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Role of Nuclear Pharmacist in Clinical Translation of Positron Emission Tomography (PET) Radiopharmaceuticals and Environmental Monitoring of Facility

Reiko Oyama

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Reiko Oyama
Candidate

College of Pharmacy
Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Jeffrey P. Norenberg, PharmD, PhD, Chairperson

Kristina M. Wittstrom, PhD

Stephen M. Moerlein, PhD

**Role of Nuclear Pharmacist in Clinical Translation of
Positron Emission Tomography (PET) Radiopharmaceuticals
and Environmental Monitoring of Facility**

BY

REIKO OYAMA

**BACHELORS OF SCIENCE
PHARMACY**

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

**Master of Science
Pharmaceutical Sciences (Radiopharmacy)**

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Albuquerque, New Mexico

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**Role of Nuclear Pharmacist in Clinical Translation of Positron Emission
Tomography (PET) Radiopharmaceutical and Environmental Monitoring of
Facility**

By

Reiko Oyama

B.S., Pharmaceutical Sciences, Nagasaki University, 1988

M.S., Pharmaceutical Sciences, UNM, 2017

ABSTRACT

There have been many changes in nuclear medicine and molecular imaging field as several radiopharmaceuticals have recently been approved and many others on the horizon. Ensuring the compliance with the increasingly aggressive national, federal, and state regulatory has become a critical piece of PET manufacturing and Nuclear Pharmacy. As a nuclear pharmacist, I have been searching for the way I can better contribute to the progress of this field with an ultimate goal of proving safe and effective healthcare to the patients. In order to do so, strengthening my expertise is essential. In this thesis, I would like to capture and narrate my professional growth, by focusing on three projects, 1) Assisting in translational work for a radiolabeled monoclonal antibody, 2) Performing Germanium analysis, and 3) Establishing an environmental monitoring program at my work place.

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I. Introduction

I am a nuclear pharmacist at Washington University School of Medicine (WUSM) Cyclotron Facility and Nuclear Pharmacy where over 35 different Positron Emission Tomography (PET) Radiopharmaceuticals (RaPhs) are produced for both clinical and research use. As a Nuclear Pharmacist I need to understand and perform a wide range of PET manufacturing and dispensing processes, which include aseptic training, media fill testing, preparation of standard operation procedures (SOPs) and batch records, and working with researchers to assist in translation of new PET RaPhs to routine production. It is also my responsibility to develop quality control (QC) methods, write quality control (QC) release specifications, assure and taking responsibility for the quality of final drug products, maintain proper documentation, and assure all activities are in compliance with appropriate regulations, including United States Food and Drug Administration (FDA), the Missouri State Board of Pharmacy, and Nuclear Regulatory Commission (NRC). Each aspect requires different skills and knowledge, but all are closely connected with the ultimate goal of providing safe and high quality drug products for human use.

During my professional career at Washington University, I have learned and acquired many skills and increased my background knowledge while performing daily tasks. However as I became a more experienced nuclear pharmacist, I realized that it is crucial to expand my learning outside of my daily work to increase my understanding of the field of nuclear medicine and molecular imaging. As this field is rapidly moving forward, I felt it was important to broaden my knowledge and become a professional whose expertise assists others in moving their clinical or research studies forward. Therefore, I

have become involved in different types of projects which would bring me new skills and experience in nuclear pharmacy in order to achieve more comprehensive growth.

For my thesis, I have focused on three projects.

1) Translational work for ^{89}Zr labeled trastuzumab:

I assisted in the translational work of conjugating the monoclonal (mAb) antibody, trastuzumab, which is marketed under the trade name Herceptin[®], to the bifunctional ligand *p*-isothiocyanatobenzyl-desferrioxamine (Df-Bz-NCS), then radiolabeling the conjugate with Zirconium-89 (^{89}Zr). This included preparation of the Chemistry Manufacturing and Controls (CMC) section for the Investigational New Drug (IND) application which was submitted for this project. I also worked with a physicist to learn how to perform human dosimetry calculations based on human image acquisitions.

2) Performing radionuclidic purity analysis for ^{89}Zr oxalate sample using a Germanium (Ge) detector:

I performed radionuclidic purity analysis for ^{89}Zr -oxalate sample using Germanium (Ge) detector. This includes analyzing the data manually and examining the cyclotron bombardment methods to keep the radionuclidic impurity low.

3) Establishing an environmental monitoring (EM) program for the 21 CFR Part 212 manufacturing process for the Washington University School of Medicine (WUSM) Cyclotron Facility and Nuclear Pharmacy:

I established the EM program for the WUMS Cyclotron Facility and Nuclear Pharmacy. The Facility is regulated under Code of Federal Regulations (CFR) Title 21 Part 212, Current Good Manufacturing Practice for Positron Emission Tomography Drugs. Additionally, I participated in the audit of the facility by FDA inspectors.

II. Description of Projects

Project 1: Translational work for ⁸⁹Zr labeled trastuzumab

a). Introduction

Breast cancer is the second most common cancer in women after skin cancer in the United States. New cases of breast cancer in 2016 are estimated to be 246,660, which consists of 14.6% of all new cancer cases, and death from breast cancer in 2016 is estimated to be 40,450.¹ Standard treatment options include surgery, radiation therapy, chemotherapy, hormonal therapy, and immune targeted therapy, and selection of therapy is based on the type and stage of the cancer, treatment goals and possible side effects. Many efforts have been made to understand the mechanism of breast cancers in order to provide better treatments. There are three different receptors identified in breast cancer cells; estrogen receptor, progesterone receptor, and HER2. Thus depending which receptor(s) is (are) overexpressed in cancer cell, the breast cancer can be classified into sub types; hormone receptor (estrogen and progesterone)-positive, HER2-positive, and triple negative, in which no receptors are overexpressed. About 80% of breast cancers are estrogen positive and about 65% of these are also progesterone positive. These estrogen-positive and progesterone-positive cancer cells proliferate in response to estrogen and progesterone, thus respond to hormone therapy better than hormone receptor negative cancer cells do. Estrogen receptor and progesterone receptor status of the tumor is assessed by immunohistochemistry test which is a staining process performed on fresh or frozen breast cancer tissue obtained by biopsy. About 25% of breast cancers are HER2 – positive. While they are less sensitive to hormone therapy compared to hormone receptor-positive breast cancers, they are very sensitive to the therapy which targets

HER2 protein. HER2-positive breast cancers have shown a distinct disease course which is characterized with recurrence after a short disease-free period and aggressive metastatic disease if not treated with HER2 targeted immune therapy.

HER2 is an abbreviation of human epidermal growth factor receptor 2, and is present on the cell surface at higher level in about 25 percent of breast cancer cells. HER2-positive cancer cells have a gene mutation, HER2 gene, which makes excessive HER2 protein. HER2-positive cancer cells tend to be aggressive and fast-growing as they receive too many stimulating signal for growth, thus leading to an overall poor patient survival.

Trastuzumab (Herceptin®) is a recombinant DNA-derived humanized mAb which binds to the extracellular and intracellular domains of HER2 protein with high affinity. It was developed to block the action of HER2, thus suppressing HER2-positive cancer.

Trastuzumab suppresses HER2 activity, thus inhibiting proliferation of cancer cells.

Trastuzumab also flag the cancer cells for destruction by immune system. Trastuzumab (Herceptin®) was approved by FDA in 1998, and has been used in treating HER2 – positive cancer. Many studies have conducted and shown that when trastuzumab is added to the treatment to HER2-positive breast cancer as an adjuvant therapy it improves patients' survival. However, trastuzumab can cause serious side effects, such as cardiomyopathy and its risk increases when trastuzumab is used for the patients who are receiving trastuzumab with anthracycline-containing chemotherapy regimens.² Its cost is also a factor to consider as it is expensive. Therefore it is critical to accurately identify the patients who can benefit from trastszumab therapy with benefit outweighing potential side effects. HER2 protein status of the tumor is currently assessed in vitro by immunohistochemistry test which detects the number of HER2 protein or fluorescence in

situ hybridization which detects the number of HER2 genes. These tests are performed on breast cancer tissue obtained by biopsy. There are several downsides of performing these tests. First of all, biopsy is an invasive method and repeated biopsy is not practical or feasible. Second, depending on the location, some location can be difficult to reach by biopsy. Third, the tumor can be heterogeneous and the result from the biopsy sample may not represent the status of whole tumor.³ Since HER2 status can change during the course of disease and after the chemotherapy, repeated assessing HER2 status of the tumor is necessary.⁴ Therefore, reliable alternative methods which can be used repeatedly to assess HER2 status on entire tumor volume is imperative in identifying the patients who can benefit from trastuzumab therapy. Positron emission tomography (PET) with radiolabeled mAb, also known as 'immunoPET', is an attractive method to non-invasively assess the patient's tumor status, and has received a lot of attention. It can provide useful information to the patient regarding the effectiveness of immunotherapy to the patient if the radionuclide is attached to the same mAbs used in therapy.

b). Translating the production from preclinical to clinical (Production and QC)

Farrokh Dehdashti, M.D, submitted an IND for ^{89}Zr - Df-Bz-NCS-trastuzumab (^{89}Zr -trastuzumab) for a phase 1 clinical study in 2013. The objectives of the studies are assessing the diagnostic quality and lesion detectability of ^{89}Zr -trastuzumab PET images, performing human dosimetry, and evaluating the relationship between ^{89}Zr -trastuzumab uptake and in vitro status of HER2. Zirconium-89 is an ideal radionuclide for labeling an intact antibody as its relatively long half- life, 78.42 hours, gives trastuzumab enough time to equilibrate in patient's body. Its half-life allows imaging the patient with PET scan up to 7 days post ^{89}Zr -trastuzumab administration, giving good tumor-to-

background contrast.⁵ Zr-89 decays via electron capture (EC) (77%) and positron decay (23%) to become its daughter nuclide Yttrium-89 (⁸⁹Y). Zirconium-89 is produced using a cyclotron most by the nuclear reaction $^{89}\text{Y}(p, n)^{89}\text{Zr}$. With its relatively low maximum beta plus energy (0.90 MeV) and low average beta plus energy (0.40 MeV), PET images with Zr-89 result in high spatial resolution. In addition, using naturally abundant ⁸⁹Y as target material helps to keep the cost of operation low. Prior to the IND submission and my involvement to this project, Suzanne Lapi, PhD, then at the Washington University, and her group conducted preclinical animal studies using ⁸⁹Zr-trastuzumab, and concluded ⁸⁹Zr-trastuzuma had shown its potential for identifying HER2 regions and helping appropriate therapy selection.⁶

I began working with Dr. Lapi's group when Dr. Dehdashti decided to submit an IND application for ⁸⁹Zr-trastuzumab by collaborating Dr. Lapi's group. I evaluated the facility environment where ⁸⁹Zr-trastuzumab production for preclinical study was ongoing at that time, and found it to be inappropriate for the production of human use RaPh since the whole labeling process was performed on the chemistry bench top in one of the University's research laboratories where the space and equipment were shared with multiple researchers and students. I recommended moving the labeling process to Washington University's GMP facility which was on the same medical campus, but it was rejected due to its cost. After more discussion and search for the appropriate place for production for human use, we decided to use one of WUSM Cyclotron Facility production area which equipped with Laminar Airflow Hood (LAFH) that provide ISO5 environment and was located in ISO 8 area. The area had been originally renovated and used as quality control (QC) laboratory for production of carbon-11 labeled PET

radiopharmaceuticals for human and preclinical studies, but it was used by researchers at that time. Prior to 2012, I had consolidated all QC work in one laboratory in another building where the majority of our PET radiopharmaceutical production was occurring.

I then proceeded to develop an ordering and receiving process for all materials needed for the production. Before my involvement, radiochemists in Dr. Lapi's group used any materials they purchased or found in their laboratories to produce ^{89}Zr -trastuzumab. I initiated the system for defining Raw Material (RM) Acceptance Sheet which consists of Identification, Description, and Specification for each material along with its log sheet and storage requirement (see Appendix A for example RM Acceptance Sheet). I then established the system to order, receive, log in the materials, and to control the RMs in an area where no one could use the materials for any process except the defined human use research project. Each time when new material is received from vendor, person who receives the material is required to write down its Purchase Order number and his/her initials for each identification, description, and specification section in order to properly accept the materials. Once the material is checked and accepted, it receives a green "Released for Use" label which has the RM number and expiration, and the initials of the personnel who performed the check in process, and stored in one specific laboratory where only personnel involved in ^{89}Zr -trastuzumab production for human use have access. I explained the importance of controlling materials and the process, and had radiochemists generate the draft RM acceptance sheets and signed them off. Establishing the RM acceptance process allowed researchers to use only materials which were purchased for ^{89}Zr -trastuzumab productions for human study. Additionally, the system added the ability to track down the materials used if needed.

Proceeding in parallel, I provided aseptic training to each of the radiochemists working on the project. I gave initial didactic training which focused on basic knowledge, such as basic microorganism, how people could contaminate the product and environment, and how to work in a LAFH using an audio visual tape and hand-outs. I also provided a written quiz at the end of the didactic training, which researchers needed to pass by obtaining no less than 70% score in order to proceed to the next step. Then I showed them how to garb, wash hands, and clean the LAFH. I designed their media fill test procedure which simulates the actual ^{89}Zr -trastuzumab labeling process by identifying the critical steps and most challenging situation, and worked with them as evaluator when each radiochemist went through three initial media fill tests.

Zirconium-89 trastuzumab was prepared as previously described by Vosjan et al.⁷

Zirconium-89 was produced by WUSM Cyclotron Facility on the CS-15 (Cyclotron Corporation, Berkeley, CA) cyclotron via $^{89}\text{Y}(p, n)^{89}\text{Zr}$. After the bombardment ^{89}Zr was separated from ^{89}Y using ion exchange chromatography and collected in an acidic solution as ^{89}Zr -oxalate with an in-house automated module.⁸ Five milligrams of Df-Bz-NCS-trastuzumab was radiolabeled with neutralized ^{89}Zr solution and incubated for 1 hour at 37 °C. The solution was collected into final product vial (FPV) through 13mm PDVF 0.2 μm of sterilizing filter (catalog number 09-720-3, Thermo Fisher Scientific, MA), and saline was added through the same sterilizing filter into the FPV to dilute the product.

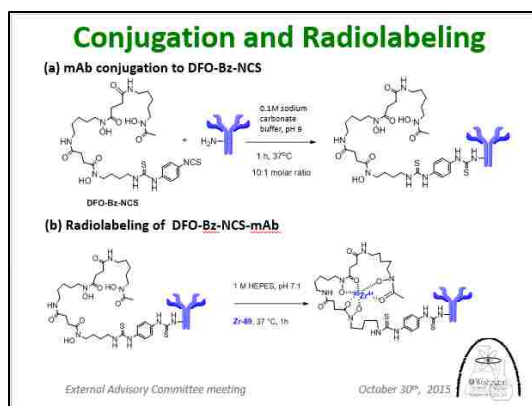


Figure 1: Zirconium-89 labeled trastuzumab chemistry scheme⁷

Conjugated trastuzumab was prepared prior to its radiolabeling process. Trastuzumab (Herceptin[®], Genentech, South San Francisco, CA) was reconstituted with bacteriostatic water, and incubated with a bifunctional chelate, *p*-isothiocyanatobenzyl-desferrioxamine (Df-Bz-NCS) (Macrocylics, Dallas, TX) for 1 hour at 37 °C. Then Df-Bz-NCS-trastuzumab was purified using Spin Desalting Columns, and sterilized through 13mm PDVF 0.2 μm of sterilizing filter (catalog number 09-720-3, Thermo Fisher Scientific, MA). A 0.5 mL of sterilized Df-Bz-NCS-trastuzumab was aliquoted into sterile microcentrifuge tubes, and stored at -80°C. The conjugation was performed in a LAFH, and I worked with the radiochemist who was performing the conjugation process in LFH and gave instructions in terms of aseptic technique during the process. The conjugated trastuzumab was assigned 1 year of expiration as radiochemists already had acquired stability data for the conjugated trastuzumab while they were doing their preclinical studies. I decided to have it re-tested every 6 months once it reached its original expiration date in order to extend the expiration date.

I also worked with the radiochemists while they prepared all other reagent solutions in the LAFH, supervising their process and aseptic technique. I assisted in generating the reagent preparation forms and reviewed them once the preparation was completed.

Once reagents were prepared and their documents were generated, I oversaw the production process in the new production area and assisted in preparing the batch records.

As mentioned previously, it was decided that the majority of the production process would take place in the LAFH which is not equipped with any radiation shield.

Zirconium-89 decays to ^{89m}Y by EC or beta plus, then ^{89m}Y decays to stable ^{89}Y by isomeric transition by emitting 909 keV photons. The half-value layer for the abundant and high energy 909 keV photons is ~10 mm, which is about twice as much as the half-value layer for 511 keV photons. Thus it was important to ensure that enough radiation shielding was provided to the radiochemist who handled radioactivity. I worked with Washington University Radiation Safety Office prior to and during validation tests, and had the radiochemist wear the fingertip dosimeter during the radiolabeling process to assess the radiation dose. The fingertip dose was found to be less than 1 mrem for the entire process.

I also established the QC Release specification that would be used to release the product.

In the QC Release specification, Filter membrane integrity test, pH, appearance and color, strength, radionuclidic purity, radiochemical purity, specific activity, and bacterial endotoxin testing were listed as pre-release test items, and radioimmunoassay and sterility testing were listed as post-release test items. I designed the 3 validation tests which consisted of normal ^{89}Zr -trastuzumab production using the highest amount of

radioactivity that was planned to use, and each test required full QC. I oversaw the production process and reviewed the batch records and QC results when each of these 3 validation tests was performed in December, 2012.

In addition to the validation tests, stability tests were performed on 3 batches in January, 2013. The product's stability was analyzed and confirmed out to 18 hours end of synthesis (EOS). Thus we decided to set the expiration of the product at 12 hours EOS which was shorter than the maximum time the product was tested, and this provided a margin of safety.

c). Preparation of the Chemistry Manufacturing and Control (CMC) section for the IND, and IND submission to FDA

Once the 3 validation tests and stability tests were successfully completed, I prepared and reviewed the CMC section for the IND prior to submission to FDA. Washington University (WU) did not conduct animal toxicity study for Zr-Df-Bz-NCS-trastuzumab. Animal toxicity studies conducted at Memorial Sloan Kettering Cancer Center (MSKCC) which concluded that 10 mg/kg of Zr-DFO-trastuzumab administered intravenously as a single dose was the No Observed Adverse Effect Level for human. The WU IND referenced the Sloan Kettering IND by obtaining a letter of access which allows the FDA to review the MSKCC IND file on behalf of WU.

The human radiation dosimetry estimates for ^{89}Zr -trastuzumab were calculated by Richard Laforest, PhD, physicist at WU, Department of Radiology, from mice biodistribution data and from MIRD S-values for Zr-89. A maximum dosage of 4.0 mCi

was proposed for human studies, assuming a worst case specific activity of 0.2 mCi/mg for the ^{89}Zr -trastuzumab.

The CMC section was submitted to Dr. Dehdashti's clinical research coordinator, who assembled the application form, CMC section, clinical trial protocol and informed consent form, and a letter of access to preclinical pharmacology and toxicology studies performed at MSKCC for IND application. The IND was submitted to FDA in March, 2013. After receiving and responding to the requests which required clarification on the QC testing section, we received the FDA's approval to proceed on April 12, 2013, although FDA requested additional information on the following items by April 19, 2013.

- 1) What is the monomer content of ^{89}Zr -Df-Bz-NCS-trastuzumab at the time of release and at the time of radiolabeling, and what is the QC specification for the monomer content?
- 2) What data do you have to support the stability of Df-Bz-NCS-trastuzumab over long term storage?

For item 1), since proteins can aggregate when mechanical stress such as shaking was added, it seems reasonable to confirm the absence of aggregation. However, we had not performed any analysis for protein aggregation for the validation test batches as the amount of trastuzumab used for each production was only 5 mg. In addition, we had just purchased a Fast Protein Liquid Chromatography (FPLC) which could be used for protein aggregation analysis in April, 2013, when we received FDA's information request. When we received FDA's information request, Df-Bz-NCS-trastuzumab which had been

prepared in December, 2012, was injected along with an IgG as a standard for 150 kDa molecular weight on the FPLC (Column: Superose 12, 10/300, UV at 280 nm, mobile phase: 50 mM sodium phosphate buffer (pH 7) containing 150 mM sodium chloride aqueous solution, flow rate at 1.0 mL/min). A single peak at ~11 minutes was observed from Df-Bz-NCS- trastuzumab injection. The result was in agreement with the elution time (volume) of the IgG. The representative chromatogram of protein standard from the column manufacture, performed using the same method, also showed IgG's retention time matched the retention time of Df-Bz-NCS-trastuzumab peak. In our response to FDA which we sent a week after receiving their information request, we assured there was no aggregation of Df-Bz-NCS-trastuzumab after 4 months of storage, and agreed to send the analysis data once we connected the radioactive detector to the FPLC system and performed analysis for ⁸⁹Zr-Df-Bz-NCS-trastuzumab.

For item 2), we were able to provide the data regarding the stability of the Df-Bz-NCS-trastuzumab for 4 months storage. We agreed to provide additional data as it became available. Later, we performed protein aggregation analyses on FPLC for Zr-89 labeled Df-Bz-NCS-trastuzumab, and confirmed there was only a small portion of aggregate (less than 2%) if any was observed in the samples. I revised the QC release specification by adding Protein Aggregation analysis as post-release testing, and set its acceptance criterion to be no less than 80% for non-aggregated protein.

d). Initiation of patient study

The following year, during January and February in 2014, another 3 validation tests were performed as we decided to add purification process after radiolabeling Df-Bz-NCS-

trastuzumab. Zirconium-89 labeled Df-Bz-NCS-trastuzumab was purified and buffer exchanged into a 1 M HEPES buffer via Zeba Spin desalting Column (Catalog number 89891-7K, Thermo Fisher Scientific, MA). Pierce Biotechnology, Rockford, IL). The purified solution was collected into a final product vial (FPV) through 0.2 μm of sterilizing filter (catalog number 09-720-3, Thermo Fisher Scientific, MA), and saline was added through the same sterilizing filter into the FPV to dilute the product. Three validation tests with stability tests were performed successfully, and an IND amendment, which also included the revision of QC release specification, was submitted to FDA in February, 2014. The first patent study was performed in March, 2014. After FDA's approval of this IND, I have been overseeing the production, signing off on each batch record, and modifying and reviewing CMC section when needed.

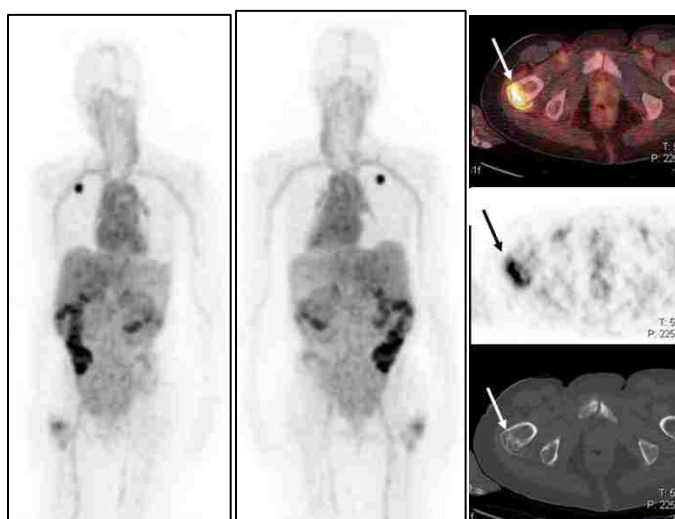


Figure 2: Anterior (left) and posterior (middle) re-projection ^{89}Zr -trastuzumab PET/CT images on day 3 in a patient with HER2-positive osseous metastasis in a right femur (arrow)

The area of intense activity in the right chest is related to administration of ^{89}Zr -trastuzumab via a port catheter. (Image courtesy of F. Dehdashti, MD Washington University School of Medicine)

Although in general the production of ^{89}Zr -trastuzumab went smoothly and final product was successfully administered to the patient, we discovered an issue regarding the dose calibrator setting in July, 2014. The research coordinator who usually received the ^{89}Zr labeled mAb had noticed the difference in dose calibrator measurements between production site and clinical site and raised a question to me. I brought the Caesium-137 source we use to calibrate the dose calibrator in the production area to the clinical area and checked the measurement at clinical dose calibrator. By talking to the research coordinator while performing the dose calibrator check, I discovered the dose calibrator's setting at clinical site was different from the setting the WUSM Cyclotron Facility was using. The WUSM Cyclotron Facility Capintec dose calibrator setting was 465 while clinical site Capintec dose calibrator setting was 525 for ^{89}Zr . I subsequently learned that in December, 2012, Washington University Radiation Safety Office and Washington University Department of Radiology (Radiological Sciences) had conducted a study to determine the optimal calibration factor for measuring ^{89}Zr patient dosages since the production of ^{89}Zr was a relatively new development and table of calibration numbers provided in the Capintec owner's manual did not include ^{89}Zr . In their final report dated December, 2012, the use of 525 setting was recommended for dose calibrators at the Washington University. Unfortunately, no one communicated that information to WUSM Cyclotron Facility, thus no one in the production group was aware of it. After I learned this information, we changed the WUSM Cyclotron Facility and Nuclear Pharmacy dose calibrator setting to 525.

e). IND amendment for changes in QC testing

We have produced a total 53 batches of ^{89}Zr -trastuzumab including test batches. There have been 2 major changes in terms of QC testing since the initiation of the study, one was changing from FPLC to High Performance Liquid Chromatography (HPLC) for protein aggregation analysis, and the other was changing the frequency of performing radioimmunoassay, a test for determining the immunoreactive fraction of radiolabeled mAb, from each batch to each new lot of Df-Bz-NCS-trastuzumab and first radiolabeled product produced by a new operator. For protein aggregation analysis, a new HPLC system was purchased in the fall of 2014. Four validation tests were performed to compare the results between FPLC and HPLC during December, 2014 and February, 2015. The test results showed that the HPLC analysis demonstrated comparable or slightly more sensitive protein aggregation detection of ^{89}Zr -trastuzumab. Based on these test results, we decided to use HPLC analysis instead of FPLC analysis.

In February, 2016, I contacted a nuclear pharmacist at MSKCC Cyclotron Facility where another type of ^{89}Zr labeled mAb for was being produced for human use under an IND study. I found that they had reduced the frequency of radioimmunoassay after they had performed 30 successful ^{89}Zr labeled mAb studies testing each with radioimmunoassay. At the Washington University, from January 2014 to February 2016, we have performed a total of 44 radioimmunoassay tests for each batch of the ^{89}Zr -trastuzumab produced, 28 for patients studies and 16 batches for test batches. Since the radioimmunoassay test met the QC Release Specification for all the patient and test batches produced, we decided to reduce the frequency of the test. We revised the QC Release Specification and changed the frequency of radioimmunoassay from each batch to each new lot of Df-Bz-NCS-

trastuzumab and first radiolabeled product produced by a new operator. The change was implemented in March, 2016 and IND amendment was submitted to FDA in the same month. Reducing the frequency of radioimmunoassay helped us in terms of personnel scheduling, as preparation and performance of radioimmunoassay took considerable time for our one analyst. I revised the QC Release Specification accordingly (see Appendix B for current QC Release Specification for ^{89}Zr -trastuzumab).

In addition to the changes in QC testing, we had encountered changes in personnel who prepared ^{89}Zr -trastuzumab. Dr. Lapi left the Washington University for another academic institution in October 2015, and WUMS Cyclotron Facility took over the production of ^{89}Zr -trastuzumab in collaboration with Buck Rogers, Ph.D. I merged all RMs used for ^{89}Zr -trastuzumab project into WUSM Cyclotron Facility RM system. I provided initial didactic aseptic training to 2 new personnel from Dr. Rogers group and worked with one of them when he performed his initial 3 media fill tests. I supervised the production procedure when 3 validation tests were performed by new production team, and reviewed the batch records. The new team has produced 15 batches of ^{89}Zr -trastuzumab since Nov, 2015, and I have been overseeing the production and signing off on each batch record. In addition, I have been working as liaison between the clinical and production group and assisting in scheduling of study and production personnel.

f). Human Dosimetry Calculation

The IND for ^{89}Zr -trastuzumab was designed to have two cohorts, Cohort 1 and Cohort 2, with the goal to assess the diagnostic quality of ^{89}Zr -trastuzumab imaging with PET.

Cohort 1 consisted of 12 adult women with HER 2 –positive primary,

recurrent/metastatic breast cancer with lesion size ≥ 1.5 cm. After ^{89}Zr -trastuzumab injection, whole-body PET images were taken at 2 time points for each patient within 7 days of injection, and these images from two time points were evaluated to identify the best time for imaging. These twelve Cohort 1 patients were asked to undergo whole body ^{89}Zr -trastuzumab imaging at 2 time points in order to collect data for estimation of normal human dosimetry. Whole body PET imaging is defining as PET data acquisition in a sufficient number of bed positions to cover from the head apex to mid-thigh. Four patients were imaged at each of the following time points:

1 and 3 days post injection

2 and 5 days post injection

4 and 6 days post injection

Cohort 2 consisted of 40 adult women with either HER2-positive or HER2-negative with at least one measurable primary or metastatic lesion, which had known HER2 status. A total of 40 patients provided a minimum of 40 evaluable lesions. Approximately twenty HER2-negative and twenty HER2-positive lesions were evaluated. Patients underwent only one PET imaging study after ^{89}Zr -trastuzumab injection, but at the optimal time established from Cohort 1, which was 5 -7 days post injection. For both Cohort 1 and 2, trastuzumab-naïve patients received 50 mg of unlabeled trastuzumab, which is less than 25% of the initial dose they would receive as initial dose if they were treated with trastuzumab, and patients who are already on trastuzumab treatment received 10 mg of trastuzumab, typically 30 min to 2 hours prior to ^{89}Zr -trastuzumab injection in order to minimize uptake of the radiotracer in normal tissues.⁹

I worked with Dr. Laforest, PhD physicist who performed the human dosimetry data analysis for the ^{89}Zr -trastuzumab using the data collected from Cohort 1 patients. As PET images were being taken for Cohort 1 patients, I manually traced the organs as regions of interests (ROIs) on the PET/CT whole body images using Siemens e.soft workstation. This program calculated the average ^{89}Zr activity concentration based on the traced region, region of interest (ROI), by me. The next step was determining the weight of each organ as it is needed to calculate the percent of injected dose distributed to organs. Measuring the total organ size from PET is usually unreliable due to low uptake in some organs which makes the boundary of the organ hard to visualize. We used the mean organ weight table from Rakesh Mandal et al¹⁰, in which organ weight is listed based on the body mass index (BMI), to calculate the organ weight for liver, spleen, and kidney, since some patients were quite obese and their organ size were markedly different from the standard organ size. Blood volume was calculated from the formula of Pearson et al.¹¹ Fat was calculated as 20 % of body weight. Muscle and red marrow weight were obtained from OLINDA/EXM (Organ Level Internal Dose Assessment/Exponential Modeling) by using adult female model for ^{89}Zr . OLINDA is approved by the FDA as a device to calculate standard size human internal radiation dose estimates. It includes S-values specific to 10 phantoms and 5 organ models for more than 800 radionuclides. Average organ activity was then decay-corrected to the ^{89}Zr -trastuzumab injection time, and activity was expressed as a percentage of injected dose (%ID), and plotted as a function of time in a Time-Activity Curve (TAC) graph.

For example:

When 80.29 MBq (1.49 mCi) was injected to the patient whose weight was 58.2 kg (BMI 21.02) at 1/7/2015 at 13:38 and the average ^{89}Zr activity was calculated to be 4032 Bq-ml from the image taken on 1/8/2015 at 9:26, the %ID for liver can be calculated as followed. (Liver weight =1400g, and decay factor=0.84 were used.)

$$(4032 \text{ (Bq/ml)} * 1400 \text{ (g)} * 100) / (80.29 \text{ (MBq)} * 10^6 * 0.84) = 8.4 \text{ (\%)}$$

Thus the liver received 8.4% of injected dose at this time point.

For each organ, all twelve patient's data for %ID were plotted as a function of time in the same Time-Activity Curve (TAC) graph and a trend line was generated. The exponential trend line was generated for spleen and blood, and a linear trend line was generated for kidney. The data plots showed constant activity concentration for liver, fat, muscle, and bone marrow, i.e. no substantial biological clearance. (See Figure 3 for each organ.)

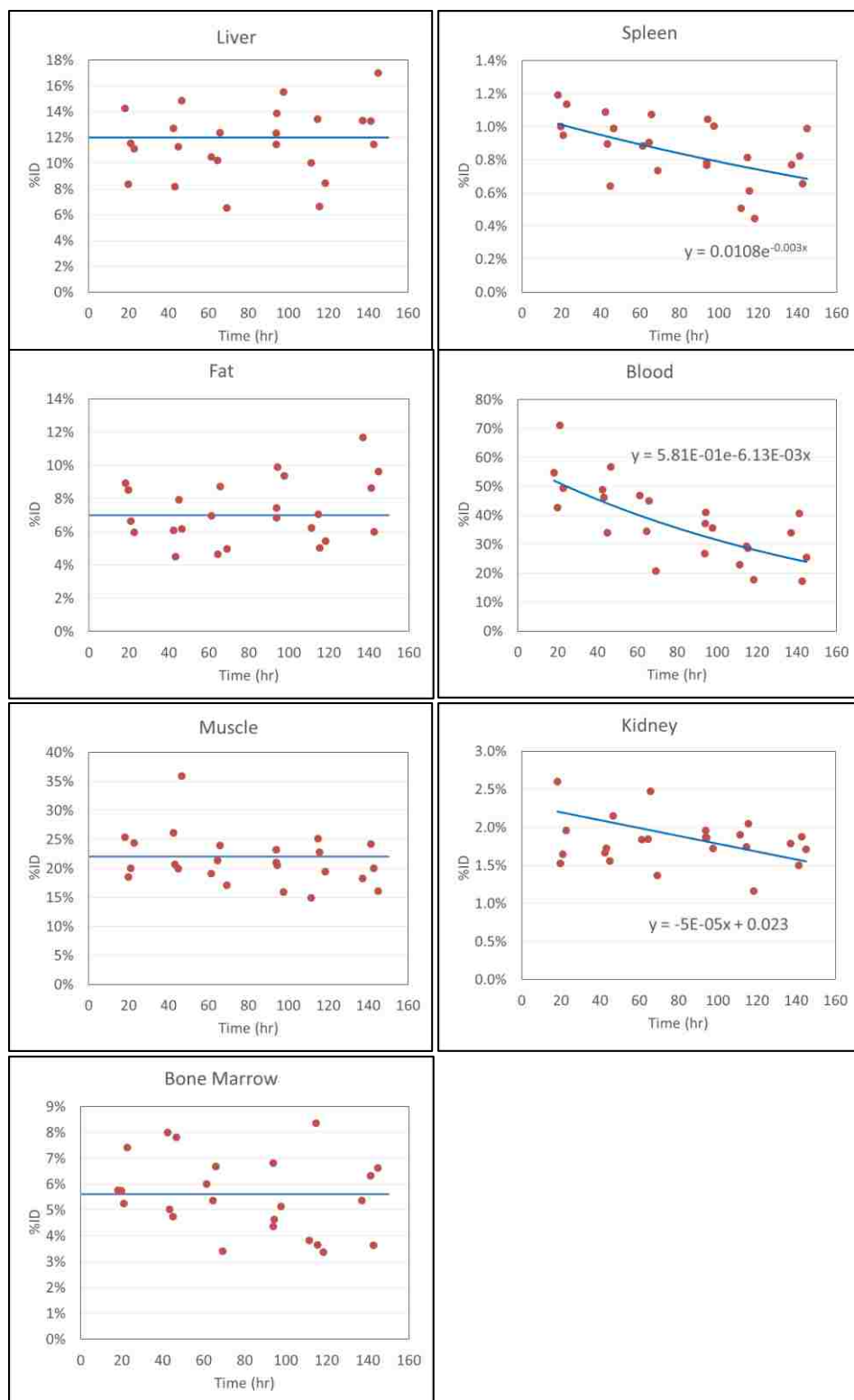


Figure 3: Graphs for Time-Activity Curves

From this biodistribution data of ^{89}Zr -trastuzumab, rapid uptake in the liver and slow blood clearance were observed. Twelve percent of the injected dose was observed in the liver, and no bladder activity was observed. For muscle, red marrow, fat, as well as liver, activity remained constant during the observed time period. For spleen and kidneys, activity was observed to slowly decrease over time.

The residence time for each organ was calculated by analytical integration of the time activity curves. This is mathematically done by dividing the %ID by the effective clearance rate (lambda effective). Lambda effective is the sum of the biological and physical lambdas. The concept was shown in a formulary below.

$$\tilde{A} = (A_0 * f_{\text{organ1}}) / \lambda_{\text{eff}}$$

Thus

$$\text{Residence time} = f_{\text{organ1}} / \lambda_{\text{eff}} = \tilde{A} / A_0$$

\tilde{A} : Cumulated activity in organ1

A_0 : Initially injected activity

f^{organ1} : fraction of injected activity in the organ1 at time 0

λ_{eff} : effective clearance rate given by $(\ln(2)/T_p + \ln(2)/T_b)$ or $(\lambda_p + \lambda_b)$

T_p : physical half-life (78.41 hr)

T_b : biological half-life

λ_p : physical decay(clearance)constant

λ_b :biological decay(clearance)constant

For this study, the residence time for liver was calculated using radioactive decay constant as total clearance constant as liver activity remained nearly constant over time.

$$\text{Residence time in liver (hr)} = f_{\text{liver}} / \lambda_{\text{eff}} = 0.12 / (0 + 0.00884) = 13.57(\text{hr})$$

However, the residence time for blood was calculated where a non-zero biological clearance was observed, the physical clearance and biological clearance constants were taken into consideration.

$$\text{Residence time in blood (hr)} = f_{\text{blood}} / \lambda_{\text{eff}} = 0.58 / (0.00613 + 0.00884) = 38.74(\text{hr})$$

Figure 4 shows the residence time for each organ.

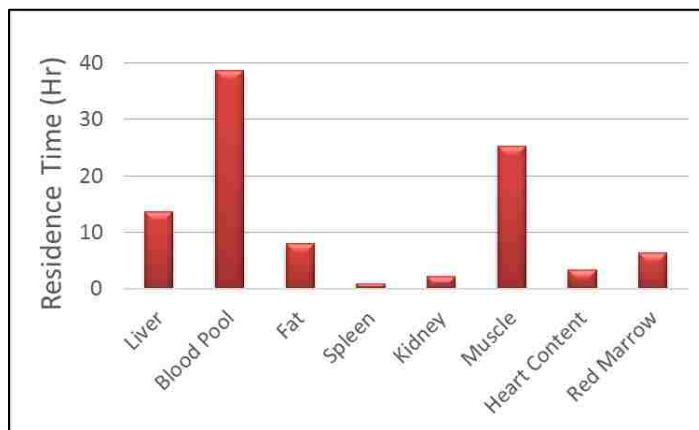


Figure 4: Zirconium-89 trastuzumab Organ Residence Time (Hr)

The total measured residence time was 98.2hr and included 3.3hr residence time assigned to the heart blood content, estimated at 8.5% body blood volume. Since the imaging agent is an antibody and thus has a long circulation time, high tracer activity is expected in the blood and consequently large total blood residence time is expected as observed. In

addition, little or no clearance is to be expected in the urine and feces. Therefore, the residence time assigned to the remainder of the body was estimated by the maximum theoretical residence time $-T_p/\ln(2)$ – minus the sum of all measured organ residence time at the exception of the blood and fat. OLINDA does not have a blood or fat source organ and thus residence blood and fat residence time were assigned to the remainder of the body (or carcass). The remainder of the body residence time was 61.6 hr.

Once residence time was calculated, those numbers were entered in OLINDA/EXM program for ^{89}Zr using the human adult female model to calculate individual organ radiation dose, the whole-body dose and the effective dose. Doses were reported in rad per mCi injected (rad/mCi) for organ radiation dose, and the effective dose (ED) was 2.25 mrem/mCi (0.61 mSv/MBq). Due to its high tracer retention, the liver is the critical organ as 12% of injected dose was observed in the liver and its residence time was 13.6 hours. The liver dose was 6.02 rad/mCi (1.63 mSv/MBq). Also it was determined that the best imaging time point was at least 4 days post ^{89}Zr -trastuzumab injection. The overall organ doses are listed in the Table 1 below.

Radiation dose is due in part by the high energy beta⁺ particle from ^{89}Zr and the emitted gammas. The self-organ irradiation is the major source of radiation to a given organ (i.e. the radiation dose in one organ is delivered by activity within this same organ). Cross-organ irradiation is due to the beta⁺ annihilation photons and the prevalent 909 keV (99%) gamma. High radiation doses is observed from the energy of the beta particle but mostly from the relatively long half-life of the nuclide.

Organ	(rad/mCi)
Adrenals	2.95
Brain	1.46
Breasts	1.54
Gallbladder Wall	3.18
Lower Large Intestine Wall	2.14
Small Intestine Wall	2.12
Stomach Wall	2.32
Upper Large Intestine Wall	2.39
Heart Muscle	4.09
Kidneys	4.56
Liver	6.02
Lungs	2.18
Muscle	1.83
Ovaries	2.19
Pancreas	2.90
Red	2.56
Osteogenic Cells	2.93
Skin	1.26
Spleen	3.18
Thymus	2.11
Thyroid	1.58
Urinary Bladder Wall	1.56
Uterus	2.14
Total	2.03
Effective Dose Equivalent	2.74
Effective Dose	2.25

Table 1. Zirconium-89 trastuzumab Organ Radiation Dose (rad/mCi)

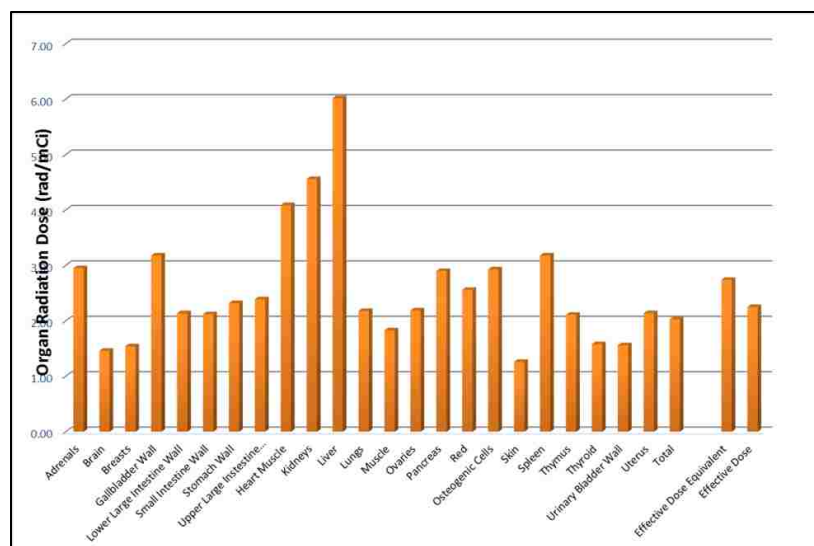


Figure 5. Zirconium-89 trastuzumab Organ Radiation Dose (rad/mCi)

In addition to the dosimetry calculation, once data from all patients in Cohort 1 were obtained from the PET/CT images, the safety of ^{89}Zr -trastuzumab was evaluated. There were no adverse effects or clinically detectable pharmacological effects observed for 12 patients.

The dosimetry data was presented at Society of Nuclear Medicine and Molecular Imaging annual meeting in 2015.¹² The research paper which discusses the safety, human radiation dosimetry, and optimal imaging time of ^{89}Zr -trastuzumab in patients was published in Molecular Imaging and Biology in 2016.¹³

g). Learning outcome from this project

This project was the first research project I was involved in, assisting the research team which consisted of radiochemists, physicians, and cyclotron staff personnel, to translate a

new radiopharmaceutical from benchtop to clinical use. It helped me learn how to work with other professionals as a team, and gave me in depth experience in terms of regulatory requirements. Furthermore, because of this project, I was introduced to the field of radiolabeled mAbs which are used for diagnostic purpose. Zirconium-89 labeled trastuzumab is the first radiolabeled mAb I worked, as I have worked only radiolabeled small molecules and peptides previously. I was able to obtain in-depth knowledge in procedure and QC testing which are specific to radiolabeled mAb, such as mAb conjugation, protein assay, radioimmunoassay, and protein aggregation testing, which is a tremendous gain as nuclear pharmacist. Moreover, I had the opportunity to work with a physicist to learn how to perform human dosimetry calculations. In addition to obtaining these new experiences and knowledge, this project generated a poster presentation and later a published paper, which are very meaningful for my academic career. Considering the project obtained IND approval from FDA and clinical studies have been successfully ongoing to achieve project's objectives, I believe I have successfully assisted the team in moving this project forward.

As previously described the human dosimetry for ^{89}Zr -trastuzumab was initially estimated from the data obtained from an animal dosimetry study. I was not involved in the human dosimetry estimation as I had not begun working with Dr. Lapi's research group when the animal study and estimated dosimetry calculations were performed. I have not yet had an opportunity to be fully involved in the process of any human dosimetry estimation using data obtained from animal study. Furthermore, I did not have an opportunity to be directly involved in pharmacology and toxicity study for this project as the Washington University did not perform the study, but referred to the data at

MSKCC. Since human dosimetry estimation, pharmacology, and toxicity study are obviously essential parts of translating any RaPhs from preclinical to clinical, getting more involved in these studies is my forthcoming plan in order to obtain the greater understanding of the translation of any RaPhs to clinical use.

Project 2: Performing radionuclidic purity analysis for ^{89}Zr -oxalate sample using Germanium (Ge) detector

a). The radionuclide of ^{89}Zr

Zr-89 is a positron emitting radionuclide which decays to ^{89}Y . In recent years ^{89}Zr has been attracting attentions from the radiology field due to its relatively long half-life of 3.3 days, which makes it suitable for binding to monoclonal antibodies, which have a longer biological half-life. This can lead to potential applications for ImmunoPET. I have performed radionuclidic purity test using a Ge detector for ^{89}Zr -oxalate sample produced at WUSM Cyclotron Facility and Nuclear Pharmacy and detailed discussion is included in this section.

b). Radionuclidic analysis for radiopharmaceuticals using Ge detector

Ge detectors are semiconductor detectors and work in a quite similar way that the ionization chambers work for gas detectors. Electrons and holes are generated by the radiation incidents for semiconductor detectors whereas electrons and ions are generated for the ionization chamber. Germanium's electrical conductivity is in between that of insulators such as glass and conductors such as metal. It has about 1 eV energy gap between the valence band and conduction band whereas the gap is equal or more than 5 eV for insulators and none for conductors. When an incident particle or photon hits the Ge atom in the detector, electrons are released from the atom and holes are created where electrons were ejected. These released electrons gained enough energy to leap the band gap and reach the conduction band. These electrons are then moved toward the electrodes by electronics and thus electronic currents are generated. Since Ge is solid material and thus 2000 to 5000 times denser compared to the gas in a gas detector, the Ge detector can

stop the photons much more efficiently compared to the gas detector. Ge detectors are also about 10 times sensitive compared to gas detectors in terms of signal strength as they need only 3 to 5 eV per ionizations while gas detectors need approximately 34 eV.¹⁴

In spite of these obvious advantages, we have not seen the wide spread of Ge detector's use due to the high initial investment and continued high maintenance costs. They require continuous cooling by liquid nitrogen in order to reduce the noise which is due to the current generated by electrons whose thermal energy is big enough to leap the conduction band at room temperature.



Figure 6: Germanium detector at Washington University School of Medicine

c). Zirconium-89 Production

A circular piece of ^{89}Y foil (Alfa Aesar, 064 mm thick, 50 x 50 mm, purity $\geq 99\%$, lot# N25A016) was encased in a Niobium target holder and bombarded with protons which were accelerated to 15 MeV at the beam current $15\mu\text{A}$ on the positive cyclotron (CS-15, Cyclotron Corporation, Berkeley, CA, USA) for 5 hours on 10/20/14. Zirconium-89 was produced via the $^{89}\text{Y}(p, n)^{89}\text{Zr}$ nuclear reaction.¹⁵ The following day, 10/21/14, ^{89}Zr was separated from ^{89}Y using ion exchange Chromatography and collected in an oxalic

solution. 38.1 mCi of ^{89}Zr was collected in a 750 μL of 1 M oxalic solution (50.8 mCi/mL) at 9:20 AM, End of Synthesis (EOS), on 10/21/14. An approximate 0.3 μL of this sample was diluted to 2 mL with Milli-Q water and used for Ge analysis.

d). Radionuclidic Purity test

Radionuclidic purity test was performed using a high purity Ge detector (Canberra, Model# GC 1818, serial no. 02955247) coupled with a spectroscopy amplifier. The system was calibrated on 7/14/16 for energy and efficiency with mixed-radionuclide ml sources standard (Eckert & Ziegler No. 152142-UP412). The data was processed by using the Genie 2000.

Prior to the sample analysis background spectrum was obtained on 10/23/14 starting at 11:44 PM by placing the 2mL of MQ water in the 2mL size centrifuge vial and placed in a sample holder which was positioned 18 cm above the Ge detector. Data was collected for an hour.

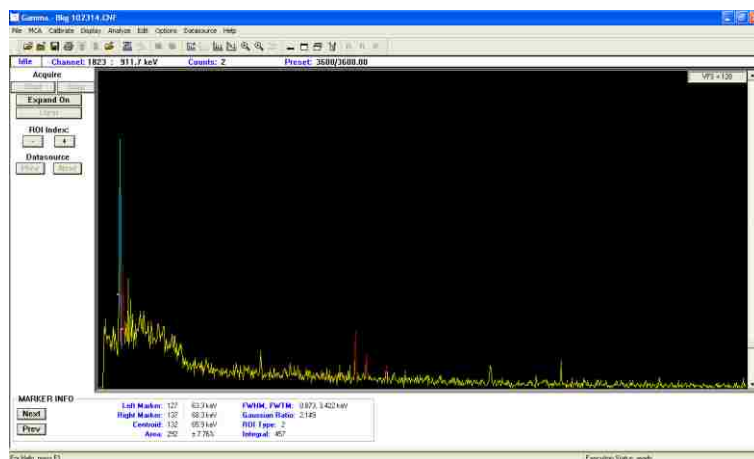


Figure 7: Ge Detector Background spectrum obtained on Oct. 23, 2014.

	Peak No.	Energy (keV)	Net Peak Area	Net Peak Uncertainty	Peak Efficiency	Efficiency Uncertainty
M	1	65.12	9.50E+01	41.32	1.06E-02	3.24E-03
m	2	66.90	1.88E+02	63.60	1.11E-02	2.95E-03
F	3	75.51	6.77E+01	13.10	1.28E-02	1.72E-03
F	4	810.80	6.10E+01	8.48	2.52E-03	9.14E-05
F	5	846.82	3.68E+01	11.04	2.42E-03	8.42E-05
F	6	909.59	1.54E+01	4.32	2.26E-03	7.35E-05

M = First peak in a multiplet region

m = Other peak in a multiplet region

F = Fitted singlet

Table 1: Peak table for background spectrum

Table 2: Peak table for Ge Detector background spectrum

A 2 mL of ^{89}Zr -oxalate sample was placed in the same sample holder, and data was collected for an hour on 10/23/14 starting at 3:38 PM (54 hours after EOS). It was recorded as Live time: 3600 second and Real time: 4069.8 second.

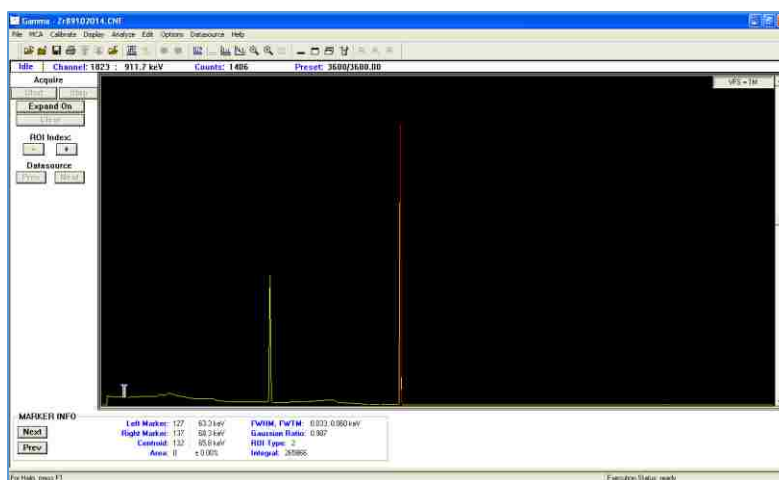


Figure 8: Ge detector Sample spectrum obtained on Oct. 23, 2014. (Full scale)

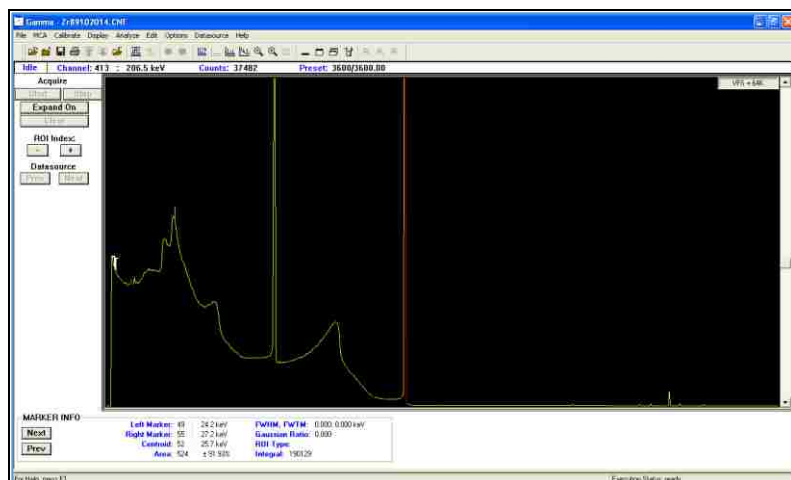


Figure 9: Ge Detector Sample spectrum obtained on Oct. 23, 2014. (Zoomed in scale)

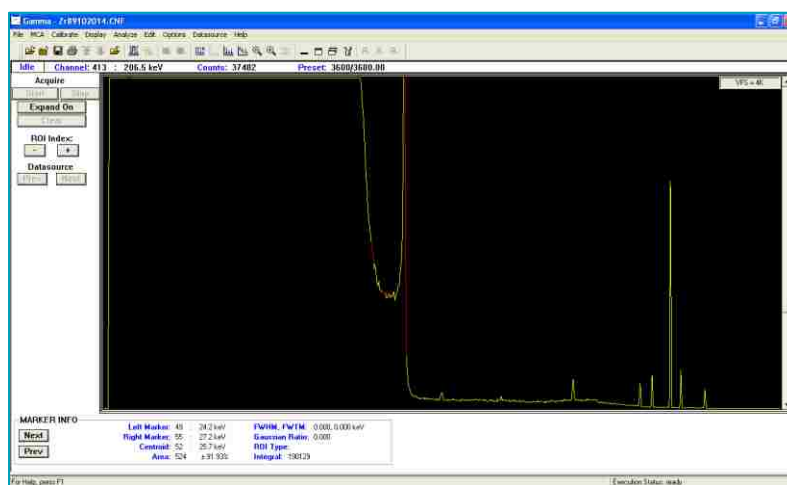


Figure 10: Sample spectrum obtained on Oct. 23, 2014. (Further zoomed in scale)

	Peak No.	Energy (keV)	Net Peak Area	Net Peak Uncertainty	Peak Efficiency	Efficiency Uncertainty
F	1	74.96	2.63E+03	353.37	1.27E-02	1.78E-03
F	2	84.77	3.62E+03	348.71	1.41E-02	8.88E-04
F	3	510.96	2.21E+06	4301.3	4.10E-03	1.69E-04
M	4	707.11	9.84E+03	1003.57	2.89E-03	1.14E-04
m	5	709.81	1.01E+04	1063.11	2.88E-03	1.13E-04
F	6	909.07	3.11E+06	2370.76	2.26E-03	7.36E-05
F	7	1202.25	3.37E+02	130.28	1.76E-03	5.85E-05
F	8	1312.53	6.76E+01	22.49	1.63E-03	5.95E-05
F	9	1419.14	2.00E+03	193.38	1.52E-03	5.94E-05
F	10	1621.04	1.29E+03	36.03	1.35E-03	6.11E-05
F	11	1657.44	1.80E+03	91.92	1.33E-03	6.31E-05
F	12	1713.14	1.29E+04	178.55	1.29E-03	6.79E-05
F	13	1744.71	2.07E+03	51.54	1.26E-03	7.16E-05
F	14	1816.86	1.29E+03	32.77	1.22E-03	8.28E-05

M = First peak in a multiplet region
M = Other peak in a multiplet region
F = Fitted singlet

Table 3: Ge detector Peak table for sample spectrum

e). Data Analysis

The sample spectrum showed two significant peaks at 511 (peak No. 3) and 909 keV (peak No. 6), which corresponded to characteristic gamma ray emissions from ^{89}Zr .¹⁶ The peak at 511 keV is the annihilation gamma ray emission due to ^{89}Zr 's positron decay, and the peak at 909 keV is actually a gamma ray emission from $^{89\text{m}}\text{Y}$ (half-life 15.7 s) as 99.0% of ^{89}Zr decays to $^{89\text{m}}\text{Y}$ by either electron capture (76.6%) or positron decay (22.3%) and 100% of $^{89\text{m}}\text{Y}$ decays to ^{89}Y by isomeric transition and emits 909 KeV gamma ray.¹⁶

The peak at 1621 keV (peak No. 10), 1657 keV (peak No. 11), 1713 keV (peak No. 12), and 1744 keV (peak No. 13) are also associated to Zr-89. As mentioned above 99.0% of ^{89}Zr decays to $^{89\text{m}}\text{Y}$, and the remaining ^{89}Zr decays to the different energy level of Yttrium such as $^{89\text{g}}\text{Y}$ by electron capture. Then they decay to ^{89}Y by emitting gamma rays at these energy level. In addition, peaks at 707, 710, 1202, 1419, 1817 keV are also associated to ^{89}Zr .¹⁶ The peaks at 707.11 and 709.81 keV appear to be the Compton scatters from 909.07 peak as the both peaks are not distinct sharp peak themselves but rather in a same broader peak. The peak at 1202.25 keV is a peak from 1713 peak which went through pair production ($1713-511=1202$). The peak at 1419.14 keV is a sum peak of 909 keV and 511 keV peaks ($909+511=1419$). The peak at 1816.86 keV is also a sum peak of 909 keV and 909 keV ($909+909=1818$). The only identified peak which is not associated to Zr-89 is the peak at 1313 keV. This is from $^{48}_{23}\text{V}$ (Vanadium-48) which was present in the same room when Zr-89 was analyzed.¹⁶ The peaks at 75 and 85 keV could not be determined well. However, the peak area from these 2 peaks are small, thus they do not affect the ^{89}Zr sample's radionuclidic purity.

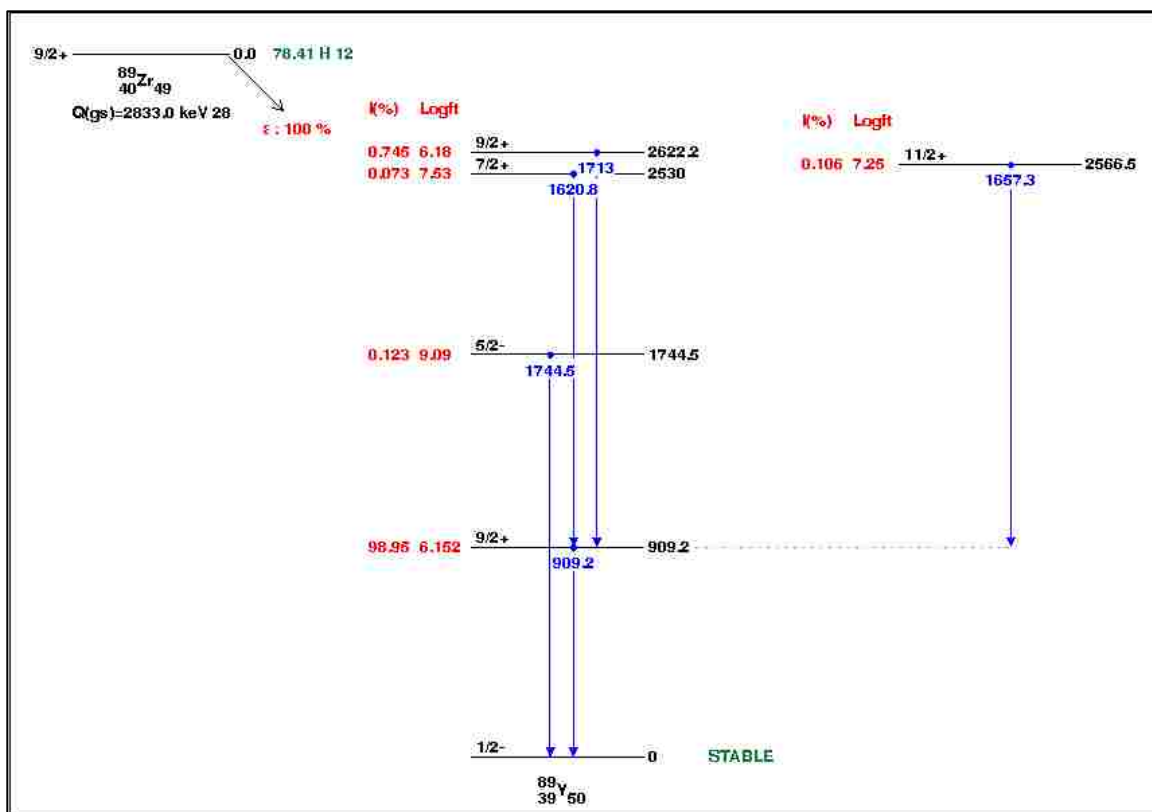


Figure 11. Zirconium-89 decay scheme¹⁶

f). Discussion

The sample's radioactivity was approximately 10 μCi at the time of analysis. This made the dead time larger than desired. The dead time was 11%, some combined peaks were observed. It could have avoided if the sample was more diluted. It is better to keep the dead time less than 5%, and I will use more diluted sample for the future analysis.

The spectrum data were also processed using the software's library function for comparison purpose. When data was processed with library, the peaks at 75, 85, 707, 710, 1202, 1419, 1621, and 1817 keV are labeled as unidentified peaks. It appears the data analysis process using library did not take the Compton scatter or peak combination into

consideration. The result using library would be more accurate if sample was diluted more in order to reduce the dead time, thus eliminating the possibility of having combined peaks.

At Washington University, ^{88}Zr and $^{87\text{m}}\text{Y}$ are listed as potential radionuclidic impurities in ^{89}Zr labeled antibody. For ^{88}Zr , it can be produced by $^{89}\text{Y}(p, 2n)^{88}\text{Zr}$ reaction. When the Yttrium target is bombarded with protons which are accelerated to 13 MeV, the possibility of producing the ^{88}Zr is very small. However if the protons are accelerated to greater than 13 MeV, the possibility of producing ^{88}Zr arises.⁸ The ^{88}Zr as impurity in ^{89}Zr would act just like ^{89}Zr in terms of radiochemistry and internal localization.

Zirconium-88 would be incorporated into Zr labeled antibody production and would have the same fate as the ^{89}Zr for localization. The ^{88}Zr decays to ^{88}Y by electron capture, and its major radiation emission are 272 keV and 399 keV gamma rays.¹⁶ Having Zr-88 as impurity in ^{89}Zr radiopharmaceutical is a concern in terms of dosimetry due to its longer half-life (83.4 days). However, as the majority of the ^{89}Zr labeled antibody as well as ^{88}Zr labeled antibody are cleared from the body over a period of weeks, the increased radiation dose due to ^{88}Zr would not be significant as long as the level of ^{88}Zr is kept low enough. At Washington University, the upper limit for the ^{88}Zr in ^{89}Zr labeled antibody is set to be 0.176 %. This means when typical dose of 2.5 mCi of ^{89}Zr labeled antibody was administered to the patient, the residual ^{89}Zr radioactivity would be 2.4 μCi after 10 half-lives (33 days). If the 2.5 mCi of ^{89}Zr labeled antibody contained 0.176% of ^{88}Zr , which is the maximum allowable concentration of ^{88}Zr , the residual radioactivity of ^{88}Zr would be 3.3 μCi after 33 days. Since majority of the Zr-labeled antibody would have been cleared from body by then, it would not cause a significant radiation risk to the patient.

For ^{87m}Y , it can be produced by $^{89}\text{Y}(p, p2n)^{87m}\text{Y}$ reaction when proton energy is 15-45 MeV. Yttrium-87m decays to ^{87}Y by isomeric transition by emitting 380.79 KeV gamma ray and its half-life is 13.37 hours.¹⁶ The possibility of having ^{87m}Y in the product is very small as all Y should be eliminated from Zr when Zr was separated from Y using ion exchange chromatography. Also, Yttrium-87m is not as problematic in terms of dosimetry compared to ^{88}Zr due to its relatively short half-life. At Washington University, the upper limit for the ^{87m}Y in ^{89}Zr labeled antibody is also set to be 0.176 %.

g). Conclusion and summary

Germanium (Ge) analysis is an important analysis to check the radionuclidic purity in the radiopharmaceutical as Ge detector is capable to detect the small amount of disintegration of radionuclide. Radionuclidic impurity in any radiopharmaceutical is a concern as the impurity causes the additional radiation dose to the patient and potential undesirable localization. At Washington University, particular attention to the proton energy is made in order to make radionuclidic impurities in ^{89}Zr as low as possible and Ge analysis is performed for every ^{89}Zr radiopharmaceutical production batch as routine quality control testing to assure the ^{88}Zr and ^{87m}Y contamination is less than 0.176%. I have performed radionuclidic purity test for the ^{89}Zr Produced on Oct. 20, 2014, and found the radionuclidic purity of this batch to be 100%.

h). Learning outcome from this project

Prior to this project, I had never used Ge detector for radionuclidic purity analysis. I knew the concept of Ge detector, but did not have in-depth knowledge. Through this project, I had the opportunity to talk to operators and a graduate student who was

knowledgeable and was frequently using the Ge detector at that time. I was able to obtain practical and theoretical knowledge about Ge detector. I was also able to educate myself by reading text books about Ge detector. Additionally, I was able to expand my knowledge about cyclotron bombardment when I was examining the methods to avoid producing impurities in ^{89}Zr . All of these helped me to obtain the deeper knowledge about Ge detector which I absolutely needed.

However, as I became more involved in this project, it became more clear that I need to gain additional knowledge about radionuclidic purity. That is especially true for setting up the maximum allowable limit for impurities. The Washington University use the same limits for radionuclidic impurities (0.176% for both ^{88}Zr and $^{87\text{m}}\text{Y}$) as the academic institution, MSKCC, used in their IND since we were referencing their IND. Although I had tried, I was not able to find the clear reasons how these limits were set. I was able to back calculate and assure that the level of impurities should not be a concern for as long as the limit was not exceeded. I would like to improve my knowledge regarding the internal dose calculation, which is needed to establish the impurity limit.

Project 3: Establishing an environmental monitoring (EM) for the 21 CFR Part 212 manufacturing processes for the WUSM Cyclotron Facility and Nuclear Pharmacy

a). Historical background of WUSM Cyclotron Facility and Nuclear Pharmacy

Washington University has a long history of pioneering work for PET tracers. The University installed the first cyclotron for medical use in the United States in 1963 under the direction of Michel Ter-Pogossian, Ph.D. Dr. Ter-Pogossian and his group developed one of the earliest PET scanners in the 1970s. Since then, under the direction of late Michael J. Welch, Ph.D. followed by Robert H. Mach, Ph.D., their research laboratory, Radiological Sciences research group, had been very active in developing novel PET tracers. The WUSM Cyclotron Facility and Nuclear Pharmacy was first opened in 2001, as part of Radiological Sciences research group, in the East Building on Washington University medical campus to provide ^{18}F -Fludeoxyglucose (FDG) for clinical and clinical research at Washington University. Sally Schwarz, M.S., R.Ph, B.C.N.P, Professor in Radiology at Washington University, Co-Director of WUSM Cyclotron Facility and Nuclear Pharmacy, has been overseeing the PET RaPh productions since the beginning of the WUSM Cyclotron Facility and Nuclear Pharmacy. She is working with an engineer who is also the Co-Director of WUSM Cyclotron Facility, and with other nuclear pharmacists. WUSM Cyclotron Facility obtained a Nuclear Pharmacy license and a Distributor's license from the State of Missouri Board of Pharmacy in 2002. As the needs for PET RaPhs grew, the number of personnel, office space, and additional space were added to meet clinical and research demands. In 2013, after Dr. Robert Mach left for another academic institution, WUSM Cyclotron Facility and Nuclear Pharmacy

became a Recharge Facility, an entity within the university that provide fee-based services to the Washington University community, under Robert McKinstry, MD.

In 2014, the WUSM Cyclotron Facility and Nuclear Pharmacy went through a big expansion when new cyclotron (TR19/9), an ISO7 GMP area for production, and a separate QC room were added to the original facility. The ISO 7 area contains 19 manufacturing hotcells, 3 dispensing LAFHC, and 2 biosafety cabinets.

In 2015, WUSM Cyclotron Facility and Nuclear Pharmacy obtained a NRC Commercial Radiopharmaceutical Distribution License to allow distribution of PET drugs to other states. We then obtained another Drug Distributor licenses in Kansas and Iowa in order to ship investigational PET RaPhs to these states as the PET RaPh manufacturing work expanded.

b). Historical background for regulations for PET RaPhs

The most commonly used PET drug today is ^{18}F -FDG which was developed in the 1970s. When the use of ^{18}F -FDG and other radiolabeled PET products which had been prepared under the practice of pharmacy became wide spread in 1990s, FDA realized the need to regulate PET drugs for clinical use. In 1995, FDA issued a Federal register notice which indicated FDA's intention to regulate PET Drug manufacturing as a traditional drug manufacturer under 21 CFR Part 211, traditional therapeutic drug manufacturing good manufacturing practice.^{17,18} However stakeholders who were then involved in PET pharmaceutical manufacturing found the FDA's proposed plan to be unworkable and urged FDA to re-consider. As a result, in 1997, specific language regarding the regulation for PET Drug manufacturing was added to the Food and Drug Modernization Act

(FDAMA), which directed FDA to take unique environment of PET Drug manufacturing into consideration and establish CGMP requirement for PET drugs.¹⁸

On December 10, 2009, 12 years later, FDA issued the final rule for title 21 CFR 212 Current Good Manufacturing Practice for Positron Emission Tomography Drugs (21 CFR Part 212 CGMP for PET Drugs) in the Federal Register.¹⁹ FDA concurrently issued the guidance titled “PET Drugs-Current Good Manufacturing Practice (CGMP)” to express FDA’s current thinking regarding compliance with the new PET CGMP requirement.²⁰ 21 CFR 212 CGMP for PET Drug described the minimum standards for PET drug manufacturing which applied to ¹⁸F-FDG, ¹³N-Ammonia, and ¹⁸F-sodium Fluoride, and became effective on 12/12/11. Upon FDA’s issue of 21 CFR 212 CGMP for PET Drugs, all PET manufacturers, both academic and commercial institution, were required to submit a New Drug Application (NDA) or Abbreviated New Drug Application (ANDA) in order to continue manufacturing these 3 PET Drugs mentioned above for clinical use after Dec., 2011. FDA later announced, using enforcement discretion, extension of the date until June 12, 2012. According to the 21 CFR 212, the provision of United States Pharmacopeias (USP), Chapter <823> then titled as “Radiopharmaceuticals For Positron Emission Tomography-Compounding” (USP 32/NF 27 version) could apply when the PET drug is produced under Investigational New Drug Application (IND) for phase 0, 1, and 2, or through approval of a Radioactive Drug Research Committee (RDRC).¹⁹⁻²¹ The PET drug producer also has the option to follow 21 CFR 212 for these cases.

c). PET RaPhs productions at WUSM Cyclotron Facility and Nuclear Pharmacy

The productions of PET RaPhs at WUSM Cyclotron Facility and Nuclear Pharmacy have been carried out under the USP, Chapter <823> prior to 12/12/11 since there was no official regulation for PET RaPhs production issued by FDA.²¹ Since Dec. 2011, the productions of ANDA drugs (¹⁸F-FDG and ¹³N-Ammonia) have been carried out under 21 CFR 212 CGMP for PET Drugs. Production of ¹¹C-Choline have been carried out under 21 CFR 212 since validation tests were performed for submission of an ANDA, and its ANDA application was submitted to the FDA in 2015. The ANDA for ¹¹C-Choline is still under FDA review, thus WUSM Cyclotron Facility and Nuclear Pharmacy has not begun its production for clinical use.

The work which we perform as practice of pharmacy has been carried out under the USP Chapter <797> Pharmaceutical Compounding—Sterile Preparations, while ensuring we comply with the regulations set by state and federal laws.²² FDA clarifies the distinction between PET Drug Production and the Practice of Pharmacy in their guidance, stating “FDA has determined that the production of a PET drug includes all operations to the point of final release of a finished dosage form, and these activities will be subject to CGMP. A PET drug product may be released to a hospital, institution, imaging facility, nuclear pharmacy (e.g., pharmacy bulk packages for use in accordance with USP Injections), or other entity or part of an entity. After a finally released PET drug product is received by the receiving facility, FDA generally regards subsequent dispensing of a patient-specific dose and use of the drug product to be part of the practice of medicine and pharmacy. FDA generally will defer to State and local authorities concerning regulation of these activities. In general, a routine FDA inspection to ensure compliance

with CGMP would focus on activities up to and including the point of final release of a PET drug product.”²⁰

WUSM Cyclotron Facility and Nuclear Pharmacy has generated numerous number of Standard Operating Procedure (SOP) and Quality Control Procedure (QCP) for each procedure the personnel need to follow. These SOPs and QCPs are revised when any changes are needed, and reviewed annually. In addition, many forms have been generated to record the activity, and these forms are routinely reviewed by upper management. All documents are controlled, and changes are tracked for record keeping purpose.

d). My involvement in facility improvement

In 2007, after becoming a licensed pharmacist in Missouri, I became involved in improving the facility, which included establishing an appropriate Environmental Monitoring (EM) system. Since then, as the work has become more centered around manufacturing of PET RaPhs for clinical and clinical research use while still providing radioisotopes to university researchers, we need to be compliant with the respective regulations becomes critical. Over the years, under the direction of the Co-Director of Cyclotron Facility who is also the Quality Assurance Manager and Pharmacist-In-Charge on our pharmacy license, I have established and improved our EM program which includes routine certification of ISO 5 Laminar Airflow Hoods, Laminar Airflow Hotcells, and the new ISO 7 room, viable particle counts testing, and air and contact plate testing. Without question, the biggest trigger for establishing and improving the EM system at our facility is issuing of 21 CFR 212 and guidance by FDA in 2009. When FDA issued 21 CFR 212 and its guidance in 2009, I reviewed the regulation and guidance word for

word and made an action plan to improve our EM system in order to improve the compliance with 21 CFR 212. Since then, I have continued to improve the system and make minor changes in EM program.

WUSM Cyclotron Facility and Nuclear Pharmacy consisted of one laboratory equipped with a negative ion cyclotron (RDS Eclipse, Siemens) which was used for RaPhs production and QC testing, and another laboratory which was used for reagent preparation. We had the LAFHs used for product vial set-up and sterility inoculations tested and certified as ISO 5 by an outside vendor since the Nuclear Pharmacy was begun in 2002. Since then the number of LAFH have increased and different types of engineering control device, such as isolator and Laminar AirFlow Hotcell (LAFHC) were added in our facility. Currently we have 7 LAFHs, 1 isolator and 4 LAFHCs in our facility. All these engineering control devices have been certified as ISO 5 every 6 months. I have made arrangements for these engineering control devices' certification, reviewed the testing results to make sure testing was performed properly. The Certification is performed by an outside vendor based on manufacturer's specification and the requirement of ISO 14644-1. ISO stands for International Organization for Standardization, and they have published more than 21,000 international standard covering all aspects of technology and business.²³ ISO 14644-1 covers the specification of air cleanliness, and classification is based on the number of airborne particulates. (See Table 4 for ISO classifications) The testing usually consists of particle count tests for inside and outside of LAF/LAFHC/Isolator, HEPA filter leak test, velocity profile and volume, face velocity, airflow smoke patterns, lightning intensity, and electrical safety. FDA's recommendation regarding the certification and testing for LAFH or LAFHC can

be found in “Aseptic Workstation” which is under Facility and Equipment in their guidance.²⁰ These test items are in line with their recommendation. Once I receive the certification report which lists the results for the test performed, I review the report and have the Quality Assurance Manager review and sign it. Then I file the report in an appropriate binder. I keep track of certification data for each ISO 5 engineering control device by using the Microsoft excel sheet, and place the updated table of certification date on the bulletin board in our office. For the general laboratory area, particle count tests are performed monthly to make sure it meets ISO 8 standard.

Class Name		Particle Count	
ISO Class	U.S. FS 209E	ISO, m^3	FS 209E, ft^3
3	Class 1	35.2	1
4	Class 10	352	10
5	Class 100	3,520	100
6	Class 1,000	35,200	1,000
7	Class 10,000	352,000	10,000
8	Class 100,000	3,520,000	100,000

²¹ Adapted from former Federal Standard No. 209E, General Services Administration, Washington, DC, 20407 (September 11, 1992) and ISO 14644-1:1999, Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness. For example, 3,520 particles of 0.5 μm per m^3 or larger (ISO Class 5) is equivalent to 100 particles per ft^3 (Class 100) ($1 \text{ m}^3 = 35.2 \text{ ft}^3$).

Table 4: ISO Classification of Particulate Matter in Room Air, Copy from USP Chapter <797>²²

As I mentioned earlier, WUSM Cyclotron Facility and Nuclear Pharmacy went through a facility expansion and added a new shipping area and ISO 7 GMP production area in 2014. These new areas are located just down the hall from the original WUSM Cyclotron Facility and Nuclear Pharmacy area which is still operational for the production of PET RaPhs for human use. One of the biggest challenges we needed to overcome during the

construction of these new areas was how to maintain a clean environment for the original WUSM Cyclotron Facility and Nuclear Pharmacy production area as PET RaPh production were carried on during the construction. During the construction, the construction area was tightly sealed with plastic partition to prevent the dust and particles from entering into the production area. The particle count tests were performed daily in LAFH, in LAFHC, general production area, and reagent preparation area to ensure the environment meets for ISO 5 standard for LAFH and LAFHC, and ISO 8 for general production area and reagent preparation area, and I reviewed the results daily until completion of facility expansion.

To routinely monitor the environment during the final product set-up or sterility sample inoculation in LAFH, we have been using air settling plates (APs) which contain tryptic soy agar for airborne microbial testing and contact plates (CPs) which contain tryptic soy agar with lecithin and polysorbate 80 for surface microbial testing while our personnel set up final product vials since 2009. FDA's recommendation regarding microbiological monitoring can be found in Facility and Equipment section in their guidance.²⁰ It states; "We recommend that microbiological monitoring (e.g., using settle plate) in the LAFW be conducted during sterility testing and critical aseptic manipulation." Also in the same guidance, Environmental and Personnel Monitoring section in Microbiological Control on Aseptic Processing and Sterilizing Filtrations section under Production and Process Control describes FDA's recommendation for microbiological testing. It states; "Environmental and Personnel Monitoring Environmental monitoring is crucial to maintaining aseptic conditions. We recommend that microbiological testing of aseptic workstations be performed during sterility testing and critical aseptic manipulation.

Methods can include using swabs or contact plates for surfaces and settling plates or dynamic air samplers for air quality.”²⁰

In our facility, APs are un-covered when personnel start his/her procedure, and then covered one hour later unless the procedure takes longer than 1 hour. Additionally one AP is used for monitor the environment outside of LAFH weekly. Personnel need to use one CP to touch the surface of workplace in LAFH at the end of procedure, and another CP to touch his/her gloved fingers. These APs and CPs are incubated at 30-35⁰C for 72 hours or up to 120 hours if the 72 hours falls on weekend, then read for the number of colonies observed. When we started using these plates for EM in 2011, we used 2 CPs, but only 1 AP to monitor the air environment during the final product set-up or sterility sample inoculation in LAFH. However, when we had an outside audit in 2012, the auditor recommended us to use 2 APs, one for each side of LAFH to better monitor the air environment. We followed his recommendation and have been using 2 APs since then. Once these AP and CP plates are read by QC personnel for the number of colonies observed at the end of the incubation period, the results are recorded in the log sheet which I review and file in an appropriate binder once log sheet is completed. QC personnel dispose the finished plates if there is no colony observed as normal trash. They notify me when the number of colonies observed exceed its alert or action level prior to taking these plates to Microbiology lab for identification of microorganism species, or dispose the plates with colony as biohazard waste if the number of colonies observed does not exceed its alert/action level (see Table 6 below). If the numbers of colonies observed on any AP or CP excluding AP used outside exceeds the action level (excluding AP used outside on room air), I remove the personnel who used the particular plate from

his/her duties which require the use of aseptic techniques, and re-train the personnel.

During the re-training session, I discuss the potential cause of the microbial contamination and give my advice as to how to improve his/her aseptic techniques. Then I work with them during the re-training media fill test until they pass three consecutive media fill tests. If the colony was found on either AP or CP but did not exceed the action level, the personnel can continue perform their job duties. Then once I receive the results from Microbiology lab, I inform the personnel who used the particular AP or CP with the results and give my comments regarding how to prevent the contamination and what actions are needed to correct the situation. If the colony on AP or CP which was used for hood surface was identified as fungus, then I make sure the LFH or LAFHC was cleaned thoroughly with sporicidal agent as well as sterile 70% Isopropanol (IPA). Currently we are cleaning LAFH, LAFHC, and Biosafety cabinet with sterile 70% IPA every time we use, and use sporicidal agent weekly as disinfecting the device with sporicidal agent takes more time, and it is not very practical to do so daily. However if I saw a trend of additional fungal colonies in the results, then I would need to take appropriate actions, which includes increasing the frequency of using a sporicidal agent, changing the type of sporicidal agent used, reviewing the method of sporicidal agent application, observing personnel during cleaning or reviewing the sampling method.



Figure 12. Pictures of WUSM Cyclotron Facility production area

(left) original PET production area, (middle) contact plate test for gloved fingers (right) Performing aseptic process in a LAFHC

During the process of establishing this EM system, I originally set action levels for each plates based on the recommendation found in USP Chapter <797> as I was not able to find the recommendation in 21 CFR 212 or its guidance, and the recommendation from USP Chapter <797> appeared to be appropriate.²² After working with an external auditor, who was a microbiologist, I added an alert level to each plate as the auditor suggested this would be a good practice. (See tables below for recommended action level for Microbial contamination listed in USP Chapter <797>, and our alert/action level listed in WUSM Cyclotron Facility and Nuclear Pharmacy SOP.) I also met the manager of Microbiology laboratory to establish the procedure for sample submission, and generated sample requisition forms, and implemented the change in our facility. In addition to the EM testing performed during final product set-up and sterility sample inoculation, we also perform EM testing for LAFHC where we withdraw QC samples from final product vial once the production is completed, and also during clinical research dose drawing. This EM testing for LAFHC is performed weekly by using 2 APs, one for inside and one for outside, and 7 CPs for various locations inside LAFHC using sterile cotton swabs.

Moreover, I implemented routine EM testing with contact plates for chemistry modules used for ^{18}F -FDG production in 2015. Today, I continuously monitor EM results, communicate personnel for Microbiology lab results, and present the past year's EM results to the group when I give annual aseptic didactic training to facilitate further discussion for improvement. Table 5 below is also referenced in USP Chapter <797>.

Classification	Fingertip Sample	Surface Sample (Contact Plate) (cfu per plate)
ISO Class 5	> 3	> 3
ISO Class 7	N/A	> 5
ISO Class 8 or worse	N/A	> 100

Table 5. Recommended Action Levels for Microbial Contamination in USP Chapter <797>²²

Alert / Action levels for each plate		
Type of Plate	Alert Level (CFU*)	Action Level (CFU*)
AP Inside	n/a	> 1
AP Outside	> 5	> 10 CFU
CP Hood	> 1	> 3
CP Finger	> 1	> 3

CFU: Colony Forming Unit

Table 6: Alert/Action Levels for each plate at WUSM Cyclotron Facility and Nuclear Pharmacy, Copy from WUSM Cyclotron Facility and Nuclear Pharmacy SOP 738 Environmental Monitoring of ISO Class 5 Laminar Airflow Hoods

When we added the new ISO 7 GMP room which consists of garbing area, intermediate area, main production area, and dispensing area, to our existing facility in 2012, I needed to assure that the area which has been qualified as ISO 7 by an external company is controlled and maintained using more stringent protocols. During my past pharmacy employment in Japan, I had worked in clean room environment on daily basis, and this assisted me in making the appropriate choices for this area. I selected the garbing materials and established the garbing procedure. I also established the cleaning procedure in the ISO 7 GMP room. During this initial stage, I repeatedly reminded our personnel that this new area required a more stringent approach in terms of garbing, cleaning, and work behavior in order to maintain the ISO7 status.

When this ISO 7 GMP room was completed, I had the room tested for air exchange rate, room pressure, HEPA filter leak test, and particle count test for inside of LAFH, LAFHC, and Biosafety cabinets as well as room itself. The test went smoothly except one HEPA filter which supplies air into the room was found to have a leak. The leak was repaired by the vendor, who was performing the testing for certification, and all LAFH, LAFHC, and Biosafety cabinets were certified as ISO5, and the room itself was certified as ISO7. The air quality in the room was found to be very close to ISO 6. Since then I have the ISO7 GMP room tested and certified as ISO 7 every 12 months, and LAFH, LAFHC, and biosafety cabinets for ISO5 every 6 months. I have been successful in establishing procedures which are appropriate for maintaining ISO 7 area since we have passed the external certification for the ISO 7 are for 2 years.



Figure 13. Images of ISO 7 GMP area certification process

(left) HEPA filter in the ceiling, (middle) HEPA filter leak test, (right) ISO5 smoke test

I also incorporated EM system into media fill tests which our personnel need to performed in our facility. I have been responsible to provide aseptic training to our personnel since 2007. The 21 CFR 212 required that PET facility must have a sufficient number of personnel with the necessary education, education, background, training, and experience to perform their assigned functions.^{19,20} USP Chapter <823> also requires all aseptic operations must be performed by operators qualified to work with aseptic techniques, and all aseptic area operators are trained and evaluated periodically through observation as well as through microbiological tests. It also states that aseptic techniques used to make sterile products are evaluated by simulations. In April, 2012, FDA also published the Guidance –Media Fills for Validation of Aseptic Preparations for Positron Emission Tomography (PET) Drugs in response to stakeholders’ requests.²⁴ In our group, APs and CPs are used during the simulation process of the final product set-up, QC sample withdrawal from final product vial, and sterility test inoculation. Alert and action level for each plate are the same as for our routine EM.

For ISO7 GMP room, I have been conducting viable particle counts testing to monitor the air borne viable detection using impactor pump (Anderson Impactor Pump) and APs we usually use for routine EM testing. See below.



Figure 14. Images of viable particle count test

(left) particle compactor (right) viable particle count test

A viable particle is a particle which contains one or more living microorganisms. For this test, active air sampling of 333 liter with an impactor is performed by collecting the air sample for 12 minutes at 28.3 ml/min at 5 different locations. Room condition can be static or active, and it is recorded on the report. All air settling plates are incubated at 30-35°C for 72 hours or up to 120 hours if the 72-hour time point falls on weekend. After the incubation, the plates are checked for the number of colonies. The results are reported to the Quality Assurance Manager. So far I have never observed colonies on any plates for the viable particle tests performed. If colonies were observed, I would take the plates to Microbiology lab for identification, and take appropriate actions.

Another EM we routinely perform is temperature check for rooms and various temperature controlled devices. The temperatures for refrigerators, freezers, incubators, and production area, and material storage area are checked and recorded by visually reading calibrated thermometer by our personnel every business day to ensure these temperatures are within the specified range. The results are reviewed and filed by pharmacist.



Figure 15. images of ISO 7 GMP area at WUSM Cyclotron Facility and Nuclear Pharmacy

(left) dispensing area, (middle) F-18 RaPh production area, (right) personnel garbing in ISO7 GMP area

e). FDA Inspections

In September, 2013, the WUSM Cyclotron Facility and Nuclear Pharmacy was inspected by the FDA as a pre-approval inspection for two ANDA drugs, ^{18}F -FDG and ^{13}N -Ammonia. The FDA Inspector conducted her inspection for 6 business days. At the conclusion of inspection, we have received FDA Form 483 which listed objectionable conditions of our operation. Only one of the items listed on Form 483 was related to EM system. The inspector noticed that there was no EM performed on the interior Lucite cover for the hotcell. The lucite cover is removed after mechanically lowering the front

window panel of the hotcell to allow operator clean the inside of LAFHC at the beginning of business day. I revised our SOP entitled “Environmental Monitoring of a Laminar Airflow Hot Cell Equipped with Manipulator Arms” to include a contact plate test with a sterile cotton swab on the Lucite cover. The response was sent to the FDA’s Director of Compliance in District Office along with other responses in October 2013. We have received FDA’s approval for both ^{18}F -FDG and ^{13}N -Ammonia in February 2014.

In March-April, 2016, the WUSM Cyclotron Facility and Nuclear Pharmacy was again inspected by the FDA as post-approval inspection for ^{18}F -FDG and ^{13}N -Ammonia and pre-approval inspection for ^{11}C -Choline. Additionally, the ISO7 area of the WUSM Cyclotron Facility and Nuclear Pharmacy was also inspected for the FDG ANDA CBC30 amendment. This time the inspection lasted for 2 weeks. There were several items related to EM listed on Form 483 for this inspection. One violation listed was that the air flow at the work surface level was not evaluated in the LAFHC bi-annual evaluation. The test results were indeed not recorded on the report, but we confirmed with the vendor who performed the certification that they always evaluate the air flow at the work surface level according to the manufacturer’s specifications. In April, 2016, we completed another LAFHC certification, and sent the report which listed the results for the air flow at the work surface level to FDA. Another EM related violation listed on Form 483 was that we did not conduct EM of LAFHC located in ISO 7 GMP room during the production of ^{11}C -Choline and ^{18}F -FDG validation batches. The EM for this LAFHC was not performed by our personnel since we had not started using this LAFHC for ANDA PET drug production for human use. Routine EM in this LAFHC was added starting on

October 2015, and we continue to conduct the EM testing weekly and the pharmacists review and sign the record. The last EM related violation on Form 483 was that we did not record the start and stop times-for open and closure times for the AP. For this, we have generated another form to record the start and stop times and pharmacists review and sign the record. The responses to these items were sent to the FDA's Director of Compliance in the District Office along with other responses in April 2016.

For both inspections I worked with the inspectors and assisted the Co-Directors. I was involved in responding to the inspectors' observations both by generating or modifying documents, implementing changes in procedures, and generating response for some of the observation items. Prior to these inspections, we had received inspections from Missouri Board of Pharmacy for our work performed under the practice of pharmacy, but not from FDA. These inspections helped me to see our operation from the FDA inspector's viewpoints. To me, these inspections are strong reminders that the ultimate goal of our operation is to provide safe radiopharmaceuticals to the public. I believe we were able to improve our operation because of these inspections.

f). Personal gain and future challenges

Prior to my involvement, there was no formal EM system in the WUSM Cyclotron Facility. I was able to establish the EM system and make numerous modifications to improve our EM system. Because our group started as a part of big research laboratory, our personnel were not accustomed to the vigorous EM system needed as a PET Manufacturer and Nuclear pharmacy. Thus the biggest challenge for establishing the appropriate EM system was to change the culture (mind set) of our personnel. I believe I

have overcome this challenge by communicating with them, and emphasizing the importance of the EM system. In other words, the success of this project depended on my leadership skill. From examining the systems I have established, I believe I have exercised leadership and made great contributions by establishing the EM system. Furthermore, to establish an appropriate EM system I needed to be familiar with the regulations, which helped me to gain more knowledge in regulations and guidelines issued by the FDA, NRC and State Board of Pharmacy. I have also gained more knowledge regarding clean room and clean room related materials. In addition, working with inspectors provided me with great experience in how to work with inspectors in order to make the inspection process go smoothly, and in looking at each item in the regulations from different points of view.

I anticipate more challenges related to EM. The challenges relate to the proposal regarding measurement of microbial contamination, which is listed in USP Chapter <1116> Microbiological Control and Monitoring of aseptic processing environments.²⁵ USP Chapter <1116> was revised in 2012, and became one of the most informational chapters from USP. The revised USP Chapter <1116> proposed the measurement of microbial contamination based on Contamination Recovery Rate (CRR) rather than the number of Colony Forming Unit (CFU). USP Chapter <1116> is general information chapter and contains no mandatory specifications as all USP chapters above 1000. USP Chapter <1116> states that the guidance provided in this chapter should be applied only to clean rooms, restricted-access barrier system, and isolators used for aseptic processing. It appears that proposal provided in this chapter, which is a significant shift from numerical levels to a more qualitative trending methodology and shows the USP

committee's current thinking and direction regarding EM. USP Chapter <1116> defines CRR: "The contamination recovery rate is the rate at which environmental samples are found to contain any level of contamination. For example, an incident rate of 1% would mean that only 1% of the samples taken have any contamination regardless of colony number."

According to the suggested initial contamination recovery rate in aseptic environment in USP Chapter <1116>, the CRR for active air sample, settle plate (4 hour exposure), contact plate or swab, and gloves and garment in ISO 5 environment are all < 0.1%. (See below.) When compared these rates to our current frequency of colonies on the EM plates, it appears to be very challenging to meet the suggested CRR of <0.1% for gloved fingers. While I understand the concept and importance of monitoring the frequency of incidence, I anticipate that we would need to pay closer attention to our procedures to improve our operation, and it would not be easy to accomplish in a short time period.

Room Classification	Active Air Sample (%)	Settle Plate (9 cm) 4 hr exposure (%)	Contact Plate or Swab (%)	Glove or Garment (%)
Isolator/Closed RABs* (ISO 5 or better)	< 0.1	< 0.1	< 0.1	< 0.1
ISO Class 5	< 1	< 1	< 1	< 1
ISO Class 6	< 3	< 3	< 3	< 3
ISO Class 7	< 5	< 5	< 5	< 5
ISO Class 8 or worse	< 10	< 10	< 10	< 10

*RABs :restricted-access barrier systems

Table 7. Suggested Initial Contamination Recovery Rates in Aseptic Environments in USP Chapter <1116>²⁵

III. Summary

Being involved in the development of new PET radiopharmaceuticals has expanded my professional experience and knowledge. As I began working with physician researchers, radiochemists, and a nuclear physicist in performing tasks required for the research projects I expanded my viewpoint. I obtained more in-depth understanding of the work needed to bring pre-clinical PET radiopharmaceuticals to clinical research use in humans, which is a tremendous gain for my career as nuclear pharmacist. Also for this project, I had the opportunity to work with physicist for human dosimetry calculation. As we know, radiation dose to the patient is unavoidable when it comes to the use of radiopharmaceuticals, and we, as professional in this field, must make sure the dose is within the acceptable level which is established based on the dosimetry data. I have been involved in writing CMC section, but I had never had hands-on experience for dosimetry as the dosimetry section is always handled by a physicist, or sometimes simply refer to the dosimetry study performed by another institution. Therefore the experience I have obtained by going through each step with physicist for ^{89}Zr -Herceptin is very important to me, and it is something that can add a great value to my academic carrier. Overall, this project gave me great personal and professional satisfaction as the work came to fruition as a poster presentation at 2015 SNMMI annual meeting, the paper was published in 2016, and project will be successfully completed in 2016.^{12,13}

Just as the use of immunotherapies has increased in the medical field, the research project involving ^{89}Zr labeled trastuzumab has increased my interest in ImmunoPET imaging. I have appreciated more fully the potential of this type of research, and became very interested in what new agents will move forward in this field.

Through the Ge analysis project, I was able to obtain hands-on experience with the Ge detector, which I would not have otherwise done while just performing my routine work. I was able to make a connection and understand the relationship between cyclotron bombardment and radionuclidic impurities production. This project helped me to realize the importance of understanding the analytical instrument and its test method as well as the importance of understanding the how the impurities would cause the additional radiation dose to the patient. As a nuclear pharmacist, the most important task is to make sure the radiopharmaceuticals we produce are safe. Thus the knowledge I obtain through this project is priceless for my career.

I also feel that I have established and improved the EM monitoring system for the WUSM Cyclotron Facility and Nuclear Pharmacy facility, and I have added to the quality assurance system in our work environment. Developing this system allowed me to demonstrate the process to the FDA inspectors during our facility inspection. I was able to provide information and answer questions that assisted in having a positive outcome for our facility. Also because of this project, I had opportunities to work with a Microbiologist, a Quality Assurance personnel from another GMP facility which is on the same Washington University medical campus. This project required me to take initiatives and work as a leader for our Cyclotron Facility, which was a great experience. I needed this opportunity to demonstrate that I understand the requirements and that I am capable of advocating for what I believe is correct. In addition because of this project, I recognized again the importance of having a proper documentation system, and working on details while not losing site of the big picture as our ultimate goal. Overall, this EM project helped me to strengthen and showcase my leadership and decision making skills.

All three projects have helped me grow professionally in different areas. In addition, I have also learned to become a leader for our group which consists of individual members who do not necessarily have background related to pharmacy, in order to improve the level of safety of our radiopharmaceutical products. Additionally, because I was working on these projects while performing routine tasks, I became better at multi-tasking, prioritizing the tasks required, and letting others perform tasks, and then I would review the results. This also allows other workers to grow in their job. Through these projects, I have learned the importance of having a better balance for my work life.

I have been trying to improve my knowledge and skills in the PET radiopharmaceutical field to become a nuclear pharmacist with specialized skills who can assist others in translating the preclinical work to humans. Without doubt, I will continue to do so in the future as well. However, from the experience I have gained from the three projects mentioned, I have now identified three specific topics to focus on in order to strengthen my expertise. They are 1). therapeutic use of RaPhs, 2) internal dose calculation for radionuclidic impurities in RaPhs in order to establish limits for human administration, and 3) additional facility improvement in terms of control of microorganisms. In a busy facility it is necessary to remain focused on daily activities, but identifying these new topics will definitely help me stay focused on these topic in order to strengthen my expertise. I also plan to keep working on my leadership skills, so I can play a leading role at my work place and the nuclear pharmacy field. I feel that PET has great potential in the healthcare system for providing high quality personalized medicine for patients both in terms of diagnosis and therapeutic benefits, and I would like to contribute to the further development of this field as a nuclear pharmacist.

IV. APPENDICES

A. Raw Material Acceptance Sheet for *p*-isothiocyanatobenzyl-desferrioxamine

(Df-Bz-NCS)

B. QC Release Specification for ⁸⁹Zr-trastuzumab

APPENDIX A. Raw Material Acceptance Sheet for *p*-isothiocyanatobenzyl-desferrioxamine (Df-Bz-NCS)

Washington University in St. Louis	
SCHOOL OF MEDICINE	
Cytogen Facility	
Raw Material Acceptance Testing	
Raw Material:	Desferrioxamine-p-NCS 10mg
MIR Code:	Rfb-021
Lot/Barcode:	Desferrioxamine-p-NCS 70014
Description:	Desferrioxamine-p-NCS is a white powder packaged in an amber bottle. Supplied as 10mg.
Specifications:	Chromatographic Purity at 270nm: >99% (max 5%)
Acceptable Suppliers:	A. Molecules Inc, 1105 Rosswood Crossing, Dallas, TX 75235 (817) 210-2540
0	
Acceptance Test:	Sample Size:
1. Identification	
Visually inspect package for proper labeling	1 Each (1x1)
2. Description	
Visual inspection matches description above	1 Total (1x1)
3. Specifications	
Check manufacturer's COA	1 Each (1x1)
Accepted by (MIR):	Test Date:
	4/22/15
2015-04-22	

Appendix B. QC Release Specification for ⁸⁹Zr-trastuzumab

TEST	ACCEPTANCE CRITERIA	PROCEDURE	TESTING SCHEDULE
Filter Membrane Integrity Test	≥ 50 psi	Bubble Point Test (see SOP 427)	Pre-release; each batch
pH	5.0 to 6.5	Narrow range pH paper (see QCP 801)	Pre-release; each batch
Appearance; Color	Clear; Colorless	Visual observation (see QCP 801)	Pre-release; each batch
Strength (Radioactivity Concentration)	0.1 – 1.0 mCi/mL	Dose calibrator (see QCP 802)	Pre-release; each batch
Radionuclidic Purity	≥ 99.5%	Gamma spectroscopy (see QCP 204)	Pre-release; each batch
Radiochemical Purity	≥ 95%	ITLC analysis (see QCP 113)	Pre-release; each batch
Specific Activity	≥ 0.2 mCi/mg	Dose calibrator assay divided by protein mass after purification of ⁸⁹ Zr-Df-Bs-NCS-Herceptin	Pre-release; each batch
Bacterial Endotoxin	≤ 175 EU/V (where V is the maximum total dose)	Chromogenic method (see QCP 501)	Pre-release; each batch
Protein Aggregation	≥ 80 % monomer	HPLC analysis (see QCP 1004)	Post-release; each batch
Sterility	Sterile (No visible growth)	Visual observation (see QCP 502)	Post-release; each batch
Radioimmunoassay	≥ 65 %	Immunoreactivity Assay (see QCP 1008)	Every new lot of conjugated product and first radiolabeled product produced by a new operator

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