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TITLE:

Sentinel Lymph Node Mapping and Biopsy for Endometrial Cancer at Early Stage with Laparoscopy

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KEYWORDS:

sentinel lymph node; mapping; biopsy; endometrial cancer; laparoscopy

SUMMARY:

This protocol describes the identification and resection of sentinel lymph nodes to make the operation as easy and minimally invasive as possible.

ABSTRACT:

Sentinel lymph node (SLN) mapping and biopsy is a promising technique for visualizing and evaluating lymph node status in cancer. This approach has been recommended for low-risk endometrial cancer (EC) patients by authoritative international guidelines, but it has not been performed broadly in China and worldwide. This work aims to describe detailed SLN mapping and biopsy procedures to promote the clinical application. SLN mapping and postoperative pathologic ultrastaging were conducted in a patient with low-risk EC using indocyanine green (ICG) dye to track the SLNs under laparoscopy and resecting them completely for ultra-staging. In conclusion, this protocol describes details of ICG injection, and SLN mapping and biopsy in EC patients based on the experiences gained during clinical practice.

INTRODUCTION:

Endometrial cancer (EC) is one of the most common diseases in gynecological oncology, and its incidence is rising[1,2]. Surgery is the first-line treatment for early-stage EC[3,4]. The evaluation of lymph node metastasis is an essential part of surgical staging in EC. The Gynecologic Oncology Group (GOG) study 33 demonstrated that lymph node metastases are associated with poor

prognosis[5].

As a new and essential technique for evaluating lymph node metastasis, sentinel lymph node (SLN) mapping and biopsy have emerged in recent years and have been recommended to be employed for patients with apparent uterine-confined EC according to the National Comprehensive Cancer Network (NCCN) guidelines for uterine cancer[6–9,10]. SLN mapping has also been extensively applied in tumors such as breast cancer[11], lung cancer[12], thyroid cancer[13], and melanoma[14]. Pathologic ultra-staging has achieved good performance in colorectal and gynecological cancer[15–17] and is recommended by the authoritative European guidelines[18]. Although principles of SLN mapping for EC staging have been provided in international guidelines[10,19], there are no detailed manipulations of surgery in other present works.

This work presents the protocol for detailed SLN mapping and biopsy with ICG in a 28-year-old female who had been clinically diagnosed with early-stage EC, thus, improving and promoting the diagnosis of patients.

PROTOCOL:

All surgery procedures related to the patient described here were approved by the Ethics Committees of Obstetrics & Gynecology Hospital of Fudan University in Shanghai, China. Informed consent was acquired from the patient.

1. Inclusion criteria for EC patients

1.1. Ensure that patients are clinically diagnosed with primary EC confined to the uterus with low-risk factors (grade 1 or 2 endometrioid carcinomas, pre-surgical endometrial lesion ≤2 cm, and myometrial invasion <50%).

1.2. Ensure that patients are not allergic to the developer (ICG).

1.3. Ensure that patients have not experienced retroperitoneal lymph node dissection for any reason before the disease.

1.4. Ensure that the patients understand the SLN surgical procedures and have signed the surgical consent forms.

1.5. Ensure that the patients cannot perform systematic lymphadenectomy but SLN mapping for surgical staging for any reason.

1.6. Ensure that the patients are enrolled in the clinical trial into the SLN mapping group if they are with intermediate-high risk EC.

2. Patient preparation

2.1. Preoperative bowel preparation

2.1.1. Provide laxatives for bowel preparation and enema to patients who have undergone multiple pelvic and abdomen surgeries.

NOTE: Intestinal preparation is not required for patients without bowel surgery history.

2.2. Preoperative diet preparation

2.2.1. Avoid solid food for 8 h before the operation, prevent a semi-liquid diet for 6 h before the procedure, and stop drinking for 2 h before the procedure.

2.3. Anesthesia

2.3.1. Use intravenous agents (e.g., propofol) to produce unconsciousness and add some neuromuscular blockers by inhalation or intravenous routes to achieve the needed depth of anesthesia. Use a combination of administrations for anesthetic maintenance to ensure the patient to be unconscious and fully relaxed with stable vital signs throughout the operation.

NOTE: The usage of anesthetics will be following the rules of the anesthesiology department of each center.

2.4. Position

2.4.1. Once the patient is anesthetized, allow the patient to be in the lithotomy position with Trendelenburg position.

2.5. Prepare the skin at the surgical site. Perform skin preparation with an antiseptic preparation (e.g., povidone-iodine) in an organized fashion from the intended site of the incision radiating out to the edges of the intended area of skin exposure, with the upper boundary of flat xiphoid process, side borders of mid-axillary lines, and lower boundary of the upper thigh (**Figure 1**).

3. Fluorescent dye preparation

3.1. Indocyanine green (ICG) preparation

3.1.1. Dissolve 25 mg of ICG into 20 mL of sterile water for a final concentration of 1.25 mg/mL and shake gently.

4. ICG injection

4.1. Use a 2 mL syringe (with the needle size of 0.6 mm x 32 mm TW LB) to inject 0.5 mL of the prepared ICG at 3 o'clock and 9 o'clock of the ectocervix at the superficial (2–3 mm) and deep (1–2 cm) cervix, respectively (**Figure 2**).

NOTE: In some cases, the ectocervix positions of 6 and 12 o'clock can also be selected. ICG should be avoided when injected into Nessler's cyst.

5. Intraoperative SLN identification

5.1. Preparation before development

5.1.1. Grasp the skin around the umbilicus with two towel forceps to elevate the abdominal wall. Make an incision of about 10 mm wide in the skin of the umbilicus and through the fascia and peritoneum.

5.1.2. Insert a 10 mm trocar through the umbilicus, producing the pneumoperitoneum with intraperitoneal pressure of about 13–14 mmHg. Insert a laparoscope (with light source and cameras) *via* the trocar.

5.1.3. Make three other small incisions of ~5 mm wide in the lower abdomen and insert 5 mm trocars through the abdominal wall into the cavity. Insert instruments for manipulation *via* the other three 5 mm trocars (**Figure 1**).

5.1.4. Identify the fallopian tubes under laparoscopy. Grasp and elevate the tubes close to fimbriae and tie a ligature around the tube with a 2-0/T silk suture.

NOTE: This step is used to prevent endometrial tumor cells from entering the abdominal cavity along the fallopian tubes from the uterine cavity.

5.1.5. Take the peritoneal washings with 100 mL of normal saline before any significant handling or manipulation of the uterus. Collect the washing fluid for cytology.

5.1.6. Open the fluorescence mode of the endoscopic fluorescence imaging system (see step 5.2). Lift unilateral pelvic infundibulum ligament and uterus to reveal the lateral peritoneum and look for the fluorescence lymphatic vessels.

NOTE: If there are no fluorescence lymphatic vessels identified, it is allowed to inject ICG repeatedly.

5.2. SLN mapping

5.2.1. Endoscopic fluorescence imaging system preparation (PINPOINT).

5.2.2. Adjust the fluorescence mode to near-infrared laser (excitation light, 805 nm) for the best field of view.

NOTE: The endoscopic fluorescence imaging system has several modes, such as HD (High

definition) white light, SPY fluorescence, PINPOINT fluorescence, and SPY CSF mode, and the surgeon can switch to another mode if necessary.

5.3. Identify SLNs under a fluorescence laparoscopy.

5.3.1. Hold an ultrasonic scalpel and toothless forceps with each hand (performed by the surgeon).

NOTE: The assistant grasps toothless forceps to cooperate with the surgeon.

5.3.2. Lift and open the peritoneum, separate the sub-peritoneal fat and connective tissues to reveal the necessary structures, including the ureter, internal iliac vessels, and obturator nerves according to the indication of green fluorescence lymphatic lines, as well as to protect them from injury.

NOTE: An ultrasonic scalpel with activation is used to cut the tissues, while an inactivated scalpel can be used for blunt dissection.

5.3.3. Observe the SLNs covered by the peritoneum about 5–10 min after ICG injection.

NOTE: Typically, SLNs present along with the fluorescence lymphatic vessels from both sides of the cervix to at least the height of the common iliac blood vessel. Occasionally, the SLNs emerge beside the abdominal aorta. PINPOINT equipment has multiple modes, including white light, fluorescence, and black and white modes. Switching between multiple modes helps to determine the location of SLNs.

6. Intraoperative SLN biopsy

6.1. Locate the SLN, the first mapped lymph node along the drainage from parametrium in each hemi-pelvis, and use multiple modes to confirm it. Fully expose the SLNs for complete resection (**Figure 3**).

6.2. Grasp and elevate the SLN by toothless forceps and perform a complete resection of the lymph node along the periphery of the lymph node in white light.

NOTE: Avoid lymph node damage, which might cause contamination with tumor cells.

6.3. Place the removed SLNs in the obturator fossa or put them into a small, simple bag. After the uterus is completely removed and taken out through the vagina, bilateral SLNs are taken out entirely through the vagina rather than through the 5 mm trocar opening to avoid fragmentation.

6.4. Send the removed lymph nodes to the pathology department for pathological ultra-staging.

6.5. During the operation, resect the enlarged or suspicious lymph nodes simultaneously and send them for pathological examination.

6.6. Record the locations of SLNs on standardized intraoperative data collection forms.

6.7. If SLN mapping fails on one or both sides, perform side-specific lymphadenectomy according to the NCCN guidelines.

NOTE: If there is no fluorescent node in the hemipelvis, continue to seek the mapped node along the lymphatic vessels until the para-aortic region due to the possibility of mapping in unexpected locations. A failed SLN mapping on either side means that any fluorescent green node is not visible with or without fluorescent green lymphatic vessels under the fluorescence laparoscope system on either side of the pelvis and para-aortic region.

6.8. Perform a total hysterectomy plus bilateral salpingectomy.

NOTE: Since this was a 28-year-old patient with early-stage endometrioid cancer who had a strong desire for fertility preservation and normal-appearing ovarian cancer and had no apparent family history of breast/ovarian cancer or Lynch Syndrome, ovarian preservation could be considered.

6.9. Indwell a drainage tube for potential residual fluid in the pelvis.

6.10. Remove the laparoscope and release the gas from the abdomen. Close the incisions in the umbilicus and lower abdomen with a single stitch by 2-0/T silk suture.

7. Postoperative SLN ultra-staging[20]

7.1. Cut the SLN along the maximum diameter and micro-sectioned 50 μm apart to get three hematoxylin-eosin (H&E) slides and one immunohistochemistry (IHC) slide with anti-cytokeratin antibody (AE1/AE3).

NOTE: Ultrastaging entails thin serial sectioning of the gross SLN. Cytokeratin IHC is not essential. No standard protocol is for ultra-staging.

7.2. Lymph node metastases were described as macrometastases (>2 mm), micrometastases (0.2–2.0 mm), or isolated tumor cells (ITCs) according to the classification of the American Joint Committee on Cancer (AJCC)[21].

7.3. Report the postoperative pathology with details about the tumor size, histologic type and grade, myometrial invasion, lymphovascular space involvement, cervical stromal involvement, number and status of per lymph nodes, and the cytology of peritoneal washings.

REPRESENTATIVE RESULTS:

The patient in the present case was a 28-year-old female with irregular vaginal bleeding for 2 years, and she was diagnosed with an abnormality of the endometrium 4 months ago. Transvaginal ultrasound examination revealed a heterogeneous endometrial thickness with an adnexal mass. Abdominopelvic magnetic resonance imaging (MRI) demonstrated a 51 mm x 56 mm x 88 mm mass with a clear boundary within the uterine cavity under a high T2W1 signal in a local hospital. Then, she was evaluated by hysteroscopy, and the endometrial lesion was dissected. The pathological results showed grade 1 endometrial cancer. This patient came to the hospital for further treatment with a strong fertility requirement. After a comprehensive evaluation (including another MRI examination that showed an irregular signal of endometrium in hospital, **Figure 4**) and signing informed consent, she experienced fertility preservation treatment. However, the second hysteroscopy in the hospital revealed the superficial EC myometrial invasion, a contraindication of fertility preservation. Ultimately, after the patient was informed of her condition, she decided to undergo a total hysterectomy + bilateral salpingectomy + SLN mapping.

The cervix was injected with diluted ICG at 3 and 9 o'clock positions superficially and deeply, respectively (**Figure 2**). Then, the lymphatic vessels and SLNs were fluorescently labeled, which allowed for their recognition under various color modes (green for fluorescence mode and blue and red for color-segmented mode) in the Pinpoint Endoscopic Fluorescence Imaging system (**Figure 3**). Subsequently, the pathology department conducted H&E and IHC staining of SLN (**Figure 5** and **Figure 6**) and ultra-staging of SLN. The staging results revealed a negative metastasis output.

FIGURE AND TABLE LEGENDS:

Figure 1: Antiseptic preparation scope of SLN mapping in EC with laparoscopy. The black arrow indicates mid-axillary lines. The numbered red circles indicate the places of four trocars on the abdominal wall. Circle No. 2 is for the 10 mm trocar. The rest of the circles are for 5 mm trocars. The distance between circles No. 2 and 3 is around four fingerbreadths.

Figure 2: Illustration of the SLN mapping in EC. (**A**) Anatomical diagram of the SLN in the pelvis. The dark green line represents the direction of the lymphatic drainage. Arrows indicate SLN lymph nodes in this case (yellow arrow presents the lymph node located between the right external and internal iliac vessels, brown arrow demonstrates the SLN in the left obturator foramen, and black arrow shows enlarged lymph node on the left iliac vein). Blue flat line indicates clock directions. (**B**) Injection spot locations in the cervix. ICG = indocyanine green. Green dots indicate the injection spots. The blue square shows the 3 and 9 o'clock positions of the ectocervix. (**C**) Enlarged cervix diagram: green dots indicate superficial and deep cervical injections.

Figure 3: Intraoperative imaging of the SLN in EC. (**A**) Fluorescence mode. The green arrow indicates the SLN under fluorescence mode. (**B**) Color-segmented fluorescence mode. The yellow arrow indicates the lymph node under color-segmented mode. (**C**) Florescence mode of sentinel lymph nodes of this case. (**D**) Color-segmented fluorescence mode of sentinel lymph nodes of

this case. HD: High definition; CSF: Color-segmented fluorescence.

Figure 4: The MRI of EC patient. (**A**) Irregular signal of the endometrium located in the lower uterus cavity (arrows) on T1WI. (**B**) Irregular signal of the endometrium (arrows) on T2WI.

Figure 5: H&E staining of EC and IHC staining of SLN. (**A**) H&E staining of myoinvasion in EC. (**B**) H&E staining of SLN. (**C**) IHC staining of AE1/AE3 (AE1/AE3 may stain myofibroblasts and smooth muscle cells and indicate the residual tumor cells) in SLN. 2.5x and 5x refer to the magnification under the microscope.

Figure 6: IHC staining of EC patients. (**A**) IHC staining of estrogen receptor (ER) with five magnifications. (**B**) IHC staining of progesterone receptor (PR). (**C**) IHC staining of P53. (**D**) IHC staining of Ki67. (**E**) IHC staining of MLH1. (**F**) IHC staining of MSH2. (**G**) IHC staining of PMS2. (**H**) IHC staining of MSH6. All the images are captured with 5x magnification.

Table 1: Comparison between tracers of blue dye, ICG, and Tc99.

DISCUSSION:

SLN mapping and biopsy is a more selective and tailored lymph node dissection approach that has been applied in the clinic for nearly 20 years. In the field of EC, SLN mapping and biopsy have been increasingly recommended by several guidelines due to their high diagnostic efficiency in early-stage EC, achieving overall and bilateral detection rates of 89%–95% and 52%–82%, respectively, with a sensitivity of 84%–100% and negative predictive value of 97%–100%[22]. The present study reports a typical EC patient who performed SLN mapping and described the SLN protocol in detail from ICG injection to SLN biopsy.

SLN mapping is feasible with various dyes. Previous research on lymph node staging of breast and endometrial cancers applied blue dye (usually methylene blue or isosulfane blue (ISB), radiocolloid, or both[23–26]), reaching good performance and accuracy. As shown in white light, the blue dyes mentioned above have been extensively used due to their convenience and the least complex equipment requirement. However, the ICG dye had higher SLN detection rates (83%) than the ISB (64%)[26]. Thus, ICG is considered a better option for successful detection. Comparison between different dyes is stated in **Table 1** according to the SGO consensus[27].

In addition to tracker dyes, injection positions also affect detection rates of SLN mapping. Dye injection in the cervix cannot wholly reflect the lymphatic drainage, though the procedure is relatively simple and popular in clinical trials[28,29]. A hysteroscopic injection requires more skill and is located near the tumor lesion. The myometrial site is challenging to access intraoperatively, resulting in a low negative predictive rate of 87.50% and a false-negative rate of 33.30%[30]. In studies using cervical injection, the sensitivity range was 62.5%–97.5%, while myometrium injection was 66.70%–94.10%[31]. A systematic review including 55 eligible studies revealed an overall detection rate of 81% (95% confidence interval is from 77 to 84)[32]. Double detection works better than single detection, regardless of the injection location[33]. In the ongoing clinical

study (NCT04276532), where 92 EC patients were enrolled, the SLN mapping detection rate was as high as 91.3%, with a total sensitivity of 73.3%. Combining cervical and fundus injections reached a higher para-aortic detection rate (40.4%) than cervical injections alone (4.4%), indicating that the combined injection is more efficient.

There are many possible reasons for insufficient SLN detection. Anatomically, the Naboth cyst and postsurgical abnormal pelvis affect mapping accuracy. Excessive adipose tissue near the cervix and cervical stenosis due to aging also lead to failed mapping. Factors such as body mass index (BMI), ISB dye use, and enlarged nodes are associated with SLN mapping failure[34]. Controversially, one prospective study including 110 EC patients reported that obesity and the presence of lymph node metastases were not associated with detection failure[35]. Lymphovascular space invasion might lead to failure of SLN mapping as well.

International guidelines consider SLN mapping and biopsy as necessary procedures[10,18]. However, there are debates about whether to conduct SLN mapping in intermediate-high risk EC patients. Patients with severe carcinoma, clear cell carcinoma, and carcinosarcoma have a higher risk of node metastasis[36]. On the contrary, some trials have determined that SLN mapping is a safe alternative to systematic lymphadenectomy because of lower complication occurrence[37,38] and equivalent overall survival[37]. More high-quality clinical trials are required to determine the optimal inclusion standards.

In conclusion, SLN mapping and biopsy are better options than systematic lymphadenectomy to make the operation as simple and minimally invasive as possible. It can even improve the pathological stage of the disease by using a pathological ultra-staging method to guide the postoperative adjuvant therapy more accurately. For maximizing the specificity and sensitivity of SLN detection, this protocol provides tips for SLN mapping and biopsy to ensure a better outcome of the surgery.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

Antiseptic preparation scope

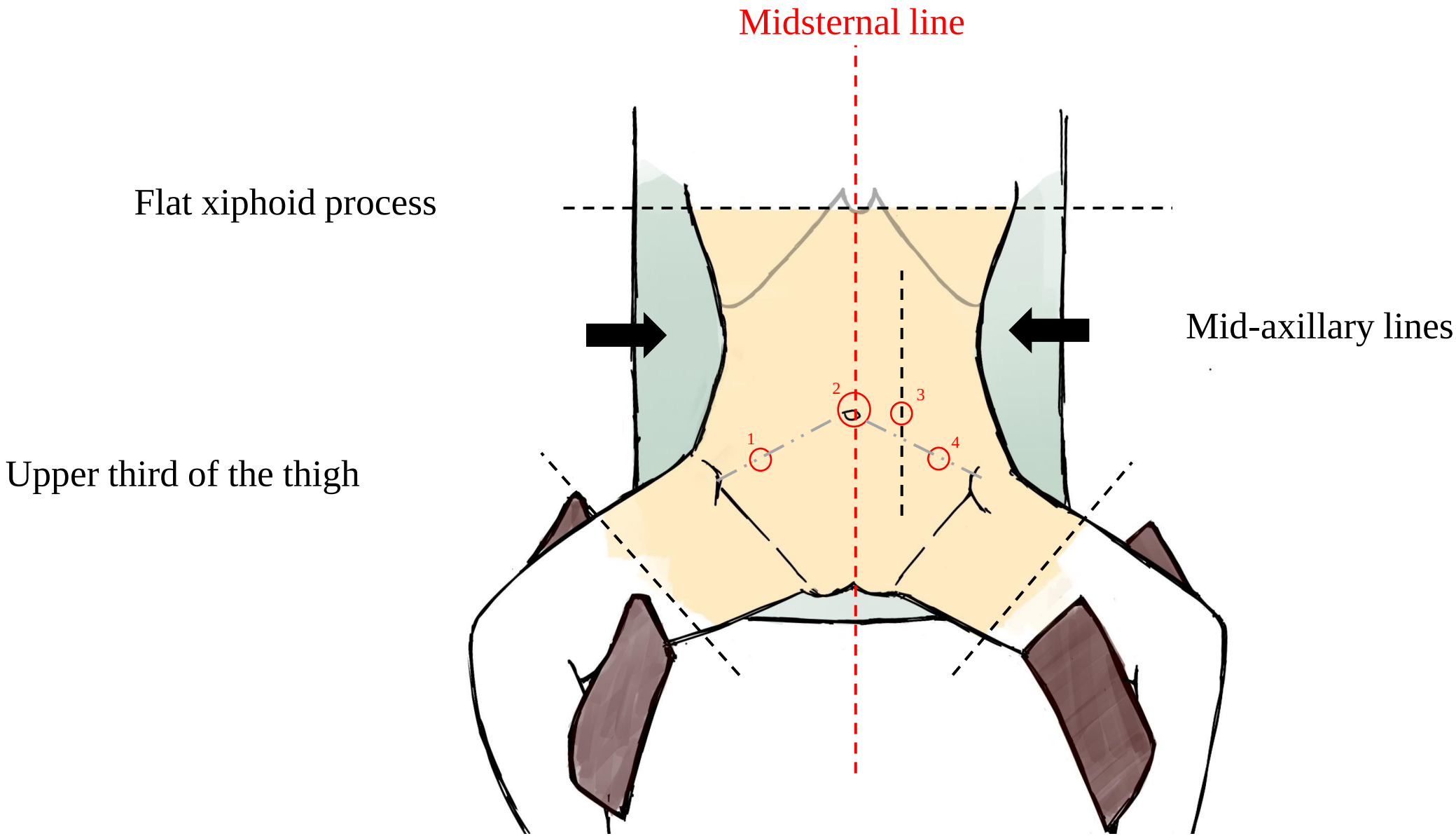


Figure 2

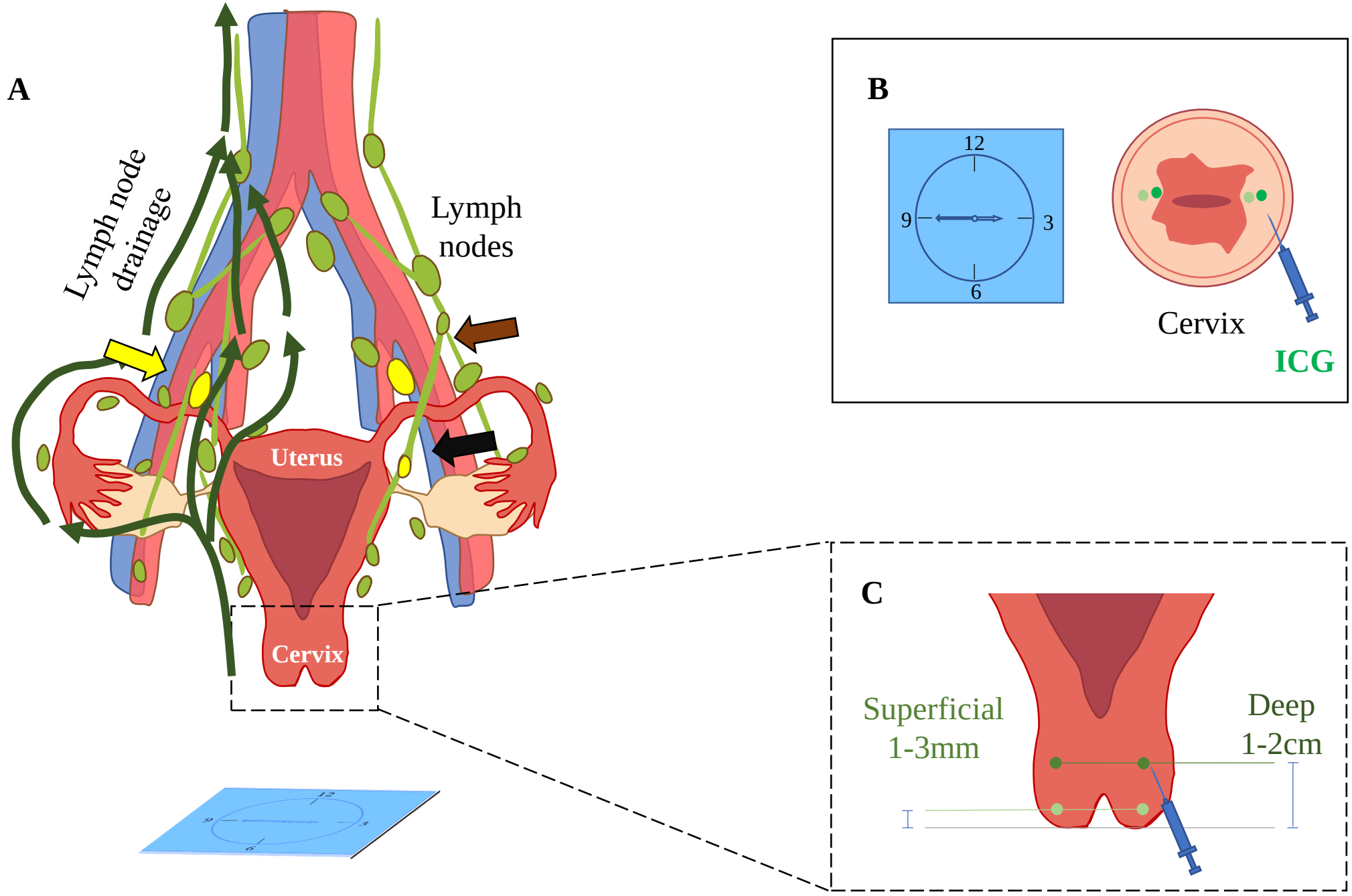


Figure 3

Figure 3

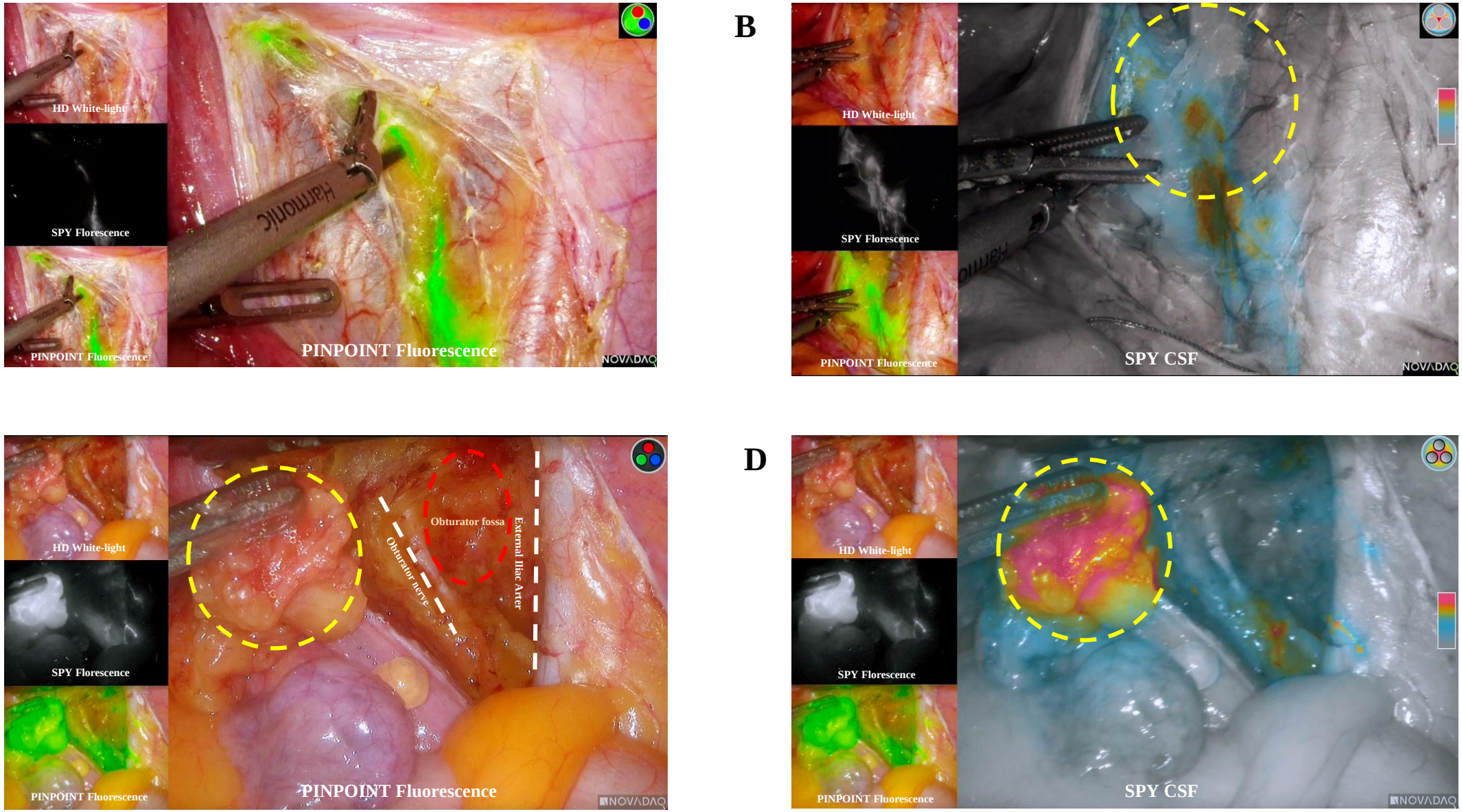


Figure 4

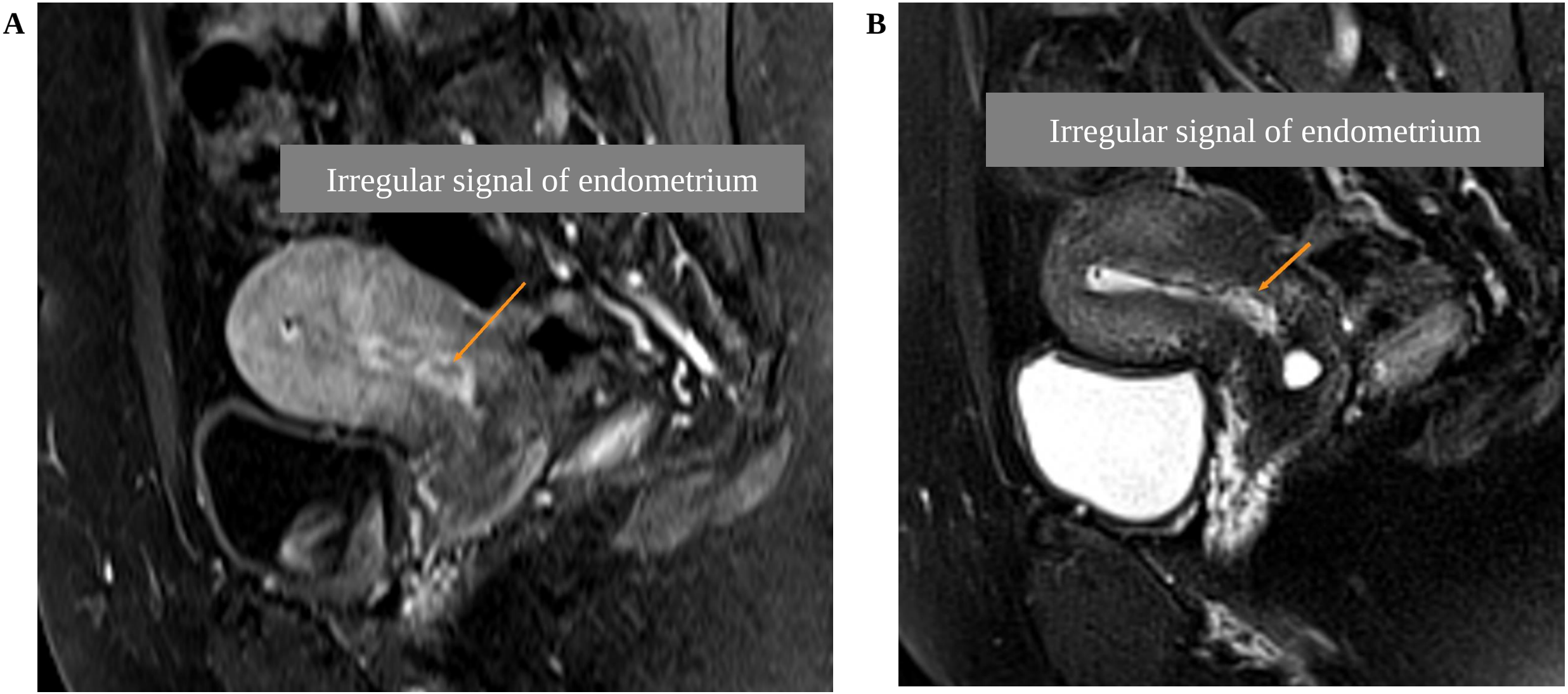


Figure 5

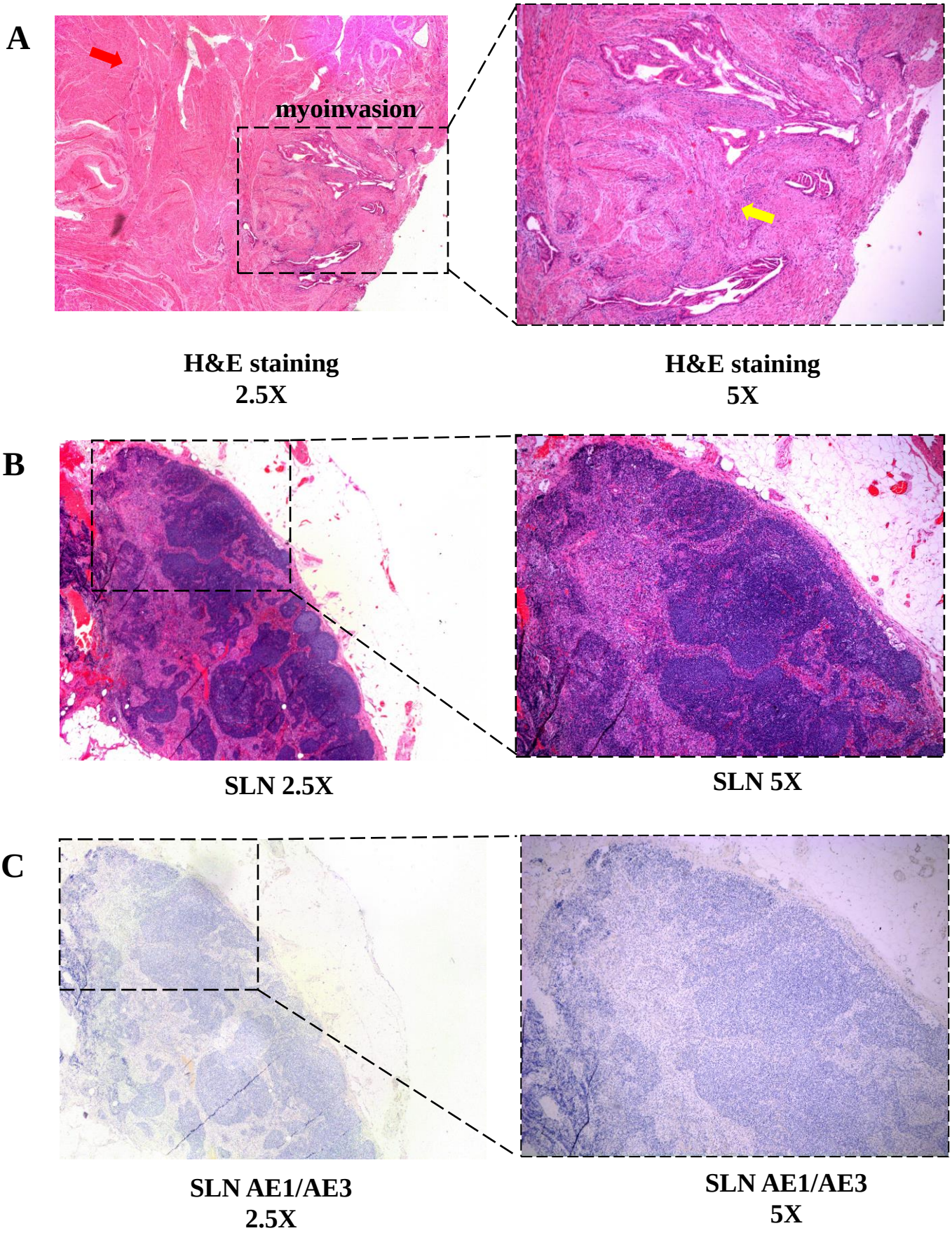
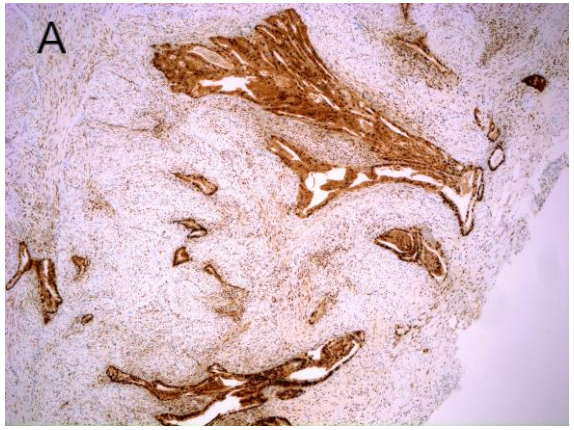


Figure 6



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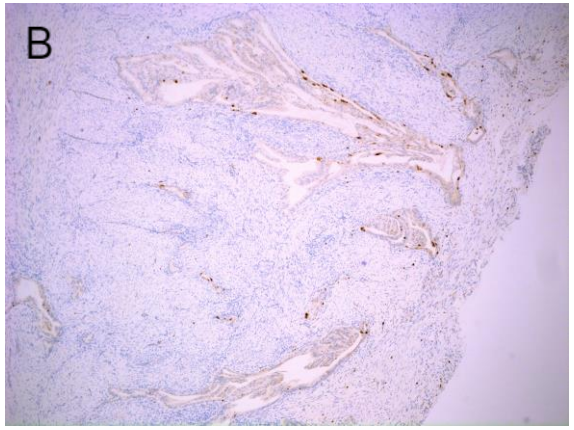

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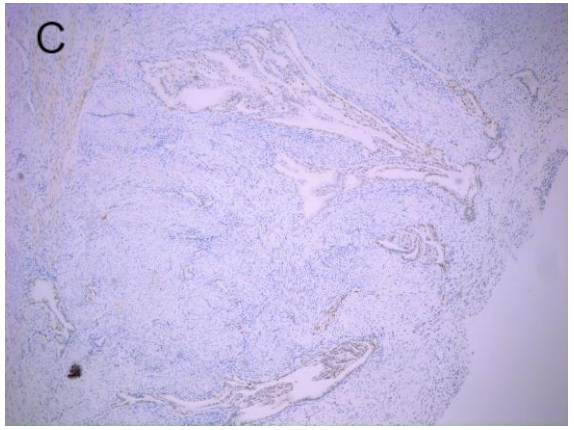

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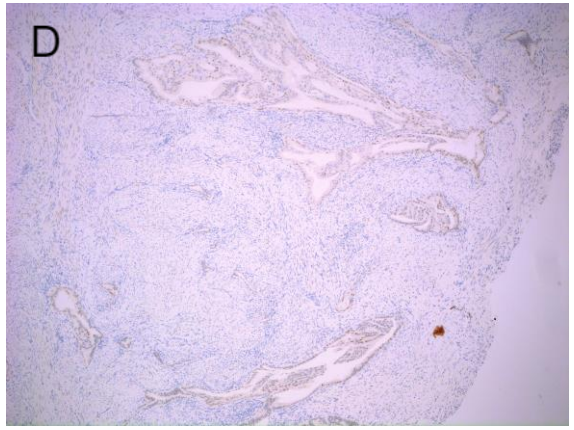

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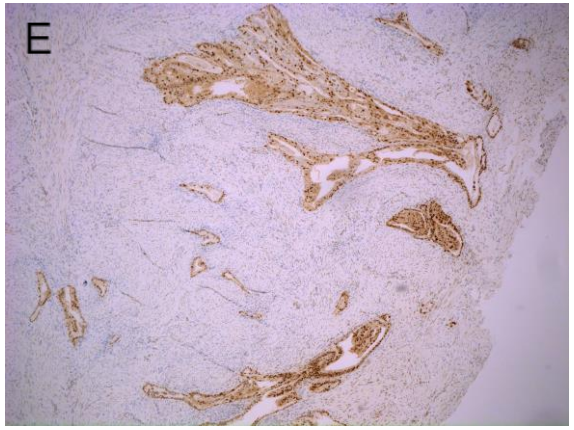

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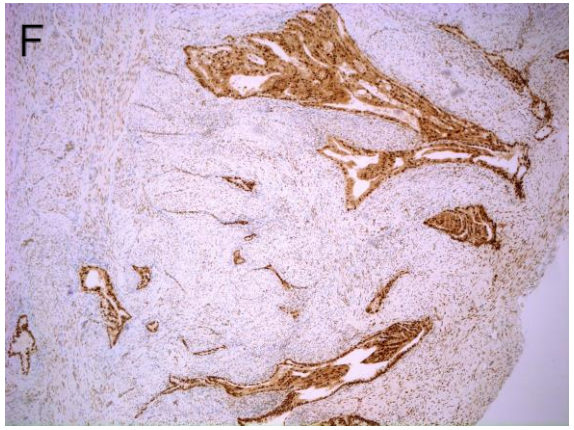

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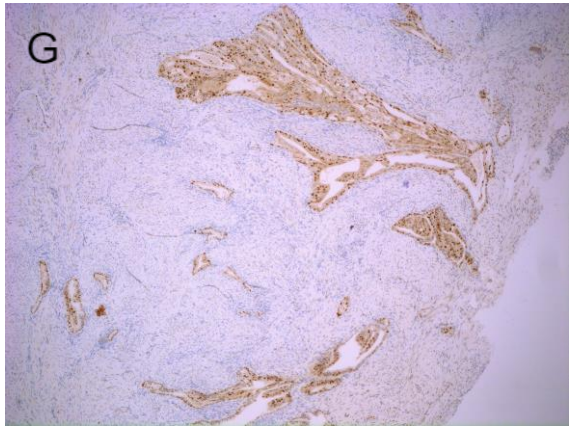

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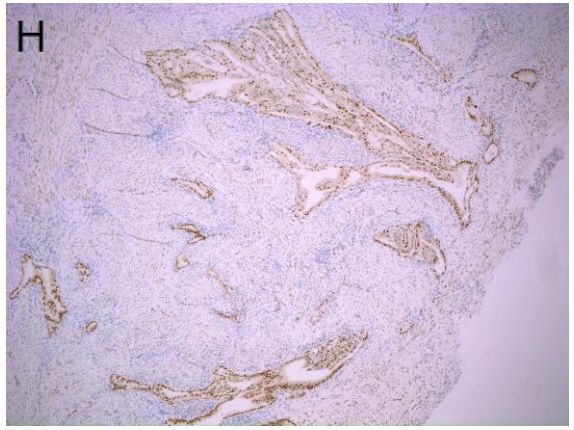

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Table 1

	Tracer	Dosage	Surgical approach	Specialized equipment
Single use	Blue: colored dyes in white light		Applicable to all	-
	Isosulfan blue	3–5 cm3 of a 1% solution		
	Methylene blue	2-4 cm3 of a 1% solution		
	ICG: water-soluble dye emitting fluorescence in NIR	2–4 mL with concentration of 0.5-1.25 mg/mL	NIR imagers are available for all	NIR imagers
Combined use	Tc99: radiolabeled colloid Tc99 with nuclear imaging / Blue & Tc99 ICG & Tc99 Blue & ICG Blue &ICG &Tc99	1 mL of 1 mCi of Tc99	Both open and laparoscopic gamma probes are available	Preoperative lymphoscintigraphy or 3D

SLN: sentinel lymph node; PA: para-aortic; NPV: negative predictive value; ICG: indocyanine

Cost & availability	SLN detection rate			Sensitivity	NPV
	Overall	Bilateral	PA SLN		
	71%	40%	1.90%	94%	99%
High expense and limited availability					
Less expensive					
-	95%	66%	12.70%	93%-100%	99%-100%
More cost and inconvenienc	93%	71%	3.20%		
	86%	57%	4.10%	95%	99%
	90%	52%	23%	100%	100%
	96%	84%	2.80%	98%	99%
	92%	76%	8%	90%	99%

green; NIR near-infrared light range; Tc99: technetium-99; 3D: three-dimens

Characteristics
Interfering with measurement of oxygen saturation
FDA approved Mapping within 10-20 min from injection Possible low detection rates due to delay mapping Risk (1.1%) of anaphylaxis Off-label use Risks of paradoxical methemoglobinemia and serotonin syndrome Off-label use Real-time visualization Signal penetration through deep tissue Superiority in obese patients Extremely low risk of adverse events (1/42,000 anaphylaxis)
It should be avoided in patients with severe iodine allergy or liver failure
Often used in synergy with a blue dye (or ICG) Advantageous in fatty nodal basins and unpredictable lymphatic drainage due to signal penetration through deep tissue

sional; SPECT/CT: single photon emission computed tomographywith integrated CT* Data of SLN de

tection rate, sensitivity, and NPV were based on studies of injection in cervix

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Yes, we have checked.

2. Line 57-59: Please support the statement with published References.

Thanks, we have deleted the statement because the revision of the prior research is being processed and no other references should be stated.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials (including reagents, instruments, software, etc.). Accordingly, in Line 129, please provide the details of the fluorescence imaging system in the Table of Materials. Please sort the Materials Table alphabetically by the name of the material.

Yes, we have deleted all commercial language from my manuscript and added them in the Table of Materials and Reagents.

4. Please ensure that the Table of Materials should include the name, company, and catalog number of all relevant materials used in the mentioned Protocol in separate columns in an xls/xlsx file.

Yes, we have checked.

5. Please add more details to your protocol steps:

Step 1/2: Please include the patient criteria before the numbered steps of the Protocol, or the major explanatory part can be moved to the Introduction or Discussion section if required. If this is to be included in the Protocol, this must be in imperative tense and in the mode of action. The Protocol should only contain discrete action steps in the imperative tense. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.

Thanks, we have made the changes.

Step 2.3: Please mention how the anesthesia was carried out.

Thanks, we have made the changes.

Step 2.4: The explanation is not clear. Please simplify. Else a diagrammatic representation can be provided to help the readers understand the position.

Thanks, we have made the changes.

6. Please highlight the Protocol steps to be filmed since many steps mentioned in the Protocol cannot be filmed due to privacy issues.

Yes, we have made the changes.

7. Please spell out the journal titles in the References.

Yes, we have made the changes.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This paper discussed the sentinel lymph node (SLN) detection for endometrial cancer. SLN detection has been widely used in low-risk EC using indocyanine green (ICG) and other tracker dyes.

Major Concerns:

This paper described the details of the SLN detection by ICG for patients with low-risk EC, which is very helpful clinically.

Thanks for the reviewer's kind suggestions. This manuscript aims to present the methods and protocol for ICG SLN biopsy in EC to improve and promote patient diagnosis. And we have made the changes about some details we missed according to the reviewer's helpful suggestions.

Minor Concerns:

1. There are several tracker dyes, including blue dye, radiocolloid, ICG and so on, with different detection rate. A table might be listed to compare these tracker dyes.

Yes, we have listed a table to compare these different tracker dyes.

2. ICG has been widely used as the tracker dye in SLN detection with a very high detection rate, whose advantages and disadvantages could be discussed in the paper.

We have compared the detection rates, sensitivities, and negative predictive values of SLN mapping with different tracker dyes, where the advantages and disadvantages of each tracker dye are also stated. Thanks for the reviewer's helpful suggestions.

Reviewer #2:

The authors of this paper describe their centre's protocol in detail for performing sentinel lymph node mapping in early stage endometrial cancer with the ICG NIR technique.

This may help other doctors who are interested in learning how to implement this novel technique in their centres.

Thanks for the reviewer's kind suggestions. It's exactly our purpose to report a typical case of an EC patient who had an SLN biopsy during surgery and describes the SLN protocol in detail from ICG injection to SLN biopsy, thus providing tips for SLN mapping and biopsy to ensure a better outcome of surgery. And we have made the changes about some details we missed according to the reviewer's helpful suggestions.

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1. Inclusion criteria for EC patients

1.1. Ensure that patients are clinically diagnosed with primary EC confined to the uterus with low-risk factors (grade 1 or 2 endometrioid carcinomas, pre-surgical endometrial lesion ≤2 cm, and myometrial invasion <50%).

1.2. Ensure that patients are not allergic to the developer (ICG).

1.3. Ensure that patients have not experienced retroperitoneal lymph node dissection for any reason before the disease.

1.4. Ensure that the patients understand the SLN surgical procedures and have signed the surgical consent forms.

1.5. Ensure that the patients cannot perform systematic lymphadenectomy but SLN mapping for surgical staging for any reason.

1.6. Ensure that the patients are enrolled in the clinical trial into the SLN mapping group if they are with intermediate-high risk EC.

2. Patient preparation

2.1. Preoperative bowel preparation

2.1.1. Provide laxatives for bowel preparation and enema to patients who have undergone multiple pelvic and abdomen surgeries.

NOTE: Intestinal preparation is not required for patients without bowel surgery history.

2.2. Preoperative diet preparation

2.2.1. Avoid solid food for 8 h before the operation, prevent a semi-liquid diet for 6 h before the procedure, and stop drinking for 2 h before the procedure.

2.3. Anesthesia

2.3.1. Use intravenous agents (e.g., propofol) to produce unconsciousness and add some neuromuscular blockers by inhalation or intravenous routes to achieve the needed depth of anesthesia. Use a combination of administrations for anesthetic maintenance to ensure the patient to be unconscious and fully relaxed with stable vital signs throughout the operation.

NOTE: The usage of anesthetics will be following the rules of the anesthesiology department of each center.

2.4. Position

2.4.1. Once the patient is anesthetized, allow the patient to be in the lithotomy position with Trendelenburg position.

2.5. Prepare the skin at the surgical site. Perform skin preparation with an antiseptic preparation (e.g., povidone-iodine) in an organized fashion from the intended site of the incision radiating out to the edges of the intended area of skin exposure, with the upper boundary of flat xiphoid process, side borders of mid-axillary lines, and lower boundary of the upper thigh (**Figure 1**).

3. Fluorescent dye preparation

3.1. Indocyanine green (ICG) preparation

3.1.1. Dissolve 25 mg of ICG into 20 mL of sterile water for a final concentration of 1.25 mg/mL and shake gently.

4. ICG injection

4.1. Use a 2 mL syringe (with the needle size of 0.6 mm x 32 mm TW LB) to inject 0.5 mL of the prepared ICG at 3 o'clock and 9 o'clock of the ectocervix at the superficial (2–3 mm) and deep (1–2 cm) cervix, respectively (**Figure 2**).

NOTE: In some cases, the ectocervix positions of 6 and 12 o'clock can also be selected. ICG should be avoided when injected into Nessler's cyst.

5. Intraoperative SLN identification

5.1. Preparation before development

5.1.1. Grasp the skin around the umbilicus with two towel forceps to elevate the abdominal wall. Make an incision of about 10 mm wide in the skin of the umbilicus and through the fascia and peritoneum.

5.1.2. Insert a 10 mm trocar through the umbilicus, producing the pneumoperitoneum with intraperitoneal pressure of about 13–14 mmHg. Insert a laparoscope (with light source and cameras) *via* the trocar.

5.1.3. Make three other small incisions of ~5 mm wide in the lower abdomen and insert 5 mm trocars through the abdominal wall into the cavity. Insert instruments for manipulation *via* the other three 5 mm trocars (**Figure 1**).

0:00:00 sec -0:00:28 sec 5.1.4. Identify the fallopian tubes under laparoscopy. Grasp and

elevate the tubes close to fimbriae and tie a ligature around the tube with a 2-0/T silk suture.

NOTE: This step is used to prevent endometrial tumor cells from entering the abdominal cavity along the fallopian tubes from the uterine cavity.

0:00:28 sec -0:00:38 sec 5.1.5. Take the peritoneal washings with 100 mL of normal saline before any significant handling or manipulation of the uterus. Collect the washing fluid for cytology.

0:00:38 sec -0:00:42 sec 5.1.6. Open the fluorescence mode of the endoscopic fluorescence imaging system (see step 5.2). Lift unilateral pelvic infundibulum ligament and uterus to reveal the lateral peritoneum and look for the fluorescence lymphatic vessels.

NOTE: If there are no fluorescence lymphatic vessels identified, it is allowed to inject ICG repeatedly.

5.2. SLN mapping

5.2.1. Endoscopic fluorescence imaging system preparation (PINPOINT).

5.2.2. Adjust the fluorescence mode to near-infrared laser (excitation light, 805 nm) for the best field of view.

NOTE: The endoscopic fluorescence imaging system has several modes, such as HD (High definition) white light, SPY fluorescence, PINPOINT fluorescence, and SPY CSF mode, and the surgeon can switch to another mode if necessary.

5.3. Identify SLNs under a fluorescence laparoscopy.

5.3.1. Hold an ultrasonic scalpel and toothless forceps with each hand (performed by the surgeon).

NOTE: The assistant grasps toothless forceps to cooperate with the surgeon.

0:03:35 sec -0:03:55 sec 5.3.2. Lift and open the peritoneum, separate the sub-peritoneal fat and connective tissues to reveal the necessary structures, including the ureter, internal iliac vessels, and obturator nerves according to the indication of green fluorescence lymphatic lines, as well as to protect them from injury.

NOTE: An ultrasonic scalpel with activation is used to cut the tissues, while an inactivated scalpel can be used for blunt dissection.

5.3.3. Observe the SLNs covered by the peritoneum about 5–10 min after ICG injection.

NOTE: Typically, SLNs present along with the fluorescence lymphatic vessels from both sides of the cervix to at least the height of the common iliac blood vessel. Occasionally, the SLNs emerge beside the abdominal aorta. PINPOINT equipment has multiple modes, including white light, fluorescence, and black and white modes. Switching between multiple modes helps to determine the location of SLNs.

6. Intraoperative SLN biopsy

0:03:55 sec -0:04:20 sec 6.1. Locate the SLN, the first mapped lymph node along the drainage from parametrium in each hemi-pelvis and use multiple modes to confirm it. Fully expose the SLNs for complete resection (**Figure 3**).

0:04:20 sec -0:05:20 sec 6.2. Grasp and elevate the SLN by toothless forceps and perform a complete resection of the lymph node along the periphery of the lymph node in white light.

NOTE: Avoid lymph node damage, which might cause contamination with tumor cells.

0:11:10 sec -0:11:35 sec 6.3. Place the removed SLNs in the obturator fossa or put them into a small, simple bag. After the uterus is completely removed and taken out through the vagina, bilateral SLNs are taken out entirely through the vagina rather than through the 5 mm trocar opening to avoid fragmentation.

6.4. Send the removed lymph nodes to the pathology department for pathological ultra-staging.

0:02:44 sec -0:03:30 sec 6.5. During the operation, resect the enlarged or suspicious lymph nodes simultaneously and send them for pathological examination.

6.6. Record the locations of SLNs on standardized intraoperative data collection forms.

6.7. If SLN mapping fails on one or both sides, perform side-specific lymphadenectomy according to the NCCN guidelines.

NOTE: If there is no fluorescent node in the hemipelvis, continue to seek the mapped node along the lymphatic vessels until the para-aortic region due to the possibility of mapping in unexpected locations. A failed SLN mapping on either side means that any fluorescent green node is not visible with or without fluorescent green lymphatic vessels under the fluorescence laparoscope system on either side of the pelvis and para-aortic region.

0:05:30 sec -0:11:10 sec 6.8. Perform a total hysterectomy plus bilateral salpingectomy.

NOTE: Since this was a 28-year-old patient with early-stage endometrioid cancer who had a strong desire for fertility preservation and normal-appearing ovarian cancer and had no apparent family history of breast/ovarian cancer or Lynch Syndrome, ovarian preservation could be

considered.

0:14:45 sec -0:14:53 sec 6.9. Indwell a drainage tube for potential residual fluid in the pelvis.

0:14:53 sec -0:14:55 sec 6.10.Remove the laparoscope and release the gas from the abdomen. Close the incisions in the umbilicus and lower abdomen with a single stitch by 2-0/T silk suture.

7. Postoperative SLN ultra-staging[20]

7.1. Cut the SLN along the maximum diameter and micro-sectioned 50 µm apart to get three hematoxylin-eosin (H&E) slides and one immunohistochemistry (IHC) slide with anti-cytokeratin antibody (AE1/AE3).

NOTE: Ultrastaging entails thin serial sectioning of the gross SLN. Cytokeratin IHC is not essential. No standard protocol is for ultra-staging.

7.2. Lymph node metastases were described as macrometastases (>2 mm), micrometastases (0.2–2.0 mm), or isolated tumor cells (ITCs) according to the classification of the American Joint Committee on Cancer (AJCC)[21].

7.3. Report the postoperative pathology with details about the tumor size, histologic type and grade, myometrial invasion, lymphovascular space involvement, cervical stromal involvement, number and status of per lymph nodes, and the cytology of peritoneal washings.

REFERENCES:

20. Barlin, J. N. et al. The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: beyond removal of blue nodes. *Gynecologic Oncology*. **125** (3), 531–535 (2012).
21. Amin, M. B. et al. in *AJCC Cancer Staging Manual, 8th edition.* Springer (2017).