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A practical guide to production and PET/CT imaging of 68Ga-DOTATATE for neuroendocrine tumors in daily clinical practice

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TITLE:

A Practical Guide for the Production and PET/CT Imaging of ^{68}Ga-DOTATATE for Neuroendocrine Tumors in Daily Clinical Practice

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KEYWORDS:

Medicine, imaging, neuroendocrine tumor (NET), positron emission tomography (PET), computed tomography (CT), labeling, gallium-68 (^{68}Ga), DOTATATE

SUMMARY:

Well-differentiated neuroendocrine tumors overexpress somatostatin receptors which can be utilized for diagnostic imaging with the radiolabeled somatostatin analog ^{68}Ga-DOTATATE. This protocol details the radiolabeling of ^{68}Ga-DOTATATE, quality control, patient preparation, and subsequent PET/CT imaging. Radiation safety and time constrictions due to the short half-life of ^{68}Ga are taken into account.

ABSTRACT:

Neuroendocrine tumors are a rare form of cancer that arise from neuroendocrine cells and can be present at almost any location throughout the body. Although heterogeneous in presentation, a common denominator among these tumors is the overexpression of somatostatin receptors. ^{68}Ga-DOTATATE is a somatostatin analog labeled with the positron emitter gallium-68 (^{68}Ga). For well-differentiated neuroendocrine tumors, ^{68}Ga-DOTATATE positron emission tomography (PET)/computed tomography (CT) imaging is used for diagnosis, determination of disease burden, and therapy selection.

This protocol details the radiolabeling of ^{68}Ga-DOTATATE, quality control, patient preparation, and subsequent PET/CT imaging. Radiolabeling of ^{68}Ga-DOTATATE is performed with a fully automated labeling module coupled to a germanium-68 (^{68}Ge)/^{68}Ga generator. Quality control of the final product evaluates radiochemical purity with instant thin-layer chromatography and

solid-phase chromatography, and pH prior to patient injection. Periodic quality control is performed to determine ^{68}Ge breakthrough, sterility, and (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) content. Patient preparation includes patient instructions, a protocol for ^{68}Ga-DOTATATE during treatment with somatostatin analogs, and intravenous administration of the radiopharmaceutical. For PET/CT imaging, the acquisition and reconstruction settings are described. For each step, radiation safety will be highlighted, as well as time constrictions due to the short half-life of ^{68}Ga.

Fully automated in-house production and quality control of ^{68}Ga-DOTATATE leads to very high success rates (95%) and produces two to four patient dosages per batch, depending on the yield of the generator. In conclusion, ^{68}Ga-DOTATATE PET/CT imaging is a noninvasive and fast method of providing information on the tumor burden of neuroendocrine tumors (NETs) while also assisting in diagnosis and therapy selection.

INTRODUCTION:

NETs are a heterogeneous group of tumors that arises from neuroendocrine cells. They can occur at almost any location in the body but are most common in the gastrointestinal tract, pancreas, and lung[1]. Although NETs are a rare disease, their incidence in the United States has risen from 1.09 per 100,000 people in 1973 to 6.98 per 100,000 people in 2012[2]. For an accurate diagnosis and staging of a NET, ^{68}Ga-DOTATATE PET/CT is the standard of care. This protocol describes the production and quality control of ^{68}Ga-DOTATATE, as well as patient preparation and the acquisition of PET/CT images.

Well-differentiated NETs are characterized by an overexpression of somatostatin receptors[1]. Somatostatin analogs that bind to this receptor can be labeled with a radioactive isotope to allow for nuclear medicine imaging. At first, iodine-123 was used with gamma camera imaging, which was soon replaced by indium-111 (^{111}In)[3–4]. ^{111}In-octreotide scintigraphy was the golden standard for nuclear medicine NET imaging for over a decade[5]. Meanwhile, technical advances were made in PET, which has a higher sensitivity and resolution than gamma camera imaging. For NETs, somatostatin analogs coupled to the positron emitter ^{68}Ga, such as ^{68}Ga-DOTATATE, were developed[6].

^{68}Ga-somatostatin receptor (^{68}Ga-SRS) PET/CT is the current modality of choice in nuclear medicine imaging of well-differentiated NETS. The superiority of ^{68}Ga-SRS PET/CT over ^{111}In-octreotide has been demonstrated in several studies[7–8]. The reported sensitivity and specificity lie around 90%–95% and 85%–100%, respectively[9–10]. A meta-analysis has shown that ^{68}Ga-SRS PET/CT leads to a change in management in 44% of cases, even if preceded by ^{111}In-octreotide scintigraphy[11]. In guidelines, ^{68}Ga-SRS PET/CT is now recommended over ^{111}In-octreotide scintigraphy for NET imaging, and it is also approved by the Food and Drug Administration and European Medicines Agency[12]. A guideline for tumor imaging with ^{68}Ga-conjugated peptides is also available[13].

This protocol details the radiolabeling of ^{68}Ga-DOTATATE (conform the quality control requirements of the European Pharmacopoeia[14]), patient preparation, and subsequent PET/CT

imaging. Radiation safety and time constrictions due to the short half-life of ^{68}Ga are taken into account.

PROTOCOL:

1. General radiation and radiopharmaceutical safety

1.1. Ensure that radioactive materials are only worked with and handled by trained personnel. The dose to hospital staff, patients, and everyone else present should always be kept as low as reasonably achievable (ALARA).

1.2. Regarding the preparation of radiopharmaceuticals, adhere to national laws, regulations, and guidelines, such as Good Manufacturing Practice (GMP).

CAUTION: The following protocol is for the ^{68}Ga-DOTATATE PET/CT imaging of adults only and is not suitable for children or pregnant women.

2. Preparations required prior to the labeling of ^{68}Ga-DOTATATE

2.1. Elute the ^{68}Ge/^{68}Ga generator with hydrochloric acid (HCl) according to the manufacturer's specifications, between 4 and 24 h prior to the start of labeling ^{68}Ga-DOTATATE.

3. Labeling of ^{68}Ga-DOTATATE

NOTE: The preparation for and labeling of ^{68}Ga-DOTATATE takes 90 min and should be started 2 h prior to patient administration, to allow for quality control. The labeling module should be placed in a lead shielding that can be closed during the labeling process to ensure radiation protection of personnel. If a registered kit is used, then the summary of the product characteristics (SMPC) must be followed or cross-validated locally with the presented protocol.

3.1. Place the ^{68}Ga labeling kit on the labeling module according to the manufacturer's specifications. Place the three manifolds on the corresponding module units. Attach the solutions provided in the ^{68}Ga labeling kit to the manifolds.

3.2. Prepare the final vial in a sterile environment, such as a downflow unit or laminar flow cabinet.

3.3. Place a nonvented 0.22 µm filter underneath a vented 0.22 µm filter and attach the nonvented filter to a sterile needle (20 G). Place the needle with the two filters attached in a 30 mL sterile vial. Place a vented 0.2 µm bent filter with a needle (22 G) in the same sterile vial as per step 3.2 to allow venting.

3.4. Attach the sterile vial with the nonvented filter to the output of the labeling module and place the vial in the lead shielding sufficient for positron emitters.

3.5. Attach the output of the ^{68}Ga/^{68}Ge generator to the input of the labeling module.

3.6. Dissolve 50 µg of HA-DOTATATE (DOTA-3-iodo-Tyr3-octreotate) or 20 µg of DOTATATE (DOTA-0-Tyr3-octreotate) peptide in 1.5 mL of 1.5 M HEPES buffer solution provided in the kit and place it in the reaction vial.

3.7. Close the lead shielding around the labeling module and start the production of ^{68}Ga-DOTATATE via the tablet computer attached to the labeling module.

3.8. Wait until the synthesis of ^{68}Ga-DOTATATE is finished (~36 min).

3.9. After labeling, remove the needles with filters from the glass vial and close the lead shielding around the vial.

3.10. Test the integrity of the nonvented 0.22 µm filter as follows.

3.10.1. Fill a syringe (10 mL) with air and place the syringe on top of the filter. Place the needle attached to the filter in a tube filled with water.

3.10.2. Force the air through the filter and needle and determine when bubbles begin to form. The air should be compressed to <20% of the original volume.

3.11. Measure the activity of ^{68}Ga-DOTATATE produced by placing the vial in a dose calibrator and note the activity reference time (ART).

3.12. In a sterile environment such as a laminar flow cabinet, remove 0.5 mL of ^{68}Ga-DOTATATE from the vial for quality control and prepare syringes for patient administration.

NOTE: Place the solution with ^{68}Ga-DOTATATE in lead shielding. In this protocol, the diluted solution does not have to be shielded due to the low levels of activity and short exposure time. However, a radiation risk assessment should be performed prior to performing quality control of the ^{68}Ga-DOTATATE.

4. Quality control of ^{68}Ga-DOTATATE prior to patient administration

NOTE: The quality control of ^{68}Ga-DOTATATE takes 30 min and should be started 30 min prior to patient administration. The described dilutions for stock solutions lead to <5% dead time in the measurement equipment. This can vary between different equipment and should be tested prior to performing quality control of the ^{68}Ga-DOTATATE. The European Pharmacopoeia describes the quality control of gallium edotreotide injections based on the following release criteria: appearance = clear and colorless; pH = 4.0–8.0; sterility; endotoxins <175 IU per administered volume; ethanol <10% v/v; radionuclide purity >99.9% of total activity; radiochemical purity >91% of total activity; absence of other impurities; HEPES <200 µg per administered volume[14].

All tests have been evaluated during the validation of the preparation method. For routine quality control, a selected subset of tests (based on trend monitoring) is performed and described below. The solid-phase extraction in this protocol has been cross-validated with and obtains the same results as the high-performance liquid chromatography method described in the European Pharmacopoeia. This was performed based on GMP.

4.1. Prepare the following solutions in advance.

4.1.1. Prepare a 1 M ammonium acetate solution by dissolving 3.9 g of ammonium acetate in 50 mL of water. The solution can be stored at room temperature for up to 2 weeks.

4.1.2. Prepare 5 mM ethylenediaminetetraacetic acid (EDTA) by dissolving 0.1 g of EDTA in 50 mL of water. The solution can be stored at room temperature for up to 1 year.

4.1.3. Prepare 50:50 methanol:1 M ammonium acetate. The solution can be stored at room temperature for up to 24 h.

4.2. Visually inspect the final ^{68}Ga-DOTATATE product to ensure that it is a colorless liquid without any particles.

4.3. Measure the pH of the ^{68}Ga-DOTATATE solution with a pH indicator strip. The pH should lie between 6.5 and 7.5.

4.4. Measure ^{68}Ga colloids through instant thin-layer chromatography (ITLC).

4.4.1. Add 500 µL of water and 20 µL of ^{68}Ga-DOTATATE to prepare a stock solution and homogenize (^{68}Ga colloids [GC]) by carefully shaking the vial.

4.4.2. Cut a strip of ITLC- silica gel (SG) glass fiber paper of at least 7 cm long and 1 cm wide.

NOTE: Only use clean ITLC-SG paper without damages. If the paper is damaged, the components traveling with the solvent can be hindered and the results will be inaccurate.

4.4.3. Add 5 µL of GC 1.5 cm from the bottom of the ITLC-SG paper and place it in a tube containing 2 mL of 50:50 methanol:1 M ammonium acetate. Ensure that the ^{68}Ga-DOTATATE does not come into contact with the liquid. Close the tubes to prevent evaporation.

4.4.4. Wait several minutes until the solvent has traveled at least 5 cm above where the ^{68}Ga-DOTATATE was applied. Cut the paper in half and place the bottom and upper halves in separate tubes (BH1 and UH1).

4.4.5. Place the UH1 and BH1 tubes in a well counter and determine the number of counts in each vial in the 400–600 keV energy window for 30 s, to determine the colloid percentage (see the calculations in steps 4.4.7–4.4.9).

4.4.6. Repeat steps 4.4.2–4.4.5 to acquire BH2 and UH2..

4.4.7. Perform a background measurement of an empty well counter and determine the number of counts in the 400–600 keV energy window for 30 s.

4.4.8. Correct the counts in the UH1, UH2, BH1 and BH2 for decay and background (determined in step 4.4.7) to obtain UH1cor, UH2cor, BH1cor, BH2cor. Δt is the time difference between the measurement of the sample and the ART (determined in step 3.11) in minutes.

$$UH1 - counts\ background = UH1cor \times 0.5^{\left(\frac{\Delta t}{68}\right)}$$

4.4.9. Calculate the number of ^{68}Ga colloids with the following formula; note that this should be less than 3%. UH1cor, UH2cor, BH1cor, BH2cor are the corrected values obtained in step 4.4.8.

$$Colloids = \frac{\left(\frac{UH1cor}{UH1cor + BH1cor}\right) + \left(\frac{UH2cor}{UH2cor + BH2cor}\right)}{2} \times 100\%$$

4.5. Determine the ^{68}Ga ions through column separation.

4.5.1. Make a stock solution by diluting 20 µL of ^{68}Ga-DOTATATE in 1 mL of 5 mM EDTA.

4.5.2. Prepare a C-18 cartridge by slowly flushing it with 1 mL of 100% ethanol, followed by 1 mL of sterile water.

4.5.3. Prepare a sample (S) by diluting 10 µL of the stock solution in 1 mL of sterile water, placing it in a well counter, and determining the number of counts in the 400–600 keV energy window for 30 s.

4.5.4. Flush the sample slowly (5–10 mL/min) through the C-18 cartridge with a syringe and collect the remaining solution (C). Rinse the sample tube with 1 mL of water and flush this through the column in the collection tube.

4.5.5. Place the collecting tube, the empty sample tube (E), and the syringe (Sy) in a well counter and determine the number of counts in each of them in the 400–600 keV energy window for 30 s. Use this to estimate the ion percentage (see the calculations in steps 4.5.7 and 4.5.8).

4.5.6. Repeat steps 4.5.2–4.5.5.

4.5.7. Correct the counts for decay and background (determined in step 4.4.7) in C, S, E, and Sy to determine C', S', E' and Sy', to determine the number of counts at the ART with the following formula, in which Δt is the time difference between the measured sample and the ART in minutes.

$$Counts\ measured - counts\ background = counts\ at\ ART \times 0.5^{\left(\frac{\Delta t}{68}\right)}$$

4.5.8. Calculate the ^{68}Ga ion percentage with the following formula; note that this should be less than 2%.

$$Ions = \frac{\left(\frac{C'1}{S'1 - E'1 - Sy'1}\right) + \left(\frac{C'2}{S'2 - E'2 - Sy'2}\right)}{2} \times 100\%$$

4.6. Determine the radiopharmaceutical purity by calculating the total amount of ^{68}Ga-DOTATATE with the following formula, which should be at least 91%.

$$Purity = (100 - colloids) \times \left(\frac{100 - ions}{100}\right)$$

5. Periodical quality control of ^{68}Ga-DOTATATE after patient administration

NOTE: This should be performed >48 h after the preparation of ^{68}Ga-DOTATATE to allow for the decay of ^{68}Ga.

5.1. Prepare the following solutions in advance.

5.1.1. Prepare HEPES reference solution by dissolving 20 mg of HEPES in 50 mL of sterile water. The solution can be stored at room temperature for up to 6 months.

NOTE: This is based on the maximum recommended HEPES dose of 200 µg per administered volume.

5.1.2. Prepare 25:75 v/v water:acetonitrile.

5.2. Determine HEPES concentration in the final product (weekly).

5.2.1. Transfer 3 µL of ^{68}Ga-DOTATATE solution in steps of 1 µL to an ITLC-SG F_{254} paper of at least 8 cm length. Use a blow dryer to dry the paper in between the 1 µL applications.

5.2.2. Repeat step 5.2.1 with the HEPES reference solution.

5.2.3. Place the strips in a solvent of 25:75 water:acetonitrile. Ensure that the applied solutions do not come into contact with the liquid.

5.2.4. Wait several minutes until the solvent has traveled to at least 2/3 of the paper length.

5.2.5. Develop the paper for at least 4 min in a closed chamber with iodine crystals.

5.2.6. Visually assess the outcome; a yellow spot should appear. The spot on the paper with ^{68}Ga-DOTATATE should be less intense than that of the reference solution.

5.3. Determine ^{68}Ge breakthrough in the final product (monthly).

5.3.1. Prepare a sample with 200 µL of ^{68}Ga-DOTATATE solution (G). Place it in a well counter and determine the number of counts in each in the 400–600 keV energy window for 30 s.

5.3.2. Repeat step 5.3.1.

5.3.3. Correct the counts in G to determine G' for decay, to determine the number of counts at the ART with the following formula, in which Δt is the time difference between the measured sample and the ART in minutes.

$$Counts\ measured = counts\ at\ ART \times 0.5^{\left(\frac{\Delta t}{68}\right)}$$

5.3.4. Calculate the ^{68}Ge breakthrough (MBq/MBq), which should be less than 0.001%, with the following formula.

$$Germanium\ breakthrough = \frac{G'1 + G'2}{S'1 + S'2} \times 100\%$$

5.4. Determine the sterility of the final product (monthly).

5.4.1. Add the remaining ^{68}Ga-DOTATATE solution to a tryptic soy broth (TSB) medium. Incubate for 14 d at 30–35 °C.

5.4.2. Check that the TSB medium is a clear liquid after the incubation period.

6. Patient preparation and administration of ^{68}Ga-DOTATATE

NOTE: The injected activity in this protocol provides good quality images with the PET/CT system available and the imaging protocol as described in section 7. With other imaging systems and protocols, the injected activity should be optimized.

6.1. In advance, send the appointment and patient folder with information about ^{68}Ga-DOTATATE PET/CT by mail to each patient. Confirm every appointment by telephone 1 day in advance.

6.2. Food and drinks are not restricted prior to ^{68}Ga-DOTATATE PET/CT imaging. Advise patients to drink an extra 1 L of water in the 2 h prior to imaging. It is also recommended patients do not bring children or pregnant women with them to the nuclear medicine department.

6.3. On the day of the ^{68}Ga-DOTATATE PET/CT, have the patients check in at the department of nuclear medicine 60 min prior to the imaging. Take a short medical history.

6.4. Inquire about the date of the last somatostatin analog administration. This is not a contraindication for ^{68}Ga-DOTATATE PET/CT but should be noted.

6.5. Place an intravenous cannula in the arm and flush it with saline to verify the placement of the cannula.

6.6. Inject 100 MBq of ^{68}Ga-DOTATATE 45 min prior to the PET/CT imaging.

7. PET/CT imaging

7.1. Place patient with the arms above their head on the PET/CT scanner. Instruct the patient to remain still throughout the exam.

7.2. Acquire a survey image and select the scan area from the pituitary gland to the mid-thigh, unless otherwise specified due to clinical indications.

7.3. Perform a low-dose CT scan with 40 mAs, 140 keV, and 5 mm slices for attenuation correction and anatomical correlation.

7.4. Perform a PET scan with 150 s per bed position, starting at the head of the patient.

7.5. Reconstruct the CT images with filtered back projection and 5 mm slices.

7.6. Reconstruct the PET images with BLOB-OS-TF, with three iterations and 33 subsets with a voxel size of 4 mm x 4 mm x 4 mm.

7.7. Send all images to the local picture archiving and communication system (PACS).

REPRESENTATIVE RESULTS:

Making use of an automated labeling system, 357 batches of ^{68}Ga-DOTATATE were produced between December 2014 and October 2018. Of the 357 produced, 17 batches failed and 340 batches were released, leading to an overall success rate of 95.2%. Of the failed batches, 11 were caused by a technical failure, whilst in six cases, the produced ^{68}Ga-DOTATATE did not meet specifications. **Figure 1** shows a flow chart of produced batches and the number of patient dosages produced. The average amount of ^{68}Ga-DOTATATE produced was 610 ± 180 MBq (expressed as mean ± standard deviation). ^{68}Ga ions are on average 0.6% ± 0.57% and ^{68}Ga colloids are on average 1.37% ± 0.69% of the produced product. The radiopharmaceutical purity was on average 98.02% ± 1.05%.

Figure 2 shows a ^{68}Ga-DOTATATE PET/CT scan without evidence of disease. The physiological uptake can be seen in the liver and spleen. ^{68}Ga-DOTATATE is excreted by the kidneys and is

therefore visible in the urinary tract. **Figure 3** shows a patient with a primary tumor in the pancreas.

In spite of careful preparations, not all acquired PET images were of optimal quality, of which two examples are given. **Figure 4A** shows an example of a patient with a lower dose of ^{68}Ga-DOTATATE due to a delay in the production of ^{68}Ga-DOTATATE, which led to less activity being present in the patient. This led to more noisy images. **Figure 4B** shows an image with a motion artifact.

FIGURE AND TABLE LEGENDS:

Figure 1: Flow chart of produced, failed, and released batches.

Figure 2: Maximum intensity projection of representative ^{68}Ga-DOTATATE of a patient with no evidence of disease. High physiological uptake of ^{68}Ga-DOTATATE is seen in the liver (yellow delineation), spleen (dark blue delineation), and adrenal gland (green delineation). Uptake in the kidneys (red delineation) is due to both physiological uptake and excretion, while the uptake in the bladder (light blue) is due to excretion only. Moderate to low physiological uptake of ^{68}Ga-DOTATATE is seen in the pituitary gland (red arrow), the thyroid gland (blue arrow), and the salivary glands (green arrow).

Figure 3: ^{68}Ga-DOTATATE PET/CT of a patient with a primary pancreatic neuroendocrine tumor. (**A**) Fused axial PET/CT image visualizing the primary pancreatic NET (green arrow). (**B**) Axial PET image visualizing the primary pancreatic NET (red arrow). (**C**) Coronal maximum intensity projection of the PET visualizing the primary pancreatic NET (red arrow).

Figure 4: Examples of suboptimal ^{68}Ga-DOTATATE PET images. (**A**) Coronal maximum intensity projection of a ^{68}Ga-DOTATATE PET in a patient who received only 42 MBq of ^{68}Ga-DOTATATE. More noise can be seen in the image, especially in the liver (red arrow). Liver metastasis is still visible (green arrow). (**B**) Coronal maximum intensity projection of a ^{68}Ga-DOTATATE PET with a motion artifact. Due to movement of the head between the PET and CT acquisitions, the reconstruction of the PET images leads to this artifact.

DISCUSSION:

This protocol describes the production and subsequent PET/CT imaging of ^{68}Ga-DOTATATE. In order for the efficient use of each produced batch of ^{68}Ga-DOTATATE, an optimal workflow with strict timing is required. Since the half-life of ^{68}Ga is 68 min, a relatively small time delay of 15 min leads to a 15% loss of radioactivity. This requires active communication between the production facility, the personnel administrating the dose to the patient, and the PET/CT technician. Also, patients should be instructed that it is critical to meet the appointment time. Furthermore, the number of patient dosages per batch is dependent on the ^{68}Ge/^{68}Ga generator's size and age and will, therefore, decrease over time. A cost-benefit analysis can be performed to determine when the generator should be replaced.

Although the sensitivity and specificity of ^{68}Ga-DOTATATE for the detection of neuroendocrine tumors are high, several limitations should be considered. First, when a NET dedifferentiates and becomes more aggressive (grade 3 NET or neuroendocrine carcinoma), somatostatin receptor expression is often lost. Tumor lesions will therefore not be detected with ^{68}Ga-DOTATATE PET/CT. In these cases, ^{18}F-FDG PET/CT, which visualizes glucose metabolism, is indicated. Second, ^{68}Ga-DOTATATE shows physiological uptake in the liver, which is also the organ in which metastases of NETs are the most common. Liver uptake is peptide dependent, but the differences between peptides are small and not clinically relevant[15–16]. The visualization of smaller liver lesions with a moderate somatostatin receptor expression will not be possible in all cases. When a clinical suspicion of liver lesions with negative findings on ^{68}Ga-DOTATATE does exist, dedicated CT or MR imaging of the liver is recommended. Third, ^{68}Ga-DOTATATE imaging is limited by the resolution of the PET system, which lies around 5 mm. Lesions smaller than 5 mm will only be detected if there is a high uptake of ^{68}Ga-DOTATATE.

The use of long-acting somatostatin analogs prior to ^{68}Ga-SRS imaging has been controversial. The current guideline recommends the discontinuation of long-acting somatostatin analogs 4–6 weeks prior to imaging because of concerns of reduced uptake in tumor lesions[13]. However, a recent prospective intrapatient comparison demonstrated that the long-acting somatostatin analog lanreotide did not reduce the tumor uptake of ^{68}Ga-DOTATATE but led to a slight increase in tumor-to-background ratios[17]. Serial ^{68}Ga-SRS PET/CT imaging performed under the same conditions, either with or without long-acting somatostatin analogs, will produce the most stable results.

^{68}Ga-somatostatin receptor imaging as described in this paper is performed with ^{68}Ga-DOTATATE, but other peptides, such as ^{68}Ga-DOTATOC and ^{68}Ga-DOTANOC, are also suitable. The three peptides show small differences in their affinity for the five different subtypes of the somatostatin receptor, but all have high specificity and sensitivity for NETs. The choice of peptide should be made according to regulatory approval, cost, and availability.

In conclusion, ^{68}Ga-DOTATATE PET/CT imaging of neuroendocrine tumors has become standard of care. This protocol describes the production, quality control, and PET/CT imaging of ^{68}Ga-DOTATATE.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

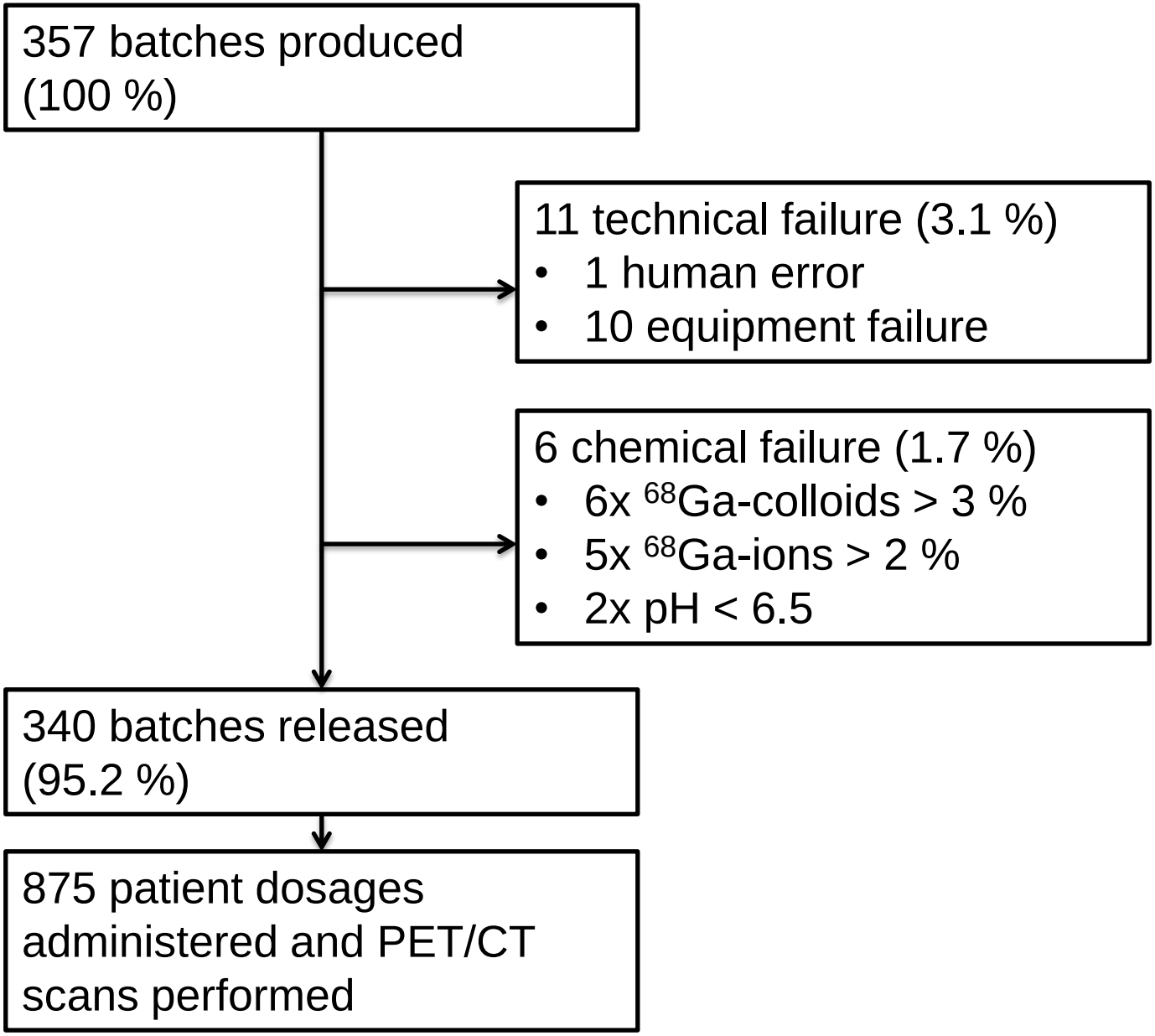
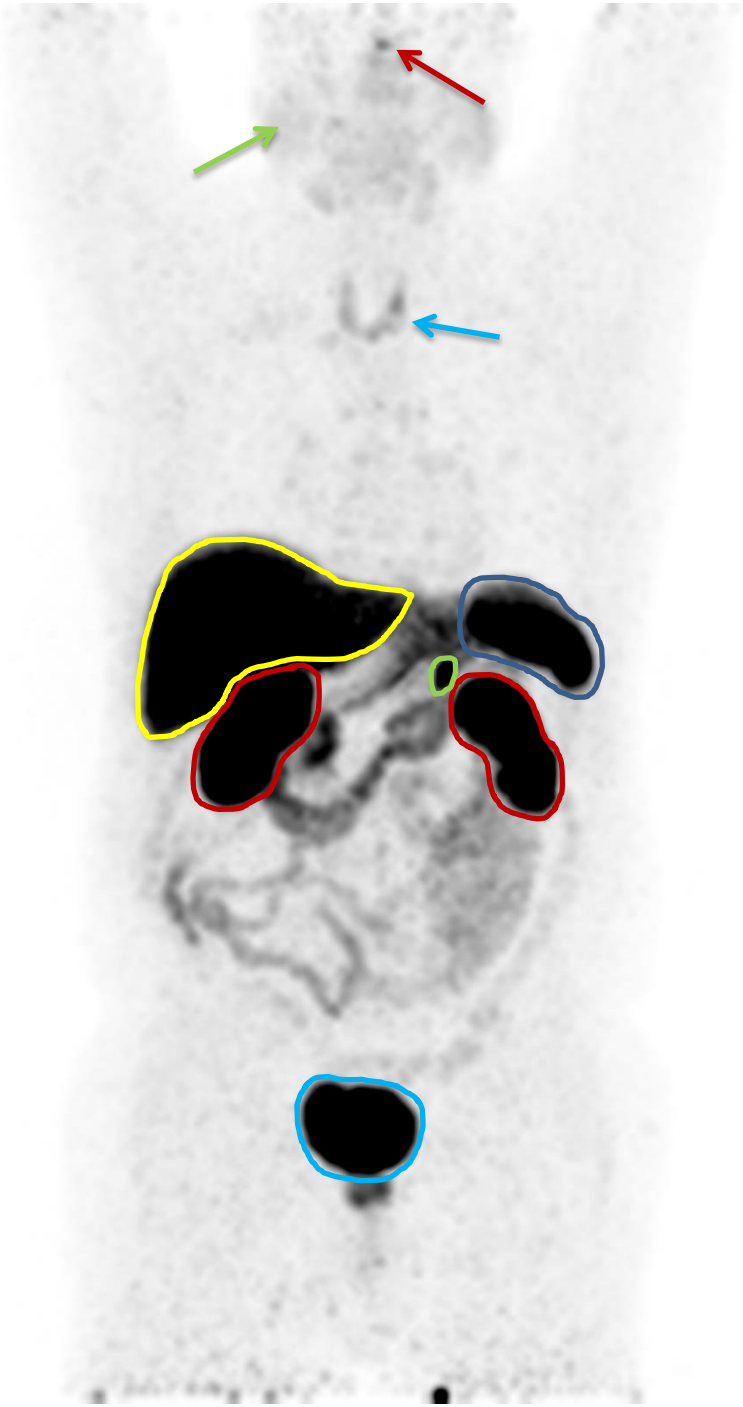


Figure 2



Arrows

Red: Pituitary gland

Blue: Thyroid gland

Green: Salivary glands

Delineations

Red: Kidneys

Dark blue: Spleen

Green: Adrenal gland

Yellow: Liver

Light Blue: Bladder

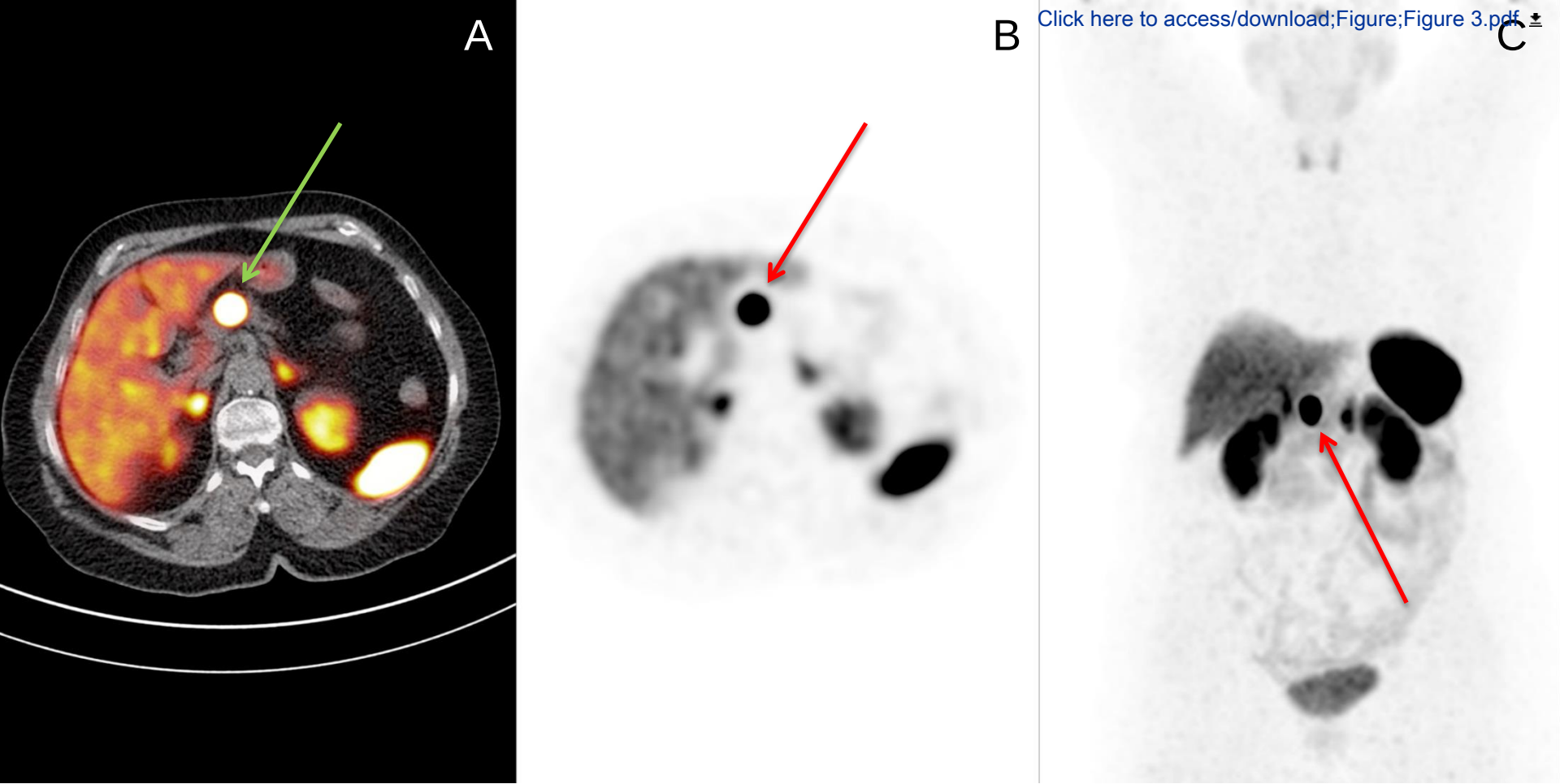
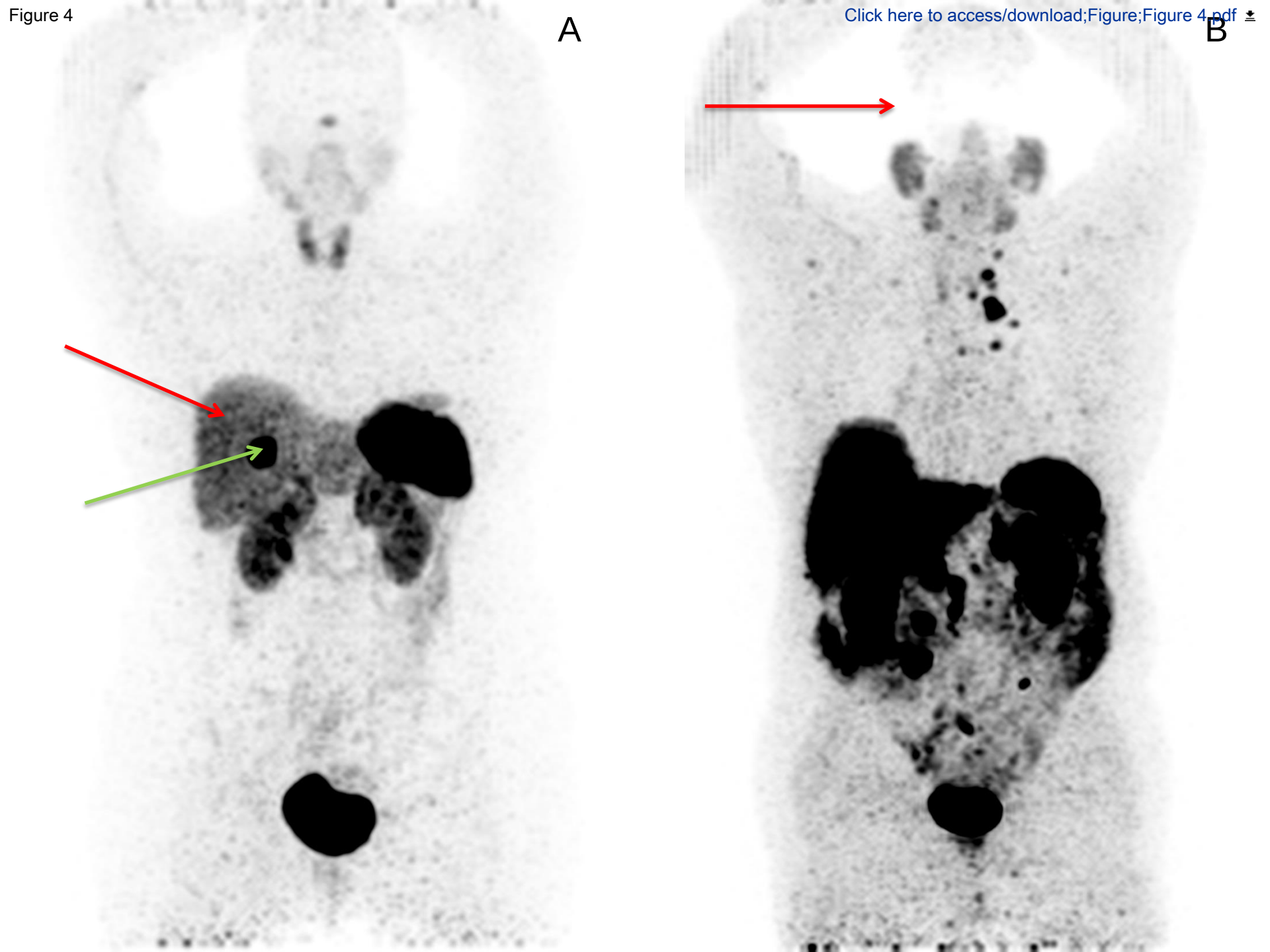


Figure 4

Click here to access/download;Figure;Figure 4.pdf



Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Acetonitrile	Biosolve	012007	> 99.9 %
Ammonium acetate	Merck	101116	≥ 98 %
Aqua / Water for injections	Braun		
Automated labeling system	Scintomics		GRP 3V
C-18 cartridge	Waters	WAT023501	Sep-Pak C18 Plus Light
Dose calibrator	Veenstra Instruments		VIK-202-5051
EDTA	Merck	324503	
Ethanol	Sigma Aldrich	32221-M	≥ 99.8 %
Ga-68 labeling kit	ABX	SC-01	
Ge-68/Ga-68 generator	Eckert & Ziegler		1850 MBq
HA-DOTATATE	Scintomics	GRPC/R-000095	
HCl 0.1M for elution	ABX	HCl-03	
HEPES	Sigma Aldrich	H3375	≥ 99.5 %
Iodine	Sigma Aldrich	207772	≥ 99.8 %, solid
ITLC-SG F254 plates	Merck	105735	TLC Silica gel 60 F254
ITLC-SG paper	Agilent	SGI0001	Glass fiber
Methanol	Sigma Aldrich	32213-M	≥ 99.8 %, Ph. Eur.
Non-vented filter	Merck	SLMPL25SS	Millex-MP filter 0.22 µm
PET/CT	Philips		Gemini TOF
pH indicator strips	Merck	109584	MColorpHast (pH2.0-9.0)
Tryptic soy broth medium	Biotrading	K111F010QK	
Vented filter	Merck	SLGV0250S	Cathivex GV 0.22 µm
Well counter	Canberra (now Mirion)		Osprey Digital Tube Base MCA Detector 76 BP76/3M-X

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Author(s): EA Aalbersberg, MM Geluk, C Young, LJ de Wit, MPM Stokkel

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CORRESPONDING AUTHOR:

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Department: Nuclear Medicine

Institution: Netherlands Cancer Institute

Article Title: A practical guide to production and PET/CT imaging of ^{68}Ga-DOTATATE

Signature: Aalbersberg Date: 11-2-2018

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Dear Editor and Reviewer,

Thank you for giving us the opportunity to revise our manuscript entitled “A practical guide to production and PET/CT imaging of 68Ga-DOTATATE for neuroendocrine tumors in daily clinical practice”. We are also grateful for the positive feedback and comments. The complete list of reviewer- and editorial comments and the suggested changes and/or rebuttals can be found below. We hope that this answers all the questions.

Kind regards,
On behalf of all the authors,
Else Aalbersberg

Reviewer #1:

1. Authors must comprise on the manuscript/references the current EANMMI 2010 procedure guidelines for tumor imaging with 68Ga-DOTA-conjugated peptides (Virgolini I et al, EJNMMI 2010;37:2004-2010).

The guideline on 68Ga peptide imaging has been added to the introduction and references.

2. Replace "somatostatin receptor" by "somatostatin receptors", "expression of somatostatin receptors" by "overexpression of somatostatin receptors",

This has been adjusted where appropriate in the short abstract, long abstract, and introduction.

3. Indicate that imaging with Ga-DOTATATE is addressed to well-differentiated NETs

It has been clarified in the abstract and introduction that Ga-DOTATATE imaging is for well-differentiated NETs only.

In the discussion this has been explained further: “when NET dedifferentiates and becomes more aggressive (grade 3 NET or neuroendocrine carcinoma) somatostatin receptor expression is often lost. Tumor lesions will therefore not be detected with ^{68}Ga-DOTATATE PET/CT. In these cases ^{18}F-FDG PET/CT, which visualizes glucose metabolism, is indicated.”

4. Whether the patient should stop long-acting somatostatin analogues 4 weeks prior to imaging with Ga-DOTATATE PET (or short-acting somatostatin analogues 48 hours prior to imaging) remains controversial. Authors must mention what is stated on the current guidelines, so that the choice can be made by centers performing the examen. However serial exams must be performed under the same circumstances.

The following text has been added to the discussion: “The use of long-acting somatostatin analogues prior to ^{68}Ga-SRS imaging has been controversial. The current guideline recommends discontinuation of long-acting somatostatin analogues 4-6 weeks prior to imaging for concerns of reduced uptake in tumor lesions.[13] However, a recent prospective intra-patient comparison demonstrated that the long-acting somatostatin analogue Lanreotide did not reduce tumor uptake of ^{68}Ga-DOTATATE but led to a slight increase in tumor-to-background ratios.[14] Serial ^{68}Ga-SRS PET/CT imaging performed under the same conditions, either with or without long-acting somatostatin analogues, will produce the most stable results.”

5. Authors must specify technical characteristics of any material and machine through the manuscript rather than a table at the end of the manuscript.

JoVE requires all manufacturer specific details to be removed from the manuscript and placed in the table of materials. Therefore this has not been changed.

6. Page 9, line 378: replace "adrenal" by "pituitary" gland, as focus of low to moderate physiological uptake of Ga-DOTATATE (regarding Figure 2).

This was indeed incorrect and has been corrected to identify the pituitary gland.

Reviewer #2:

1. Please refer to Ph. Eur . 9: Gallium edotreotide Injection and explain release criteria are based on monograph.

68Ga ions identification is decribed by SPE. In monograph is described by liquid chromatography. As stated by guide for the elaboration of monographs on radiopharmaceutical preparation (EDQM 2018, 2.3.5.) methods should be validated, please show that the used of SPE is validated and can be used for determination of 68Ga ions.

Radiopharmaceutical purity should be >91% and is based on all measured 68Ga impurities. Formula (4.6.1.) is only considering colloids and not non labeled 68Ga ions. Additionally no proves are given that there is no radiolysis (HPLC analyses) which should be taken into account in this formula as well . Please discuss on this item.

A note has been added to the text to explain the monograph:

"Note: The European Pharmacopoeia describes quality control of gallium edotreotide injection based on the following release criteria: appearance clear and colorless, pH 4.0-8.0, sterility, endotoxins < 175 IU per administered volume, ethanol < 10% v/v, radionuclide purity > 99.9% of total activity, radiochemical purity > 91% of total activity, absence of other impurities, HEPES < 200 µg per administered volume. All tests have been evaluated during validation of the preparation method. For routine quality control a selected subset of tests (based on trend monitoring) is performed and described below. The solid phase extraction in this protocol has been cross-validated with- and obtains the same results as the high performance liquid chromatography method described in the European Pharmacopoeia. This was performed based on GMP (good manufacturing practice)."

The full cross-validation of this protocol is beyond the scope of this manuscript, and therefore, these results are not shown. However, the text now explains that the described

method has been fully validated.

2. Page 3: 3.5. mentioned that HA-DOTA-TATE was used. Since SomKit is registered in Europa and NetSpot in US, no HA-DOTA-TATE can be use unless there is a specific medical need. Please mention whether this protocol can be used to label these kits.

The protocol has been validated for both HA-DOTATATE and DOTATATE. In step 3.5 this both peptides are now mentioned.

If a kit is used, then the summary of product characteristics (SMPC) must be followed or cross-validated locally with the presented protocol. This has been added as a note to the manuscript.

3. Please follow nomenclature rules as been published by Coennen et al.

The nomenclature rules have been followed. However, DOTATATE is commonly used as such in literature, and therefore referred to as DOTATATE.

4. Please check: ml, ul, µl, l throughout the text -> should be mL, µL, L

This has been checked and changed throughout the manuscript.

5. 4.1.1-3: were solution prepared under aseptic conditions with sterile excipience? Where are storage conditions based on?

These solutions do not have to be prepared under aseptic conditions with sterile excipients. The solutions used or not administered to patients, and therefore sterility is not required. Storage conditions are based on general storage conditions for laboratory solutions and based on the conditions in the GLP certified laboratory at our institution.

6. 4.3.1: pH must be between 6 .6-7.5. in Ph Eur this is between 4.0 and 8.0 why this small range?

The labeling kit used in this protocol utilizes a buffer that ensures a pH of 7. If the pH is outside the range of 6.5-7.5, this indicates an error in the production process. Trend monitoring indicates that if the pH is outside the specified range, other quality control variables (^{68}Ga colloids and ions) have a high risk of also lying outside of specifications.

7. 4.4.7 and 8, 4.5.8. please take into account the background

The performance of a background measurement has been added to the protocol and the formulas now include a background correction.

8. 4.5.4. please indicate flowate (mL/min)

The flow rate (5-10 mL/min) has been added to the text.

9. 5.1.1.: please mention that here described HEPES solution is based on maximum recommended dose in mL

This has been added to the text.

10. 5.3. please make clear that there is a difference in 68Ge breakthrough between eluate for generator and final product.

It has been clarified that the quality control described in 5.3 is for the final product.

11. 5.3.4. please add required units of breakthrough % (MBq/MBq)

The units have been added to the text.

12. 5.4: Sterility: Ph Eur describes endotocine release test , please add mus be less than 175/V IU/mL

This has been added to the text.

13. 6.2: "to wear…. Department. Please consider if this important to add in this protocol?

The sentence “to wear comfortable clothes without metal components, and to leave any jewelry at home” has been removed.

14. 6.6. is 100 MBq proven efficient? No patient mass dependency?

100 MBq provides sufficient image quality at our department with the available PET/CT camera and the image protocol (time per bed position) as described. This is also within range of the recommended dose in the guideline (Virgolini, et al.), which is 100-200 MBq depending on local equipment and protocols. This has been added in a note: “Note: The injected activity in this protocol provides good quality images with the PET/CT system available and the imaging protocol as described in part 7. With other imaging systems and protocols, the injected activity should be optimized.” The dose is indeed not dependent on patient mass.

15. Representative results: please add standard deviation to numbers.

Standard deviations are added to the text.

16. how much delay of injection (also fig 4)

There was no delay of injection, but a delay in the production of ^{68}Ga-DOTATATE which led to less activity being available. This has been clarified in the text.

17. Discussion: Is liver uptake related to HA-DOTA-TATE? please comment on this in discussion.

There is no significant difference in liver uptake between DOTATATE and HA-DOTATATE (Brogsitter et al 2014). However, small but clinically irrelevant differences between different peptides do exist (Sandstrom et al 2013). This has been added to the discussion.

18. Line 417 and 18: Please add references for this statement

Line 417-418 is the conclusion of our manuscript, therefore no reference is needed.

19. Table of equipment: what was the size of the 68Ga generator? MBq?

The ^{68}Ga generator was 1850 MBq. This has been added to the table of materials.

20. Which well counter was used?

The well counter has been added to the table of materials.

Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

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

The manuscript has been proofread and corrected.

2. Please rephrase the Introduction to include a clear statement of the overall goal of this method.

The goal of the protocol has been added to the introduction.

3. Please use the micro symbol µ instead of u. Please abbreviate liters to L to avoid confusion.

The abbreviations have been corrected.

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Commercial language has been removed from the manuscript and placed in the Table of Materials.

5. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

This protocol describes a routine clinical procedure. No approval from the human research ethics committee is required.

6. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

This has been done.

7. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.

7a. 3.1: Please describe the manufacturers specifications. We need specific details for filming.

This has been added.

7b. 3.2.1: Please provide the size of the sterile needle and capacity of the vial.

This has been added.

7c. 3.2.2: Where is the Millex-25 0.2 µm bent filter placed? Also specify the size of the needle.

This has been added.

7d. 3.6: How to start the production? Is a button pushed or is it controlled by a software? How long does this process take?

This has been added.

7e. 3.8.1: Please specify the size of the syringe.

This has been added.

7f. 3.9: Please describe how to measure the activity of produced 68Ga-DOTATATE.

This has been added.

7g. 4.4.1: Please describe how to homogenize the stock solution.

This has been added.

7h. 6.1: What are the inclusion and exclusion criteria for the participating patients?

This is not a study but a clinical protocol. ^{68}Ga-DOTATATE PET is indicated for any patient with a well-differentiated NET as discussed in the introduction and described in the referred guidelines. In the discussion several exceptions are discussed.

8. References: Please do not abbreviate journal titles.

The full journal titles are mentioned.

9. Table of Materials: Please sort the items in alphabetical order according to the name of material/equipment.

This has been done.