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Cancer Imaging Program

National Cancer Institute

Standard Operating Procedure

GEN-Q118

A Color Spot Test for the Detection of Kryptofix® [2.2.2]

1. **Purpose:** To describe the quality control procedure for detecting Kryptofix® [2.2.2] in the final product solution.

2. **Scope:** The color spot test described in this procedure will be performed at the sites manufacturing radiopharmaceuticals. It will be used to verify that the residual Kryptofix® [2.2.2] in the final product is below the 50 µg/mL limit in accordance with the FDA guidelines.

3. **Responsibilities:**
   3.1. *Manufacturing Sites*: To follow this procedure as written and to ensure that all results, control information, and raw data are documented as specified in dedicated Quality Control Laboratory Notebooks and recorded on the appropriate testing forms.

4. **References**
   4.1. USP 28 ‹621› Chromatography
   4.2. USP 28 ‹821› Radioactivity
   4.3. USP 28 ‹823› Radiopharmaceuticals For Positron Emission Tomography—Compounding
   4.4. **GEN-Q015**, "Investigating Out of Specification QC Test Results"
   4.5. FDA Draft Guidance PET Drug Products—Current Good Manufacturing Practice (CGMP)—September 2005 (CDER)

5. **Forms**
   - **Form FQC-###**, "Final Product QC Test Results"
   - **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"

SOP**: GEN-Q118** Date:

Printed Name	Signature	Date
Author:		
Regulatory Approval:		

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6. Policies

6.1. The FDA has proposed a maximum permissible level of 50 µg/mL of K-[2.2.2] in 2-[^{18}F]FDG, therefore this maximum permissible level will also apply to the final product.

6.2. Store the pre-coated acidic reagent strips in vented glass jars to reduce buildup of iodine vapors.

6.3. Always wear gloves when handling the coated reagent strips.

6.4. Iodoplatinate reagent is not specific for Kryptofix® [2.2.2]; it reacts with tertiary amines in general, therefore a false-positive reaction is possible. However, a negative color reaction does indicate that Kryptofix® is within acceptable limits (i.e. < 50 µg/mL).

7. Materials and Equipment

7.1. 50 µg/mL standard solution of Kryptofix® [2.2.2]

7.2. Chloroplatinic acid, 6-hydrate (reagent ACS)

7.3. Potassium Iodide (reagent ACS)

7.4. Deionized water

7.5. Product-Specific Solution Matrix

7.6. Concentrated Hydrochloric Acid

7.7. Silica Gel 60 TLC plates (200 µm thick, plastic-backed), Merck

8. Procedures

8.1 Preparation of Stock Iodoplatinate Reagent

8.1.1 Mix 5 mL of 5% chloroplatinic acid in water with 45 mL of 10% potassium iodide in water.

8.1.2 Dilute this mixture with an additional 100 mL of deionized water.

8.1.3 Label this as the Stock Iodoplatinate Reagent.

8.2 Preparation of the Acidic Iodoplatinate Reagent

8.2.1 Mix one part concentrated hydrochloric acid with 10 parts of the Stock Iodoplatinate Reagent.

8.2.2 Label the reagent as the Acidic Iodoplatinate Reagent.

8.3 Preparation of Iodoplatinate Indicator Strips

8.3.1 Immerse pre-cut strips of thin-layer silica gel separately into the iodoplatinate reagent solutions for 5 to 10 seconds to saturate each strip of silica gel completely.

8.3.2 Place the strips on blotting paper and allow to dry overnight at room temperature.

8.3.3 Store the coated strips at room temperature in a vented glass jar until time of use.

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NOTE: Prepare only the number of strips that may be used within a month.

8.3.4 Label the jar with the date that the strips were prepared.

8.4 Preparation of Kryptofix® Standard

8.4.1 Weigh out 5 mg of Kryptofix® in a glass vial, with a Teflon®-lined cap.

8.4.2 Add 1 mL of ethanol to make a 5 mg/mL stock solution and mix thoroughly.

8.4.3 Label the vial as 5 mg/mL of Kryptofix® Stock Solution.

8.4.4 Store this stock solution at 2-8°C in a dark container protected from light until ready to use.

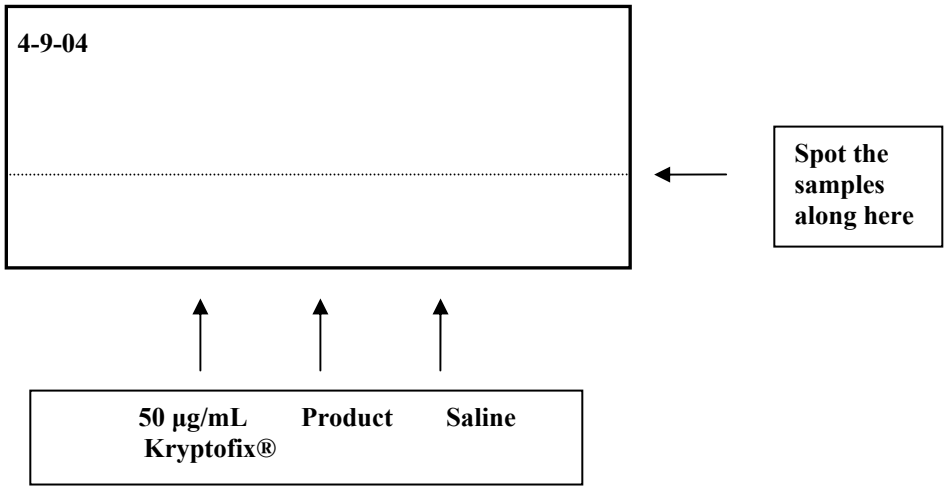
8.4.5 When ready to use transfer 10 µL of the Kryptofix® Stock Solution into a glass vial with a Teflon®-lined cap and add 1 mL of the appropriate Product-Specific Solution Buffer and mix thoroughly. Label this vial as the 50 µg/mL Kryptofix® Standard Solution. Protect this solution from the light as well by placing aluminum around the container.

8.5 Running the Color Spot Test

8.5.1 Using a syringe or micro-pipette apply approximately 1-2 µL of the 50 µg/mL Kryptofix® standard, the lot of FMISO to be tested, and the saline negative control on the pre-coated TLC plate, as illustrated in Diagram 1.

8.5.2 Allow the spots to dry for approximately 5 minutes.

Diagram 1: Preparation



8.5.3 Read the results of the test; the presence of Kryptofix® [2.2.2] is indicated by a blue-black spot created by the Kryptofix®

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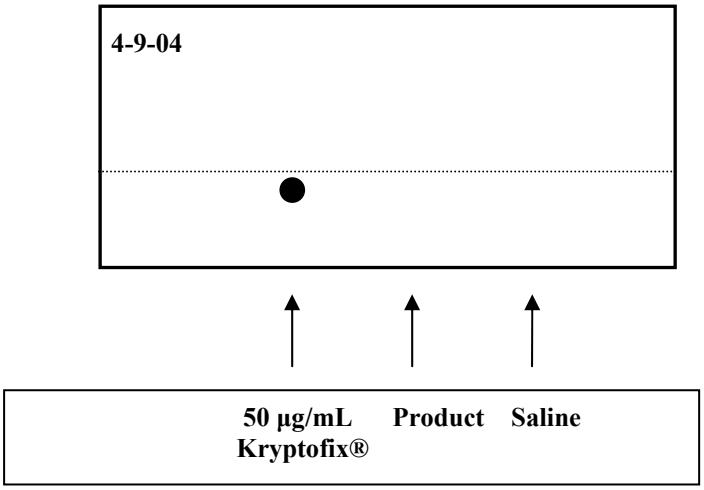
reaction. The sensitivity of this test is as low as 2 µg/mL of Kryptofix® in solution.

Diagram 2: Reading the Results



8.6 Interpretation and Reporting of the QC Results

8.6.4 If the size and intensity of the spot obtained from the test solution, the final product, is *less than* that of the standard, Kryptofix® Solution, then the sample *passes* the specification for the Kryptofix® test.

8.6.2 If the size and intensity of the spot obtained from the test solution, the final product, is *equal to or greater than* that of the standard, Kryptofix® Solution, then the sample *fails* the specification for the Kryptofix® test.

8.6.3 Record the results of this test for the final product sample on both, the appropriate corresponding **FQC Form**, and in the QC Laboratory notebook.

NOTE: The final product must meet the release specification outlined in 8.7.1 in order for the product to pass this quality control testing requirement.

8.6.4 If the product fails to meet the criteria, reject the product and conduct an investigation as per **NCI-Q015**, "Investigating Out of Specification QC Test Results, and record the results of the investigation on the **Form IOS-001**."

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