FINAL SCRIPT: APPROVED FOR FILMING

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Title: Automated Preparation of [^{68}Ga]Ga-3BP-3940 on a Synthesis Module for PET Imaging of the Tumor Microenvironment

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 28
Number of Shots: 55

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

REQUIRED:

1.1. **Cyril Fersing:** This research focuses on the development of an optimized automated radiolabeling protocol for the preparation of [^{68}Ga]Ga-3BP-3940. This compound is an experimental radiopharmaceutical for PET imaging of the tumor microenvironment.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 1.*

What are the most recent developments in your field of research?

1.2. **Cyril Fersing:** Targeting the tumor microenvironment has gained significant attention in recent years. In the field of radiopharmaceuticals, a key challenge is to develop efficient and safe radiolabeling protocols for their preparation.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.11.1., 2.12.2*

What are the current experimental challenges?

1.3. **Cyril Fersing:** The main experimental challenge in producing ^{68}Ga-labeled investigational radiopharmaceuticals is implementing a preparation protocol that is adapted to both the compound being radiolabeled and the synthesizer used.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What significant findings have you established in your field?

1.4. **Cyril Fersing:** In the field of experimental gallium-68 radiopharmaceuticals, we highlighted that carefully optimizing the automated preparation conditions for a given compound can significantly improve the radiolabeling process.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 6.*

How will your findings advance research in your field?

1.5. **Cyril Fersing:** This protocol addresses the lack of automated gallium-68 radiolabeling of 3BP-3940 and offers a turnkey method for its preparation using a given synthesizer, enabling synthesis in just twenty minutes. Its GMP compliance enables radiolabeling of pharmaceutical-grade 3BP-3940, supporting the clinical application of this innovative radiotracer.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.1, 3.6.2.*

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions:

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Cyril Fersing:** We believe that publishing in JoVE could enhance the visibility and impact of our research. More specifically, we hope this work will help highlight the very particular profession of radiopharmacist-researcher and inspire interest among pharmacy students.

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

1.7. **Cyril Fersing:** Our primary expectations following this work relate to the training of radiopharmacy students. Nevertheless, the increased visibility associated with this publication could also foster new collaborations and funding opportunities.

1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the radiation safety officer of University of Montpellier

Protocol

2. Preparation of the Synthesis Cassette and Its Installation Procedure

Demonstrator: Cyril Fersing

2.1. To begin, unwrap the [68Ga]Ga *(gallium-sixty-eight)* cassette envelope **[1]**. After checking for any damage, tighten each Luer connection on the cassette **[2].** NOTE: The VO has been edited.

2.1.1. WIDE: Talent opening the cassette envelope and removing its contents.

2.1.2. Talent tightening Luer connection by hand.

~~2.1.3. Talent removing spike caps from the cassette.~~ Author's NOTE: This shot overlaps with other subsequent ones. It should therefore be deleted.

2.2. Using a 5 milliliter Luer Lock syringe fitted with a 21-gauge needle, withdraw 5 milliliters of absolute ethanol from the reagent kit and pass it very slowly over the C_{18} *(C-eighteen)* cartridge **[1]**. Then, using the same syringe, withdraw 5 milliliters of water for injection and pass it very slowly over the same cartridge **[2]**.

2.2.1. Talent using Luer Lock syringe and needle to draw ethanol from the reagent bottle and slowly passing ethanol through the C_{18} cartridge.

2.2.2. Talent drawing water for injection using the same syringe and passing it over the cartridge.

2.3. Position ramp A of the tubing set on the synthesis module **[1]**, and turn the two latches to hold it in place **[2]**. Connect the free end of the vertical A1 tubing to a 19-gauge needle and insert it into the waste vial **[3]**.

2.3.1. Talent placing ramp A onto the synthesis module.

2.3.2. Talent locking the latches to secure ramp A.

2.3.3. Talent connecting tubing A1 to a needle and inserting it into the waste vial.

2.4. Insert a venting needle into the waste vial and orient it backward behind the shielded container that will receive the evacuation vial **[1]**. Place a 0.22-micrometer filter in position A4 **[2]**.

2.4.1. Talent inserting venting needle into the waste vial and orienting it backward behind the shielded container.

2.4.2. Talent placing 0.22-micrometer filter into position A4 on the module.

2.5. Connect the horizontal A1 tubing to the pressure sensor located at the bottom left of the module front panel **[1]**.

2.5.1. Talent connecting the A1 tubing into the pressure sensor port.

2.6. Using a male-to-male adaptor, connect a 30-centimeter extension line in the horizontal A5 position, ending in a 0.22-micrometer terminal filter and an 80-millimeter 20-gauge needle **[1]**.

2.6.1. Talent connecting extension line in the horizontal A5 position, ending in a 0.22-micrometer terminal filter and an 80-millimeter 20-gauge needle.

2.7. Insert the 20-gauge needle into a sealed, sterile evacuation vial **[1]**. Then, add an aeration needle **[2]** and place the vial in its shielded container **[3]**.

2.7.1. Talent inserting needle into a sealed, sterile evacuation vial.

2.7.2. Talent placing aeration needle to the vial.

2.7.3. Talent placing the vial in its shielded container.

2.8. Place the tubing line connecting vertical A1 to vertical C1 behind the retaining hooks above ramp B **[1]**.

2.8.1. Talent placing the tubing line behind retaining hooks over ramp B.

2.9. Connect horizontal manifolds A2 and B1 using a short extension line after removing the adapter mounted in position A2 **[1]**. NOTE: The VO has been edited.

2.9.1. Talent connecting manifolds A2 and B1 with a short tubing extension line after removing the adapter mounted in position A2. Author's NOTE: This shot has to be slightly modified.

2.10. Position ramp B on the synthesis module **[1]** and secure it by turning the two latches **[2]**.

2.10.1. Talent placing ramp B on the synthesis module.

2.10.2. Talent locking the ramp B in place.

2.11. Connect the pre-conditioned C_{18} cartridge to the horizontal C2 position, making sure the adapter linking horizontal valve B5 to C2 is kept on the left side **[1]**.

2.11.1. Talent connecting C18 cartridge to the horizontal C2 position and adjusting adapter (linking horizontal valve B5 to C2) placement to the left side.

2.12. Position ramp C on the synthesis module before securing it by locking both latches **[1]**.

2.12.1. Talent placing ramp C on the synthesis module.

2.13. Using a male-to-male adaptor, connect a 50-centimeter extension line from horizontal C5 to the gallium-sixty-eight generator **[1]**.

2.13.1. Talent attaching the extension tubing from C5 to the generator port.

2.14. Place the stained-glass reaction vial of the tubing set in the module oven **[1]**, then carefully place the tubing from vertical A5 to vertical C5 in the peristaltic pump **[2]**. Close the pump **[3]**, and after verifying that the tubing is properly positioned, pass the tubing through the activity sensor on the left-hand side of the pump **[4]**.

2.14.1. Talent placing reaction vial into the oven compartment.

2.14.2. Talent placing the tubing from vertical A5 to vertical C5 in the peristaltic pump.

2.14.3. Talent closing the pump.

2.14.4. Talent passing the tubing through the activity sensor on the pump's left.

3. Reagents Installation Procedure

3.1. Connect the 250-milliliter water for injection bag to C4 tubing using the spike adaptor and hang the bag on the designated hook located on the right side of the module **[1]**.

3.1.1. Talent attaching water for injection bag using spike adaptor and hanging the bag on the right-side hook of the module.

3.2. Using a 3-milliliter three-piece Luer Lock syringe with a 20-gauge needle, withdraw 1.5 milliliters of 10 milligrams per milliliter L-methionine solution **[1]**. Connect the syringe to horizontal C1 tubing and hang it in the designated slot on the right-hand side of the module **[2]**. Leave approximately 2 milliliters of air between the liquid surface and the syringe plunger seal to ensure complete transfer **[3]**.

3.2.1. Talent drawing L-methionine solution into syringe.

3.2.2. Talent connecting the syringe to horizontal C1 tubing and mounting it in the right-hand slot.

3.2.3. Close-up of the air gap between the liquid surface and the syringe plunger seal inside the syringe.

3.3. Using a 1-milliliter syringe with a 20-gauge needle, withdraw 750 microliters of the 10 milligrams per milliliter L-methionine solution and inject it into the 0.9 percent sodium chloride vial after disinfecting the septum **[1]**.

3.3.1. Talent withdrawing L-methionine solution using a syringe and injecting it into NaCl vial.

3.4. Using a 10-milliliter three-piece Luer Lock syringe with a 20-gauge needle, withdraw the contents of the 0.9% sodium chloride and L-methionine vial **[1]**, adjust the volume to 8.6 milliliters **[2]**, remove the spike in position B4 and connect the syringe in B4 instead **[3-TXT]**.

3.4.1. Talent withdrawing solution from vial.

3.4.2. Talent adjusting syringe volume to 8.6 milliliters.

3.4.3. Talent removing spike from B4 and connecting syringe to B4. **TXT: Leave about 2 mL of air between the liquid surface and the plunger seal**

3.5. After disinfecting the septum, withdraw the contents of the 60 percent ethanol vial using a 3-milliliter three-piece Luer Lock syringe with a 20-gauge needle **[1]**. After confirming that the volume is at least 1.5 milliliters, remove the spike from position B5, and connect the syringe in its place **[2]**.

3.5.1. Talent disinfecting septum and drawing ethanol from orange-capped vial.

3.5.2. Talent removing spike from B5 and inserting syringe into B5. **TXT: Leave about 2 mL of air between the liquid surface and plunger seal**

3.6. Using a low dead volume 1-milliliter syringe fitted with a 20-gauge needle, withdraw 0.25 milliliters of 0.8 molar sodium acetate buffer solution and inject it into the vial containing 30 micrograms of 3BP-3940 *(three-B-P-three-nine-fourty)* **[2]**. Solubilize it by successive injection and aspiration cycles **[3]**. Withdraw the 0.25-milliliter solution into the same syringe, remove the needle, and place the syringe into position B3 **[3-TXT]**.

3.6.1. Talent withdrawing sodium acetate buffer into syringe and injecting it into the vial containing 3BP-3940.

3.6.2. Talent performing multiple inject/aspirate actions to dissolve 3BP-3940.

3.6.3. Talent withdrawing the 0.25-milliliter solution into the same syringe, removing the needle, and placing syringe in B3. **TXT: Leave about 0.25 mL of air between the liquid surface and plunger seal**

3.7. Click **Run Synthesis** when all reagents are placed on the ramps and all required information is correctly recorded in the software **[1]**.

3.7.1. Talent clicking on **Run Synthesis** button and the synthesis process begins. *Videographer: If this shot is done on a computer screen, please make sure the*

computer screen is clearly visible in the frame.

4. Dispensing and Quality Controls of [^{68}Ga]Ga-3BP-3940

4.1. Transfer the terminal vial to an appropriate shielded cell to prepare it for radioactivity measurement and patient dose preparation **[1]**.

4.1.1. Talent moving the terminal vial into a shielded cell.

4.2. Measure the radioactivity of the terminal vial using a properly calibrated dose calibrator and record the preparation on the computer **[1]**.

4.2.1. SCREEN: 4.2.1.-Activity.tif

4.3. After labeling the vial correctly, place it inside an appropriate shielded container **[1]**. Using aseptic and radiation protection techniques, withdraw approximately 0.5 milliliters of the sample from the terminal vial for quality control testing **[2]**.

4.3.1. Talent placing the labeled vial in the shielded container.

4.3.2. Talent withdrawing a sample using a syringe from the terminal vial while wearing protective gloves and using shielding.

4.4. Examine the sample visually to assess clarity and color **[1]**. Assess the pH by applying a drop of the product onto a pH paper strip **[2]**.

4.4.1. The withdrawn sample.

4.4.2. Talent applying drop onto pH strip and comparing color against reference chart.

4.5. Deposit a drop of the product solution on each of the two iTLC-SG plates to measure the radiochemical purity by radio-TLC *(radio T-L-C)* **[1]**. Allow the plates to develop in the appropriate mobile phases **[2]**, and read them using the radiochromatograph **[3]**. Integrate the resulting radiochromatogram by measuring the area under the curve of the product signal and the impurity signal **[4]**, then calculate the radiochemical purity according to the formula shown on the screen **[5]**.

4.5.1. Talent applying product drops to both iTLC-SG plates.

4.5.2. Talent inserting the plates into solvent chambers for development.

4.5.3. The radiochromatograph reading of the developed plates.

4.5.4. SCREEN: 4.5.4.-TLC.tif *Video Editor: Highlight the red curve when the VO says "product signal" and the green curve when the VO says, "and the impurity signal".*

4.5.5. TEXT on PLAIN BACKGROUND:

$RCP\ (\%) = 100 - \%AUC_{impurity\ 1} - \%AUC_{impurity\ 2}$

4.6. To measure radiochemical purity using radio-HPLC *(H-P-L-C)*, inject approximately 50 microliters of the sample into the HPLC vial **[1]**. Load the vial into the HPLC autosampler at the correct position and start the analysis sequence **[2]**. After the sample has been injected, remove the vial from the autosampler before returning it to the shielded container to minimize exposure **[3]**.

4.6.1. Talent pipetting and injecting sample into HPLC vial.

4.6.2. Talent placing the vial in autosampler and starting the analysis sequence.

4.6.3. Talent retrieving the vial from the autosampler before returning it to the shielded container post-injection.

4.7. At the end of the run, integrate the radiochromatogram by measuring the area under the curve for the product and impurities signals as shown previously, and calculate the radiochemical purity using the given formula **[1]**.

4.7.1. TEXT on PLAIN BACKGROUND:

$RCP\ (\%) = 100 - \Sigma(\%AUC_{impurities})$

Results

5. Results

5.1. A comparison of the effectiveness of different anti-radiolysis agents in preserving radiochemical purity during crude [^{68}Ga]Ga-3BP-3940 *(gallium-three-B-P-three-nine-fourty)* synthesis is shown in this figure **[1]**.

5.1.1. LAB MEDIA: Figure 5.

5.2. Methionine at 10 milligrams per milliliter achieved the highest radiochemical purity of 94.7 percent **[1]** compared to gentisic acid **[2]** and ascorbic acid **[3]**.

5.2.1. LAB MEDIA: Figure 5. *Video Editor: Highlight C.*

5.2.2. LAB MEDIA: Figure 5. *Video Editor: Highlight A.*

5.2.3. LAB MEDIA: Figure 5. *Video Editor: Highlight B.*

5.3. The final product collected in the terminal vial retained an average of above 75.1 percent of total radioactivity, confirming high yield and efficient collection **[1]**.

5.3.1. LAB MEDIA: Figure 6. *Video Editor: Only show the bar chart and highlight the tallest bar.*

5.4. Total synthesis-related losses averaged only about 24.9 percent, distributed among the waste vial, SPE *(S-P-E)* cartridge, and reaction vial, indicating low waste generation **[1]**.

5.4.1. LAB MEDIA: Figure 6. *Video Editor: Only show the bar chart and highlight the small three bars.*

5.5. The average final activity of the radiolabeled compound across the three test batches was above 737 megabecquerel, indicating consistent synthesis performance **[1]**.

5.5.1. LAB MEDIA: Figure 6. *Video Editor: Only show the table and highlight 737.0 ± 2.8 at the bottom right cell.*

Pronunciation Guides:

1. **Luer**
Pronunciation link: https://www.merriam-webster.com/medical/Luer%20syringe
IPA: /ˈlʊər/
Phonetic Spelling: LOO-er

2. **C18 Cartridge**
Pronunciation link: No confirmed link found
IPA: /si eɪˈtiːn ˈkɑːrtrɪdʒ/
Phonetic Spelling: SEE eighteen KAR-trij

3. **Methionine**
Pronunciation link: https://www.merriam-webster.com/dictionary/methionine
IPA: /məˈθaɪəˌniːn/
Phonetic Spelling: muh-THY-uh-neen

4. **Gentisic Acid**
Pronunciation link: https://www.merriam-webster.com/medical/gentisic%20acid
IPA: /dʒɛnˈtɪsɪk ˈæsɪd/
Phonetic Spelling: jen-TISS-ik ASS-id

5. **Ascorbic Acid**
Pronunciation link: https://www.merriam-webster.com/dictionary/ascorbic%20acid
IPA: /əˈskɔːrbɪk ˈæsɪd/
Phonetic Spelling: uh-SKOR-bik ASS-id

6. **Peristaltic Pump**
Pronunciation link: https://www.merriam-webster.com/dictionary/peristaltic%20pump
IPA: /ˌpɛrəˈstɔːltɪk pʌmp/
Phonetic Spelling: pair-ih-STAWL-tik pump

7. **Radiochromatograph**
Pronunciation link: https://www.merriam-webster.com/medical/radiochromatography
IPA: /ˌreɪdiəʊˌkrəʊməˈtɒgrəf/
Phonetic Spelling: RAY-dee-oh-KROH-muh-tog-raf

8. **Radio-TLC**
Pronunciation link: No confirmed link found
IPA: /ˌreɪdiəʊ tiː ɛl siː/
Phonetic Spelling: RAY-dee-oh TEE-EL-SEE

9. **Radio-HPLC**
Pronunciation link: https://www.merriam-webster.com/dictionary/HPLC
IPA: /ˌreɪdiəʊ eɪtʃ piː ɛl siː/
Phonetic Spelling: RAY-dee-oh AITCH-PEE-EL-SEE