



# A feasibility study (ICG-10) of indocyanine green (ICG) fluorescence mapping for sentinel lymph node detection in early breast cancer

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## Abstract

**Background:** There is now increasing evidence to support the use of indocyanine green (ICG) for sentinel lymph node (SLN) detection in early breast cancer. The primary objective of this feasibility study (ICG-10) was to determine the sensitivity and safety of ICG fluorescence imaging in sentinel lymph node identification when combined with blue dye and radiocolloid.

**Methods:** One hundred women with clinically node negative breast cancer (95 unilateral; 5 bilateral) had sentinel lymph node (SLN) biopsy using blue dye, radioisotope and ICG. One patient was excluded from analysis and sensitivity, or detection rate, of ICG alone, and in combination with blue dye and/or radioisotope, was calculated for the remaining 104 procedures in 99 patients.

**Results:** Transcutaneous fluorescent lymphography was visible in all 104 procedures. All 202 true SLNs, defined as blue and/or radioactive, were also fluorescent with ICG. Detection rates were: ICG alone 100%, ICG & blue dye 95.0%, ICG & radioisotope 77.2%, ICG & blue dye & radioisotope 73.1%. Metastases were found in 25 of 201 SLNs (12.4%) and all positive nodes were fluorescent, blue and radioactive. The procedural node positivity rate was 17.3%.

**Conclusion:** The results of this study confirm the high sensitivity of ICG fluorescence for SLN detection in early breast cancer. The combination of ICG and blue dye had the highest nodal sensitivity at 95.0% defining a dual approach to SLN biopsy that avoids the need for radioisotope.

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## Introduction

Methods for accurately staging the axilla continue to evolve, but remain dominated by sentinel lymph node biopsy (SLNB) which is now widely practiced and accepted as a standard of care in the UK. This has been supported by early UK-based clinical trials<sup>1,2</sup> and national training programmes<sup>3</sup> which promoted dual localisation with blue dye and radiocolloid as the optimal technique. This permits more confident identification of the sentinel lymph node (SLN) and is associated with a shorter learning curve, higher identification rate (>90%) and a low false negative rate (5–10%).<sup>4</sup>

Many units in the UK currently use either blue dye alone for SLNB, or perform blue dye-assisted axillary node sampling because of lack of access to radiocolloid or funding. The blue-dye assisted variant of axillary node sampling aims to harvest at least 4 nodes and increases the chance that the true sentinel node(s) have been harvested.<sup>5,6</sup> Use of radiocolloid can be expensive and does involve radiation protection measures as well as exposing healthcare workers to a cumulative radiation dose. Moreover, these particular radioisotopes are formed as a bi-product of the nuclear industry and in 2009 worldwide shortages of radioisotopes were reported. Localisation techniques using a combination of blue dye and a non-radioactive tracer of comparable accuracy to blue dye and isotope therefore warrant further investigation.

There is now increasing evidence to support the use of indocyanine green (ICG) for SLN detection in early breast cancer. Previous studies, either using ICG alone,<sup>7</sup> or more

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recently in combination with blue dye,<sup>8–10</sup> have shown that it is extremely safe, without allergic reactions following subareolar injection, with SLN identification rates greater than 90%. Furthermore injection of ICG allows real-time transcutaneous lymphography with direct visualisation of the lymphatic pathway(s) which can be traced to the axilla to facilitate SLNB.

ICG is a popular reagent which is usually injected intravenously and is currently approved for clinical usage. It absorbs light in the near infra-red range (maximally at a wavelength of approximately 800 nm).<sup>11</sup> This agent also emits fluorescence which is generated by contact of ICG with plasma proteins. The fluorescence signal is captured by a PDE camera composed of a series of light emitting diodes (LEDs) producing light at a wavelength of 760 nm (which activates ICG), a lens and a filter. The detector is a charge coupled device (CCD) camera which filters out wavelengths below 820 nm. The fluorescent signals are transcribed into a black and white image which is continually observed on a TV monitor or laptop computer.

The primary objectives of this feasibility study (ICG-10) were to determine the sensitivity and safety of ICG fluorescence imaging in SLN identification in early breast cancer when combined with blue dye and radiocolloid. Triple localisation of the SLN using a combination of blue dye, radiocolloid and ICG is reported within this study and the tracer characteristics for individual nodes were carefully documented as well as the percentage of patients in whom the sentinel lymph node was identified with each method. This permits a direct comparison of sensitivities between the different tracers. This paper describes results from the full cohort of 100 patients following a planned interim analysis of the first 50 patients.

## Patients and methods

### Patient eligibility

Between April 2010 and August 2011, a total of 100 clinically node negative patients with core biopsy proven invasive breast cancer scheduled to undergo SLNB with blue dye and radioisotope as part of their breast cancer surgery, were recruited to the ICG-10 study. The study was conducted within a single institution (Cambridge Breast Unit, Addenbrooke's Hospital) and all SLNB procedures were performed by two surgeons (JRB & GCW). All patients gave informed consent to participate in the study which had approval from the MHRA (Medicine and Healthcare products Regulatory Agency; Eudract number 2009-016743-18) and the local Research Ethics Committee. Patients with previous invasive breast cancer or axillary surgery, hypersensitivity to iodine or indocyanine green, hyperthyroidism and patients who were either pregnant or lactating were excluded from the study. Patients with

non-invasive cancer were not included to avoid a potential reduction in the node positivity rate.

### Study design

This was an open, non-randomised study of 100 patients scheduled to undergo routine SLNB using a combination of blue dye and radioisotope for localisation. ICG was administered as a third tracer and the number of sentinel nodes for each patient recorded numerically and whether they were blue, hot or fluorescent. The sensitivity of ICG fluorescence was defined as the number of SLNs detected by conventional methods (blue dye, radio isotope or combination) that were also fluorescent with ICG. For the purposes of this analysis, only those nodes which were blue and/or hot were designated as sentinel but palpably suspicious nodes at operation were recorded together with any fluorescent staining characteristics.

### Sentinel lymph node biopsy

Routine SLN identification within the authors' unit is currently performed using dual localisation with both patent blue dye and radioisotope (Technetium<sup>99</sup> nanocolloid, 20 MBq). The isotope is injected intradermally at the nipple on the morning of surgery and 2 ml of 2.5% blue dye (1 ml intradermally, 1 ml subcutaneously) is injected after induction of anaesthesia. After injection of blue dye the breast is massaged for 5 min. SLNs are harvested by direct visualisation of blue lymphatics and nodes as well as using a hand held gamma probe to detect radioactive nodes.

All 100 study patients had dual localisation with blue dye and radioisotope as above but in addition during surgery, 2 ml 0.5% ICG was also injected (1 ml intradermally, 1 ml subcutaneously) at the edge of the areola, at the 2 o'clock position (left breast) or 10 o'clock position (right breast). The ICG was injected separately immediately before the blue dye and prior to breast massage. Fluorescent subcutaneous lymphatics were visualised using a PhotoDYNAMIC Eye (PDE) camera (Hamamatsu Photonics, Hamamatsu, Japan) and traced to the axilla where occasionally nodes could be seen percutaneously (Figs. 1–3). SLNs

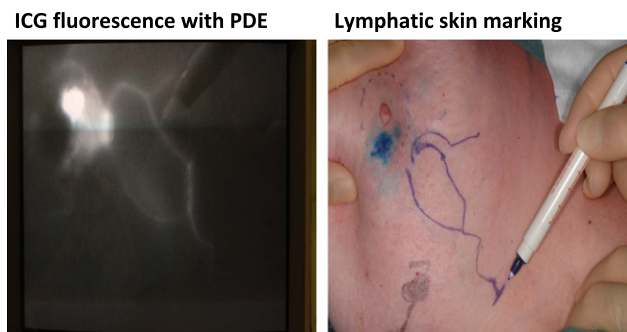
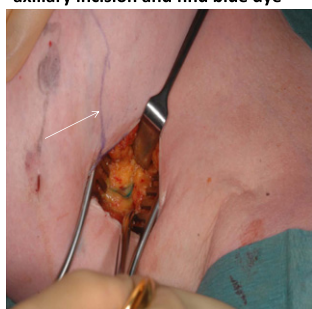


Figure 1. Lymphatic mapping following injection of ICG.

Lymphatic mapping helps plan axillary incision and find blue dye



Blue lymphatic traced to first sentinel lymph node

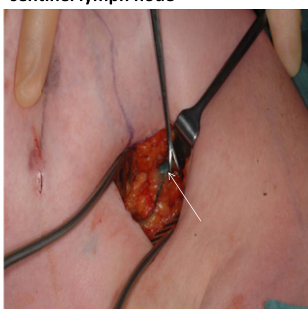


Figure 2. Use of ICG fluorescence and blue dye for sentinel node detection.

detected by blue dye and/or radioisotope were also examined with the PDE camera for fluorescence following excision (Fig. 4).

Any immediate toxicity following injection of ICG and any adverse events and reactions during the study period were recorded during surgery and at the two-week follow up appointment.

#### Histopathology of sentinel lymph nodes

All SLNs underwent pathological evaluation according to local protocol including serial sectioning at 2–3 mm followed by routine staining with haematoxylin and eosin (H&E).

Immunohistochemical assessment using CAM5.2 was only performed if suspicious cells were seen on H&E stained sections. Metastases were reported as macrometastases (>2 mm), micrometastases (>0.2 mm; ≤2 mm in size or <0.2 mm within lymph node parenchyma), and isolated tumour cells (ITCs, ≤0.2 mm).

## Results

#### Patients

A total of 100 patients with biopsy proven invasive breast cancer underwent SLNB (95 unilateral, 5 bilateral)

Blue SLN 1 & 2

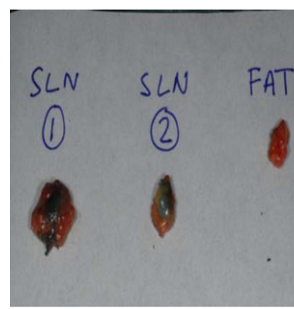


Fluorescent SLN 1 & 2



Figure 3. SLN 1 & 2 highlighted by blue dye & ICG fluorescence.

Blue SLNs 1 & 2



ICG fluorescence in SLN 1 & 2 but not fat



Figure 4. ICG fluorescence of SLN 1 & 2 and fat.

as an operative axillary staging procedure. One unilateral case was a benign adenomyoepithelioma on definitive pathology despite an initial core biopsy being interpreted as positive for invasive cancer. The final analysis was therefore based on a total of 104 procedures in 99 women with an average age of 60 years (range 34–81 years). Of the 104 cases, 53 were screen-detected and 51 presented symptomatically as a palpable lump. The median tumour size for combined modes of detection was 17 mm (range 4–45 mm). Amongst these 104 cancers, 15 were grade I, 59 grade II and 28 grade III (Table 1). In addition, there was one case each of an encysted papillary carcinoma and high grade ductal carcinoma in situ with micro-invasion only on final histology.

#### Sentinel lymph node biopsy

A total of 242 lymph nodes were removed in 104 procedures. Of these, 201 were true SLN's as defined by the study protocol (blue and/or hot). The remaining 41 'non-sentinel nodes' were either palpably suspicious and removed surgically or found after dissection of excised SLN's. Therefore this category includes a subgroup of nodes which would by convention be termed sentinel. There were 2 patients in whom fluorescent but non-hot/blue nodes

Table 1  
Patient demographics.

	Number of patients	%
Age		
<50	13	13.0%
50–69	70	70.0%
70+	17	17.0%
Screen-detected		
Yes	53	51.0%
No	51	49.0%
Tumour size (mm)		
<20	59	
20–50	44	
Grade		
I	15	14.7%
II	59	57.8%
III	28	27.5%

were identified. The first case was amongst an earlier cohort and underwent immediate axillary dissection whilst the second case occurred later on in the study and further axillary surgery was omitted (70 year old patient with a 20 mm grade II IDC and 1 out of 4 nodes intensely fluorescent). There were 10 procedures in which sentinel nodes were fluorescent but either blue only (9) or hot only (1). Therefore identification of the SLN was based on both blue colouration and radioactivity for the majority of procedures (92/104).

The nodal retrieval rate was 2.33 nodes per axillary procedure and the number of nodes excised per procedure was as follows: 1 ( $n = 24$ ); 2 ( $n = 39$ ); 3 ( $n = 26$ ); 4 ( $n = 9$ ); 4+ ( $n = 5$ ). The mean number of nodes detected with each method was 1.93 (201/104), 1.84 (191/104) and 1.5 (156/104) for fluorescence, blue dye and radioisotope respectively.

### ICG fluorescence

Transcutaneous fluorescent lymphography was visible in all 104 procedures but axillary nodes were seen percutaneously (prior to skin incision) in fewer than one-quarter of cases (Fig. 1). The proportion of nodes detected with each tracer agent (blue dye, radioisotope or ICG) alone or in combination are shown in Table 2. These sensitivity calculations are based on 201 nodes that were blue and/or hot. All 201 “true” sentinel nodes were ICG positive, 191 were blue, 156 were radioactive and 147 were both blue and radioactive. Therefore 100% of these nodes were ICG positive, 95.0% were both ICG positive and blue, 77.6% were both ICG positive and radioactive while 73.1% of these nodes were identified with all three tracer methods. The proportion of nodes detected with a combination of blue dye and ICG (95.0%) compared favourably with blue dye and isotope (73.1%). It should be noted that these sensitivities relate to nodal staining characteristics and not overall patient sensitivity rates. The procedural detection rates were 99% (103/104) for blue dye, 91.3% (95/104) for radioisotope and 100% for ICG fluorescence. The majority of the ‘non-sentinel nodes’ (36/41) were fluorescent with 1 patient having 3 non-fluorescent nodes and 2 patients with a single non-fluorescent node each.

### Lymph node positive patients

A total of 25 nodes contained metastases amongst the 201 SLN’s including 16 (8.1%) with macrometastases and

9 (4.6%) with micrometastases. One SLN had isolated tumour cells only. All 25 positive SLN’s (100%) were blue dye positive, radioactive and fluorescent and all node positive patients had metastatic disease in the first SLN excised. There were 2 patients with fluorescent ‘non-sentinel’ nodes containing metastases and in each case there was tumour in either a single SLN (1/1) or in both SLN’s (2/2) retrieved. There were no cases of fluorescent ‘non-sentinel nodes’ containing metastases in the absence of tumour in the cognate blue and/or hot SLN’s. Eighteen patients in total had one or more positive lymph nodes yielding procedural and node specific positivity rates of 17.3% (18/104) and 12.4% (25/201) respectively.

### Safety

There were no serious adverse events or reactions during this study. A total of 9 patients had minor generalised skin reactions which occurred after injection of ICG, but these were not necessarily attributable to the fluorescent tracer itself.

### Discussion

Although SLNB now constitutes an accepted standard of care for staging the axilla in clinically node negative patients, standardisation of methodology remains poor and variation in details of practice is common. Combined localisation techniques with both blue dye and radioisotope maximise performance indicators and are recommended for all patients. Results from the largest SLNB trial reveal an overall false negative rate of 9.8% with higher rates when only a single sentinel node is removed as opposed to 2–3 nodes.<sup>4</sup> Furthermore, at a median follow up of 97 months, there are no statistically significant differences in loco-regional recurrence nor overall survival between SLNB negative patients randomised to either no further axillary surgery or completion axillary lymph node dissection (ALND).<sup>12</sup> A randomised trial comparing blue dye alone with blue dye and isotope reported equivalent identification rates for these two techniques and questioned the need for co-localisation with radioactive colloid.<sup>13</sup> Nonetheless, when blue dye alone is used, surgeons tend to perform a blue dye-assisted sampling with harvesting of at least 4–5 nodes rather than 2–3 nodes as for a ‘pure’ SLNB.<sup>6</sup>

There are potential drawbacks associated with use of radioisotopes, including radiation exposure, mandatory licensing and limited availability. These issues have prompted exploration of alternative tracer agents such as ICG which could replace radioactive colloid and be more widely embraced by the breast surgical community. It seems unlikely that significant improvements in sensitivity and accuracy of SLNB will result from minor adjustments of technique using current tracer agents. The use of a fluorescence-based tracer introduces the modality of lymphography; visualisation of the subcutaneous lymphatics can provide a real time navigation system which assists the

Table 2

Detection rate of SLNs ( $n = 116$ ) by tracer agent.

SLN detection	Detection rate	Detection rate + ICG fluorescence
Blue dye	95.0% (191/201)	95.0% (191/201)
Radioisotope	77.6% (156/201)	77.6% (156/201)
Blue dye & radioisotope	73.1% (147/201)	73.1% (147/201)
ICG fluorescence only	100% (201/201)	



surgeon with surgical localisation as well as confirming sentinel status once a node(s) has been identified.<sup>7</sup> Thus there is potential for both guiding the surgical procedure and improving overall sensitivity.

The ICG fluorescence system appears to be safe and of low toxicity with few side effects and allergic reactions.<sup>7</sup> The incidence of the latter is estimated at 1 in 10,000 which compares favourably with 1 in 1000 for blue dye.

Japanese researchers have applied the principle of fluorescence imaging to SLNB mapping in breast cancer with identification rates in excess of 90% for ICG alone.<sup>14</sup> Subsequent practice has favoured a combination of ICG with either blue dye or radioisotope which allows both conventional and fluorescent visualisation of the lymphatic vessels and nodes. These have all shown high levels of nodal recognition by fluorescence with few nodes (<5%) being classified as blue and/or hot but not fluorescent.<sup>9</sup> This is in keeping with the results from this study where all 201 nodes that were blue and/or hot were also fluorescent with ICG. A small study of 30 women undergoing SLN biopsy with axillary dissection suggested that ICG fluorescence permitted both imaging of lymphatics and sentinel nodes (detection rate of 97%). False negative rates for ICG and radioisotope were 8% and 23% respectively.<sup>15</sup>

This is the largest study to report on the use of all 3 tracer agents for SLN identification. This study design was selected to assess the feasibility of ICG as a tracer agent without compromising overall accuracy and potentially increasing false negative rates. Deliberate removal of nodes was guided by conventional criteria (i.e. nodes which were hot and/or blue) as well as palpably suspicious nodes identified at operation. During node dissection, ICG can leak into the axillary tissues and produce non-specific staining. Adjacent non-sentinel lymph nodes can become fluorescent which could increase the nodal yield. However, the average nodal count was 2.33 which is not significantly different from a comparison group of clinically node negative patients undergoing routine SLNB with blue dye and isotope.<sup>16</sup>

Japanese workers have reported on results of fluorescent imaging in clinically node negative patients using a combination of blue dye and ICG. Detection rates for fluorescence mapping using a combination of blue dye and ICG exceed 99% compared with rates of 83%–93% for blue dye alone<sup>9,10</sup> and nodes with tumour deposits >0.2 mm were all fluorescent.<sup>9</sup>

A prospective study found identification rates of 98.7% and 96.9% for ICG and radioisotope respectively when individually combined with blue dye.<sup>17</sup> Once again, the mean nodal count for fluorescence mapping [3.7; range 1–11] was almost twice that for blue dye [2.0; range 1–5]. Node positivity rates and false negative rates were equivalent for the two techniques, but ICG was notably cheaper in the longer term.

All studies investigating ICG fluorescence imaging for SLNB have consistently shown near 100% identification

rates and confirmed that combination with either blue dye or isotope is the optimal technique.<sup>18</sup> In particular, when separate lymphatic tracts pass towards the axilla rather than convergence to one lymph duct, two tracer agents may act in a complementary manner to maximise the chance of detecting those nodes with a high probability of harbouring metastases.<sup>8</sup>

In the present study, the mean number of nodes detected with each method does not suggest that fluorescence mapping is associated with harvesting of an excessive number of nodes. A conjugated form of ICG adsorbed onto serum albumin (ICG:HSA) has been explored in an attempt to limit the number of fluorescent nodes. Mieog and colleagues reported use of ICG:HSA as a third tracer in 24 breast cancer patients undergoing conventional localisation with blue dye and isotope. All SLNs were fluorescent and the average nodal count was 1.45 which is comparable to the present study using ICG alone.<sup>19</sup> A further study involving 49 consecutive breast cancer patients has compared ICG alone (first 28 patients) with ICG:HSA (next 21 patients). There was no statistically significant difference between the number of SLNs detected with these 2 tracers, suggesting that conjugation of ICG to a carrier molecule may be unnecessary.<sup>20</sup>

Within this feasibility study, there were no patients with metastases in fluorescent only positive nodes and a procedural node positivity rate of 17.3% is consistent with half the cancers being screen-detected and preoperative axillary ultrasound routinely undertaken.

ICG fluorescence imaging provides an additional dimension to SLNB; subcutaneous lymphatics are seen within seconds of tracer injection and following gentle massage lymphatic vessels appear radiating outwards from the subareolar plexus. Usually these lymphatics converge towards a single duct in the region of the axillary tail (Fig. 1). These then delve more deeply into the axillary tissues at the “fluorescent line”<sup>9</sup> which lies approximately 1–2 cm proximal to the hairline. These subcutaneous lymphatics guide the site of the skin incision which is always made at or beyond the “fluorescent line”. Once the axillary incision is made, fluorescence can provide further navigation within the axillary tissue though spillage of ICG during surgical dissection can obscure lymphatic pathways and cloud nodal definition. Nonetheless, fluorescence navigation permits a more orderly and sequential dissection but, unlike radio-colloid does not quantify tracer uptake when standard imaging equipment (PDE and CCD) is employed. However, the detection system can be adapted to incorporate quantification of the fluorescent signal and this may increase confidence in identification of true sentinel nodes.

In this study the sensitivity for ICG fluorescence and blue dye (95.0%) compares very favourably with that for blue dye and radioisotope (73.1%) and suggests that a dual tracer approach using ICG and blue dye may be a potential way forward for SLNB detection. By combining the lymphatic mapping benefit of ICG fluorescence with the

ability of blue dye to directly visualise lymphatics in the axilla the detection of SLNs can be optimised (Fig. 3). The lack of adverse events in this study supports the high safety profile for ICG use in many areas of medicine.

In summary, this study confirms high sensitivity of ICG fluorescence for SLN detection in breast cancer either alone or in combination with blue dye and/or radioisotope. For many breast units that are using blue dye alone, or who have logistical problems with use of radioisotope, ICG fluorescence mapping using PDE technology can provide a safe and effective additional method for SLN detection. With further refinements and experience with this technique it may be possible to rely on ICG alone as a single tracer agent for SLN identification.

### Conflict of interest statement

None.

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