Alisha DSouza, Ph.D.

Senior Review Editor

Journal of Visualized Experiments

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Dear Dr. DSouza

Thank you for your email [dated 9 August] enclosing the editors’ comments. Our responses are given in a point-by-point manner below. Change to the manuscript is shown in track the changes within the manuscript to identify all of the edits.

**Response to the comments from the Editorial Comments**

**Comment 1**: This sounds a bit awkward. Consider deleting this?

**Response**: Thank you for your suggestion. We have changed the article title to **“Semi-quantitative assessment using [18F]FDG tracer in patients with severe brain injury”**

**Comment 2**: The manuscript will benefit from thorough language revision as there are a number of grammatical errors throughout. Please have a proficient English-speaker thoroughly review the manuscript and edit any errors.

**Response**: Thank you for your suggestion. This manuscript was checked by Edanz Research Editing Group (www.edanzediting.com/ac) again

**Comment 3**: Some steps were edited for clarity. Highlighting was adjusted to meet JoVE’s style requirements.

**Response**: I really appreciate your editing and highlighting. I agree all parts.

**Comment 4**: Reference?

**Response**: In accordance with editor ’s comment, we have added the **REFERENCE 17 (p.3, lines 101)**.

**Comment 5**: Unhighlighted due to lack of filmable content.

**Response**: I agree it.

**Comment 6**: Please ensure that the hot lab is accessible for filming. Please mention notes of caution regarding appropriate shielding and personnel protection.

**Response**: Our hot lab is accessible for filming. We conjecture that the cameraman will be exposed to radiation about 10Sv, which means 5-10 percent of simple X ray. In accordance with editor ’s comment, we have added the **PROTOCOL 2.1. (p.3, lines 130-132)**

⇒Be sure to check the radiation monitor in the hot lab and use the portable radiation dosimeters to check the radiation levels of each person before they enter the hot lab.

**Comment 7**: Unclear what is to be done and what we would show, please clarify.

**Response**: In accordance with editor ’s comment, we have added the **PROTOCOL 2.1. (p.3, lines 130-132)**

⇒Be sure to use the automatic program to check the mobility of the pumping system in the FDG synthesizer and to ensure that air does not leak from the reagent kit.

**Comment 8**: What is checked for?

**Response**: In accordance with editor ’s comment, we have added the **PROTOCOL 2.2. (p.4, lines 133-134)**

⇒2.2. Check the volume of [16O]-water and [18O]-water and the volume of helium, hydrogen, and nitrogen in the gas tank. Check the tap water temperature for primary cooling under 25 degrees and that that for secondary cooling is under 22 degrees.

**Comment 9**: What is done to prepare the cyclotron and gas tank?

**Response**: In accordance with editor ’s comment, we have added the **PROTOCOL 2.2. (p.4, lines 134-135)**

⇒Check the tap water temperature for primary cooling under 25 degrees and that that for secondary cooling is under 22 degrees.

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**Comment 10**: What is done here? In order to film this, exact actions must be described. Please elaborate. Mention any relevant settings.

**Response**: In accordance with editor ’s comment, we have added the **PROTOCOL 2.3. (p.4, lines 138-145)**

⇒2.3. Begin preliminary irradiation of [16O]-water in the cyclotron (1 h after start). Check the monitor to be sure that 2–3ml of [16O]-water Is irradiated in optimal conditions (e.g., 20 A, 5 min) in the target area of the cyclotron. After irradiation, enter the vial of [16O]-water into a radioisotope dose calibrator and measure the level of radioactivity (see table of materials).

Note: The radioactive decay should be calculated using the formula: N(t) = N(0) × (1/2)t/T

N(t) is the number radioactive nuclei at t = t seconds; N(0) is the number radioactive nuclei at t = 0 seconds; T is the half-life.

**Comment 11**: What is done here? In order to film this, exact actions must be described. Please elaborate. Mention any relevant settings.

**Response**: In accordance with editor ’s comment, we have added the **PROTOCOL 2.4. (p.4, lines 147-148)**

⇒2.4. Begin irradiation of [18O]-water in the cyclotron (1 h 30 min after start). Set bombardment time for up to 20 min and the energy of the impinging protons to 16.5 MeV.

**Comment 12**: Prepare how exactly? Do you simply mean, load 2-3 ml 18O water? Where is the water loaded? In what kind of container?

**Response**: In accordance with editor ’s comment, we have added the **PROTOCOL 2.5.1. (p.4, lines 153-154)**

⇒2.5.1. After irradiation, use helium gas to transfer 2–3 ml of the [18O]-water from the cyclotron to the polypropylene receiver of the FDG synthesizer.

**Comment 13**: How? What I done here? What would we film?

**Response**: In accordance with editor ’s comment, we have added the **PROTOCOL 2.5.1. (p.4, lines 156-158)**

⇒2.5.2. Hook syringes onto the corresponding syringe drivers, pressurize reagent vials, dissolve the 1,3,4,6–Tetra–O–acetyl–2–O–trifluoromethanesulfonyl––D–mannopyranose in 1 vial (7 ± 0.2 ml) of acetonitrile ( purity ≥ 99.5%), and rinse the cassette with acetonitrile.

The cameraman can film the procedure on the monitor of the FDG synthesizer.

**Comment 14**: What is the precursor?

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.1. (p.4, lines 157)**

⇒1,3,4,6–Tetra–O–acetyl–2–O–trifluoromethanesulfonyl––D–mannopyranose

**Comment 15**: What volume? 1 vial is vague.

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.1. (p.4, lines 157-158)**

⇒1 vial (7 ± 0.2 ml)

**Comment 16**: %?.

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.1. (p.4, lines 157-158)**

⇒purity ≥ 99.5%

**Comment 17**: There appear to be missing steps before this as there is some discontinuity..

**Response**: In accordance with editor ’s comment, we have added highlighted color area of **PROTOCOL 2.5.4.**

The cameraman can film the procedure on the monitor of the FDG synthesizer.

**Comment 18**: %?.

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.5. (p.4, lines 173)**

⇒purity ≥ 99.5%

**Comment 19**: Unclear. Is this in the synthesizer?.

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.5. (p.4, lines 173-174)**

⇒A nucleophilic substitution reaction occurs at 85 °C in the FDG synthesizer.

**Comment 20**: Ultrapure? Distilled? De-ionized?

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.6. (p.4, lines 176)**

⇒26 ml of distilled water

**Comment 21**: Ultrapure? Distilled? De-ionized?

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.6. (p.5, lines 180)**

**⇒**using 10 ml, 10 ml, 13 ml, and 13 ml of distilled water

**Comment 22**: There is a discontinuity in the highlighting. Please ensure continuity between highlighted steps. Please highlight relevant steps for filming from 2.5.3-2.5.9.

**Response**: In accordance with editor ’s comment, we have added highlighted color area from **PROTOCOL 2.5.4. - 2.5.9.3.**

**Comment 23**: If relevant, cite a reference for how this is done. Please add a bit more details to describe what is done (for filming purposes).

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.3. (p.5, lines 207-208)**

⇒Measure the radioactivity and half-life using a radioisotope dose calibrator the same as step 2.3.(see table of materials) (criterion: 105–115 min).

**Comment 24**: Unclear what is meant. Please describe the actions in detail.

**Comment 25**: Unclear what we would film here. Please describe all the steps..

**Response**: In accordance with editor ’s comment, we have edited for clarity and changed the **PROTOCOL 2.5.10.3. -2.5.10.5 (p.5, lines 207-216)**

2.5.10.3. Measure the radioactivity and half-life using a radioisotope dose calibrator the same as step 2.3.(see table of materials) (criterion: 105–115 min).

2.5.10.4. Dispense 0.5 ml from the vial.

2.5.10.5. Perform a radiochemical purity test via carbohydrate analysis. Use 3.9 × 300 mm columns for high-performance liquid chromatography (see table of materials) to detect the peak radioactivity (over 95).

Note: A single peak means high purity.

**Comment 26**: How is this done? What would we film here?

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.6. (p.5, lines 219-220)**

⇒Measure the　residual 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (see table of materials) (< 40 ppm) using test paper (see table of materials)

**Comment 27**: How is this done? What would we film here? All steps need to be described in the text.

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.6. (p.5, lines 220 - p.6, lines 221)**

⇒Measure the endotoxins with the appropriate endotoxin-measuring device through absorbance measuring (see table of materials) (0.25 EU/ml).

**Comment 28**: Unclear what the incubation conditions would be.

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.6. (p.6, lines 221)**

⇒finding no bacteria after 8 days at 37°

We think that we had better set the humidity in future

**Comment 29**: Food and water?

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL3.1. (p.6, lines 233-234)**

⇒Do not stop taking water. Patients should fast starting 7 hours before image acquisition.

**Comment 30**: Which parameters? Please describe.

**Response**: In accordance with editor ’s comment, we have deleted the words.

**Comment 31**: Place where?

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 3.2. (p.6, lines 236-238)**

⇒Secure a 22–24 G needle with 5 ml heparin sodium (10 units/ml) on the lower limbs before entering the radiation-controlled area.

**Comment 32**: Mention volume and needle gauge.

**Response**: In accordance with editor ’s comment, we have changed the **PROTOCOL 3.4. (p.6, lines 244-245)**

⇒Recheck the patency of the intravenous route by drawing blood with a 10 ml syringe without a needle.

**Comment 33**: Please add the step where the ROIs/VOIs are drawn.

**Response**: In accordance with editor’s comment, we have changed the following text in the **PROTOCOL 4.4** **(p.7, lines 300-303)**

⇒Be sure to draw a border around the targeted VOI on the browser using the three-dimensional sphere, excluding other targets, extraocular muscles, and the scalp because they tend to disturb the set SUV threshold. Check the target area on axial, coronal, and sagittal slices.

**Comment 34**: Unclear what is being said. Please revise for grammar.

**Response**: In accordance with editor’s comment, we have changed the following text in the **PRPTOCOL 4.8** **(p.7, lines 312-313)**

⇒4.8. To sterically visualize glucose metabolism of the whole brain surface, use the software (see table of materials) to set a color map for the [18F]FDG-PET/CT images based on blood glucose.

**Comment 35**: Reference?

**Response**: In accordance with editor’s comment, we have added the **REFERENCE 17 (p.8, lines 319)**

**Comment 36**: Reference?

**Response**: In accordance with editor’s comment, we have added the **REFERENCE 20 (p.8, lines 329)**

**Comment 37**: It looks like the opposite is the case in 2c. Please indicate the right and left hemispheres in the figures

**Response**: Thank you for your suggestion. In accordance with editor ’s comment, we have changed the **Figure 2C** and indicate the right and left hemispheres

**Comment 38**: Please mark the right and left sides of the brain on the panels.

**Response**: In accordance with editor ’s comment, we have added the marking of the right and left sides of the **Figure 2**

**Comment 39**: The text and markings are too small to see clearly. Please increase the line weights and font sizes. Please translate the marking to English as well to allow a reader to interpret this.

**Response**: In accordance with editor ’s comment, we have changed the font size language and of the **Figure 2**. The font size of **Figure 2A** was fixed in this program, so I enlarged the picture

**Comment 40**: Unclear what is meant, needs revision.

**Response**: In accordance with editor’s comment, we have changed the following text in the **DISCUSSION** **(p.9, lines 374-377)**

⇒Second, attention should be paid to the tube for 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane because it can easily become stopped up by crystallization of 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane.

**Comment 41**: Unclear what is meant, needs revision.

**Response**: In accordance with editor’s comment, we have changed the following text in the **DISCUSSION** **(p.9, lines 378-379)**

⇒Third, the hook of syringes (PROTOCOL 2.5.2) should be handled carefully because it tends to be broken.

**Comment 42**: But you previously say that the main reason behind your protocol is that MRI has artefacts from metal implants. This is a bit confusing

**Response**: In accordance with editor’s comment, we have changed the following text in the **DISCUSSION** **(p.10, lines 404-405)**

⇒In the future, this protocol should be modified for use with advanced PET/CT imaging.

**Comment 43**: Please do not abbreviate journal titles. Please follow this format: Godbolt A. K., Deboussard C. N., Stenberg M., Lindgren M., Ulfarsson T., Borg J. Disorders of consciousness after severe traumatic brain injury: a Swedish-Icelandic study of incidence, outcomes and implications for optimizing care pathways. Journal of Rehabilitation Medicine. 45 (8), 741-748, (2013).

**Response**: In accordance with editor’s comment, I have corrected the references throughout the manuscript.

We wish to thank the editor again for the valuable comments.

We look forward to a publication of our manuscript in Journal of Visualized Experiments.

**Tomohiro Yamaki M.D., Ph.D.**,

3-30-1 Isobe, Mihama-ku, Chiba 261-0012 Japan, Division of Neurosurgery and Division of PET imaging

Rehabilitation Center for Traumatic Apallics Chiba (National Agency for Automotive Safety and Victims’ Aid)

Email: [t-yamaki@chiba-ryougo.jp](mailto:akiranak@chiba-cc.jp); yamakitomohiro@hotmail.com

Phone: +81-43-277-0061; Fax: +81-43-277-2259