospital



Reason for Discordance	Nodes
• Tissue allocation bias	10
• Low copy number	8
• High volume ITC	2
• Low expression CK19?	1
• Inhibition	1
• Not retested	1
• Contaminated/sample mix-up (all OSNA++/no met cases)	3

Table 2. Discordant Case summary by repeat OSNA x 3, PCR CK19mRNA and Western Blot.



Tissue Allocation Bias: tumour only present in one slice of node

179 lymph nodes from 89 patients were investigated by both OSNA and histopathology. Overall concordance for this technical phase was 85.5%. Results per lymph node are shown in table 1. The two major causes for discordant results were due to tissue allocation bias (all 7 OSNA -/micromet) or low copy numbers (borderline results just above the CK19mRNA copy number threshold for "+", 8 of 12 OSNA+/no met) (table 2).

Nodes N=179	HISTOPATHOLOGY				
	Positive		Negative		
	Macro	Micro	ITC	No Met	
OSNA	++	21	6	0	3
	+	6	4	2	12
	-	2	7	5	111

Table 1. Results of technical phase per lymph node. Concordant results are represented in colour. 'Macro' = macrometastasis >2mm; 'micro' = micrometastasis 0.2-2mm; 'ITC' = isolated tumour cells; 'no met' = no metastasis.

Patients N=101	HISTOPATHOLOGY				
	Positive		Negative		
	Macro	Micro	ITC	No Met	
OSNA	++	16	0	0	0
	+	0	1	1	2
	-	1	3	2	75

Table 3. Early results of intraoperative phase per patient. Concordant results are represented in colour.

Our early results from the 'intraoperative phase' using SLNs only show a **concordance or 93.0% per patient** and **95.2% per node** from 101 patients with 189 SLNs (table 3). This improvement in concordance results from completion of the learning curve.