**Radiolabeling and Quality Control of $^{99m}$Tc-rituximab**

The reduction and radio-synthesis of $^{99m}$Tc-rituximab were shown in Supplement Figure 1.

Firstly, Rituximab (10 mg, 70 nmol) was dissolved in 1 mL of phosphate buffer solution (PBS) (pH =7.4, 10 mM), and 0.3 mL of the solution was taken out to react with 25 μL of 10% (V/V) 2-mercaptoethanol (2-ME) for 15 min in dark. The reaction system was then purified by a PD10 column to obtain 2 mL reduced Rituximab, named Rit-SH, and the solution was divided into 0.2 mL each vial, and the samples were stored at -20 $^{o}$C for further use.

When radiolabeling was performed, 0.2 mL solution of reduced Rituximab (Rit-SH) was warmed to room temperature (RT), and was added 10 μL of 100 mg/mL glucoheptonic acid, 15 μL of 1 mg/mL $SnCl_2$ and 370-470 MBq $Na^{99m}TcO_4$. The reaction mixture was shaken at RT for 10 min. $^{99m}$Tc-Rituximab was then purified by a PD-10 column. The radiotracer solution was then diluted to approximately 74 MBq/mL (2 mCi/mL) with saline and was filtered with a 0.20-μm Millex-LG filter (EMD Millipore). The synthesis of Rit-SH and radiolabeling with $^{99m}$Tc were described previously and were performed under good-manufacturing-practice conditions with daily quality control. Each patient was injected with 37 MBq (1.0 mCi) of $^{99m}$Tc-Rituximab.

Radiochemical purity (RP) of the tracer was always greater than 99% tested by Radio-TLC and HPLC.

The rapid radiochemical purity was tested by two kinds of analysis system by Radio-TLC. System 1: the supporter was ITLC-SG, the developer was 0.9% saline solution; System 2: the supporter was ITLC-SG, the developer was 0.01 M pH 7.4 PBS solution. Since the PD-10 column has been used for the purification of $^{99m}$Tc-rituximab from $^{99m}$Tc-colloid or free $^{99m}$Tc. In both Radio-TLC system, most radioactivity was associated with $^{99m}$Tc-rituximab (Rf = 0.0~0.1), with less than 1% free $^{99m}$Tc detectable (Rf = 0.9~1) shown in supplement figure 2.

For HPLC system, agilent bio SEC-3 (4.6mm×300mm, 3μm) was used as the chromatogram column, phosphate-buffered saline solution (pH7.4, 0.01mol/L) as the elution. The washing rate was 1.0ml/min. HPLC analysis results of $^{99m}$Tc-rituximab and the co-injection of Rit-SH were shown in supplement figure 3. The retention time of $^{99m}$Tc-rituximab is 6.69 min and co-injection of Rit-SH is 6.19 min. And the radiochemical purity is more than 99%.

**Standard SLN Biopsy Criteria**

The criterion for inclusion of patients include: (a) definite diagnosis of breast cancer by fine needle aspiration or excision biopsy and histopathology examination; (b) no lymph nodes enlargement palpated in axillary; (c) no suspicious lymph node metastasis in axillary as verified by ultrasound; (d) no distant metastases.

The criterion for exclusion of patients include: (a) patients had major surgery on breast or axillary with

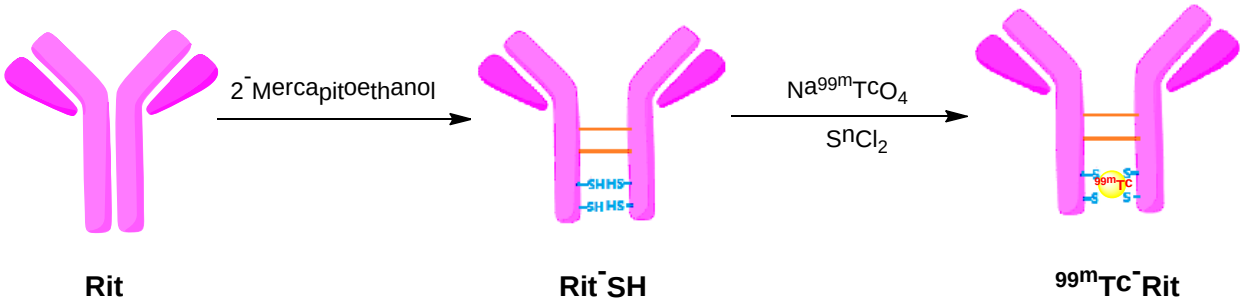
lymph backflow destruction; (b) multi-center or multi-focal breast cancer lesion; (c) confirmed metastasis on axillary lymph nodes; (d) patients had radiotherapy or chemotherapy history.

**Lymphatic Mapping Protocol of $^{99m}$Tc-Rituximab**

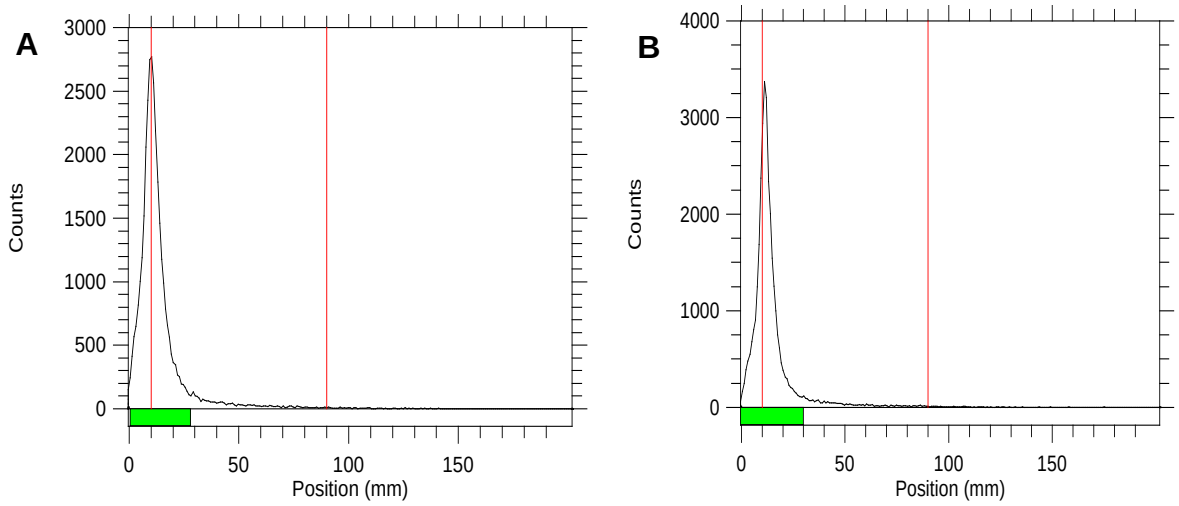
Guided by ultrasound, all patients had peritumoral subcutaneous injections of $^{99m}$Tc-rituximab (37MBq, 0.5ml) 2 to18 hours before surgery. Lymphoscintigraphy was acquired by a SPECT (Siemens, e.cam, Germany) with the patient positioned supine in the anterior view and the ipsilateral lateral view to determine the location and number of SLN (Supplement Figure 4).

Acquisition parameters: low energy high resolution parallel hole collimator, energy peak 140Kev, 20% window width, 128×128 matrix, zoom=1. After axillary incision, SLNs were identified by a handled gamma detecting probe (Cystal, USA).

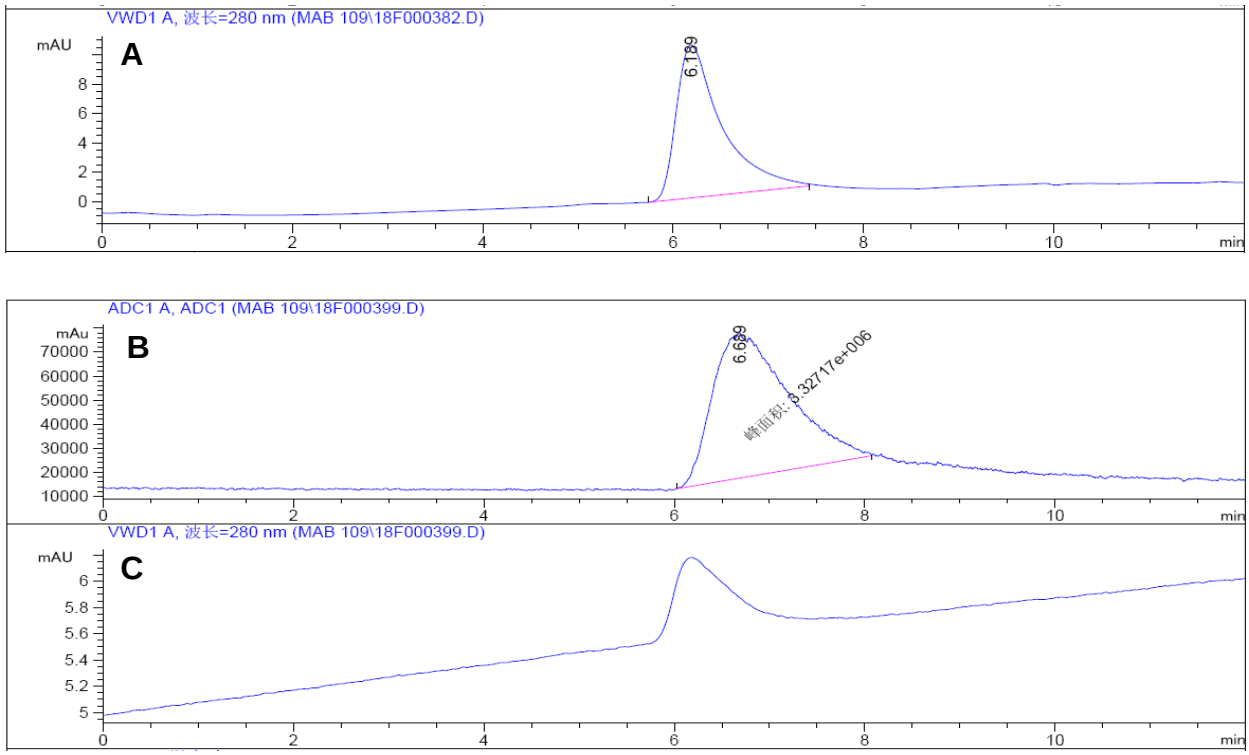
All radioactive nodes with a count rate $\geqslant$10% of the hottest node were removed. All SLNs were evaluated by frozen-section analysis. During the learning curve period, all the patients underwent an axillary lymph node dissection (ALND). After that, only patients with positive frozen sections immediately underwent an ALND. The SLNs and non-SLNs were all analyzed by hematoxylin-eosin staining.



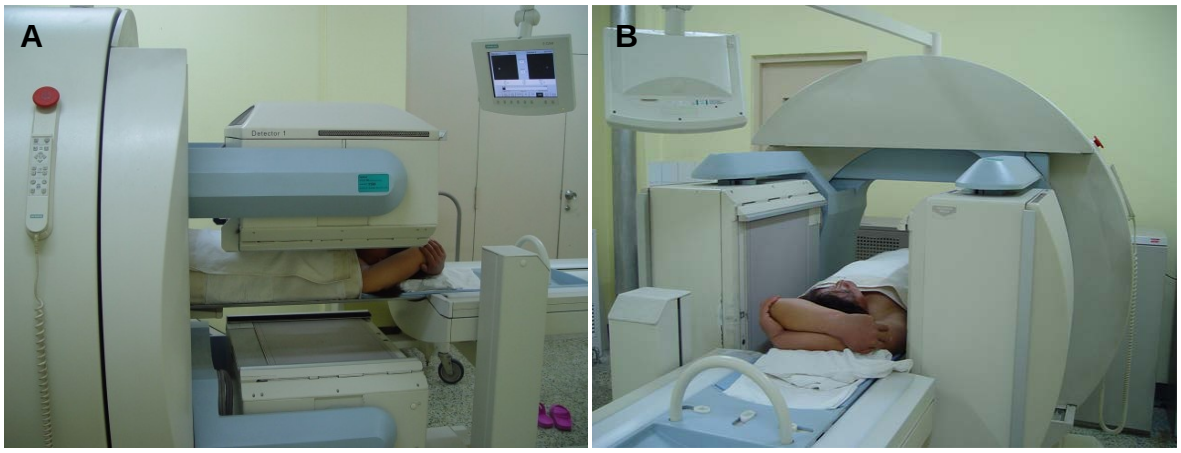

Supplemental Figure 1: Schematic diagram of the synthesis of $^{99m}$Tc-rituximab.



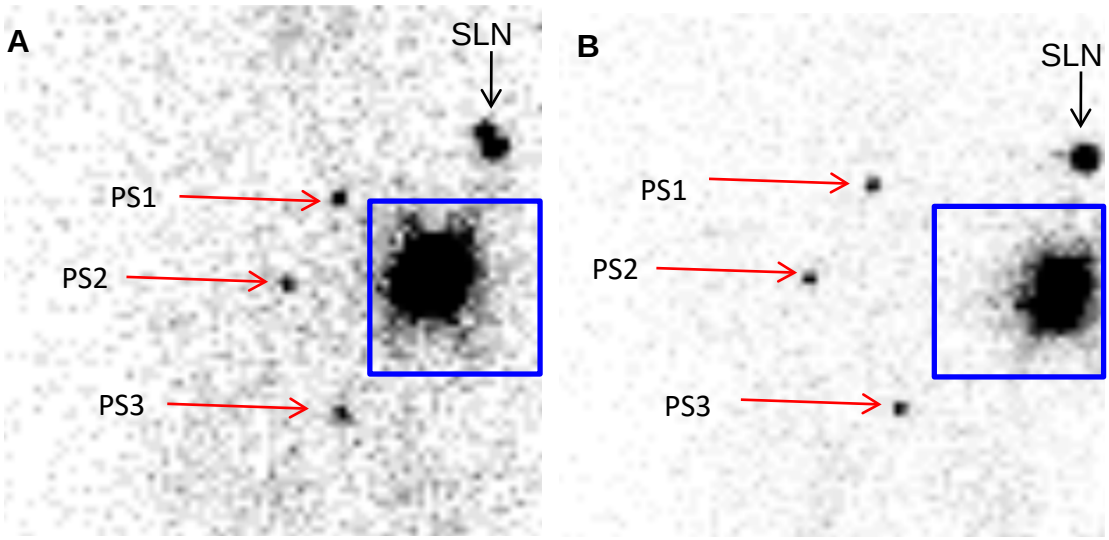

Supplemental Figure 2: Radio-TLC analysis of $^{99m}$Tc-rituximab. A. 0.9% Saline as elution and ITLC-SG as supporter. Rf value of $^{99m}$Tc-rituximab 0.0. Radiolabeling yield >99%; B: 0.01 M pH7.4 PBS as elution and ITLC-SG as supporter. Rf value of $^{99m}$Tc-rituximab 0.0. Radiolabeling yield >99%.



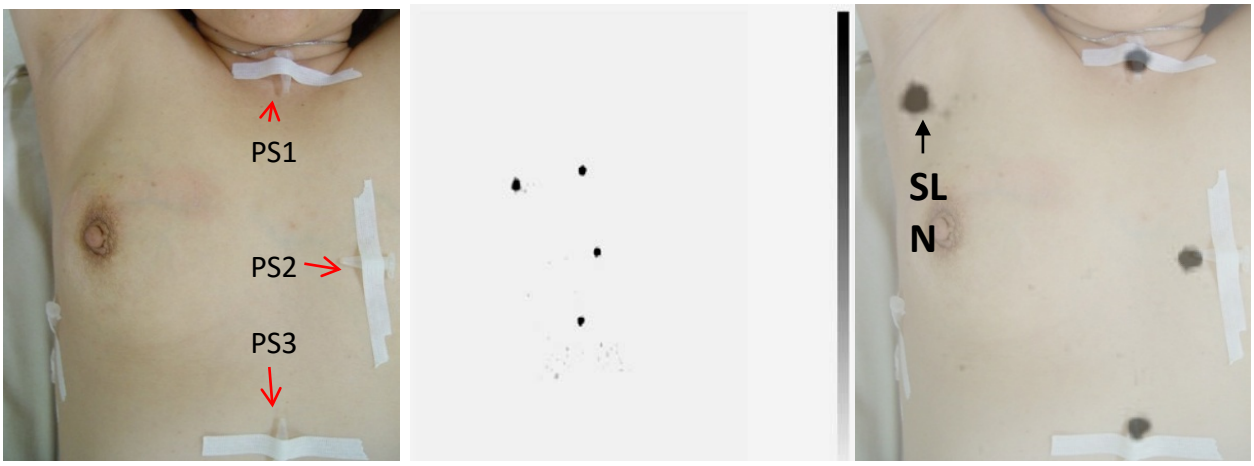

Supplemental Figure 3. Radio-HPLC analysis of $^{99m}$Tc-rituximab. A. Rituximab-SH detected by UV 280 nm detector with 6.19 min retention time; B. $^{99m}$Tc-rituximab detected by gamma detector with 6.69 min retention time; C. Coinjection of Rituximab-SH with $^{99m}$Tc-rituximab detected by UV280 detector in 6.18 min retention time.




Supplemental Figure 4. Actual pictures of (A) anteroposterior and (B) lateral position of using $^{99m}$Tc-rituximab tracer for SLN lymphoscintigraphy.

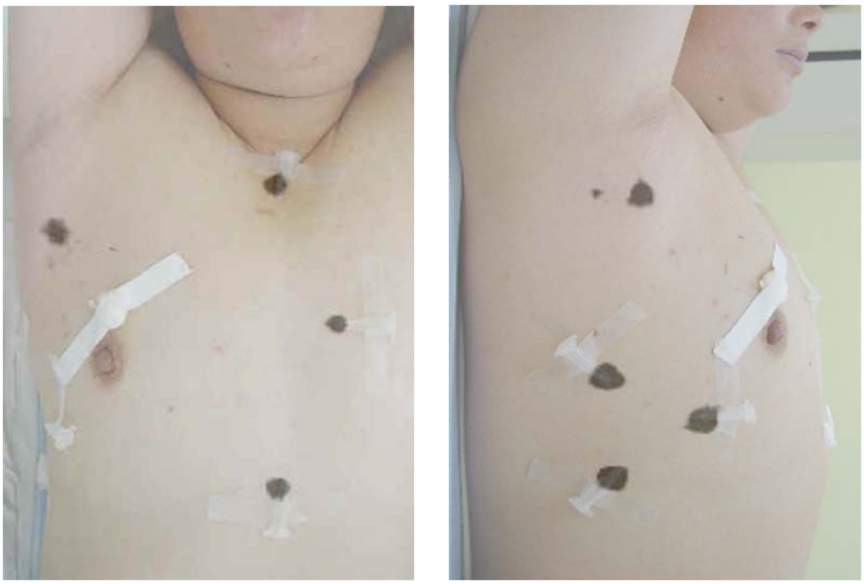



Supplemental Figure 5. Comparison of (A) 4 hours and (B) 18 hours Lymphoscintigraphy after injection of $^{99m}$Tc-rituximab. Blue square outlines the injection point, which always has been removed in our formal report. Point Sources 1: PS1; Point Sources 2: PS2 ; Point Sources 3: PS3.




Supplemental Figure 6. Case report 1. The lymphoscintigraphy of a patient anteroposterior position. This is similar to the position on the operating table. On the anterior view three point sources (PS) are put at sternal notch, xiphisternum and parasternum on the contra side. And the injection point has been removed from SPECT imaging in the report.


Supplemental Figure 7. Case report 2. The lymphoscintigraphy of a patient in lateral position. And the injection point has been removed from SPECT imaging in the report.



Supplemental Figure 8. Case report 3. The Lymphoscintigraphy of a patient in (A) anteroposterior and (B) lateral position. This is a formal case report. The injection point has been removed from this report.