1p/19q Deletion in Gliomas, FISH, Tissue - Standard Operating Procedure (SOP)

1. PURPOSE

The purpose of this SOP is to provide a detailed and standardized method for detecting 1p/19q co-deletion in gliomas using Fluorescence In Situ Hybridization (FISH) technique on tissue samples.

1. SCOPE

This procedure applies to all medical laboratory scientists and technicians who are trained and qualified to perform FISH for the identification of 1p/19q deletions in glioma tissue specimens.

1. RESPONSIBILITY

- Qualified laboratory personnel are responsible for performing this procedure.
- The laboratory supervisor is responsible for ensuring that personnel are trained and that quality control measures are implemented.

1. REFERENCES

- Vendor FISH kit instruction manual (e.g., Abbott Molecular, Dako)
- CLSI guidelines for FISH
- Relevant scientific literature on glioma 1p/19q deletions

1. SPECIMEN REQUIREMENTS

Preferred Specimen:

- Formalin-fixed, paraffin-embedded (FFPE) tissue block representative of the glioma

Acceptable Specimen:

- Unstained slides with tissue sections (4-5 micron thick) cut from a FFPE tissue block, up to a maximum of 10 slides

Unacceptable Specimen:

- Tissues fixed in non-formalin based fixatives
- Decalcified tissues

1. EQUIPMENT AND SUPPLIES

- FISH probes specific for 1p36 and 19q13 loci and respective centromeric controls

- FISH hybridization buffer
- FISH wash buffer
- FISH mounting media with DAPI (4',6-diamidino-2-phenylindole)
- Fluorescent microscope equipped with appropriate filters (DAPI, SpectrumGreen, SpectrumOrange)
- Hybridization chamber
- Thermocycler or hybridization oven
- Humidified chamber
- Xylene, ethanol, and deionized water
- Microscope slides, cover slips
- Forced-air drying oven
- Positive control tissue

1. PROCEDURE

Day 1:

A. Pre-Treatment of Slides

1. Deparaffinization and Rehydration:

- Place slides in xylene for 3 times, 5 minutes each.
- Rehydrate in a series of ethanol washes (100%, 85%, 70%) for 2 minutes each.
- Rinse twice in deionized water.

1. Enzyme Digestion:

- Apply pepsin solution to slides.
- Incubate in a humidified chamber at 37°C for 10 minutes.
- Rinse in 2X saline-sodium citrate (SSC) buffer for 5 minutes at room temperature.
- Dehydrate slides in an ethanol series (70%, 85%, 100%) for 2 minutes each.
- Air-dry slides.

B. Denaturation and Hybridization:

1. Denaturation:

- Apply denaturation buffer (70% formamide/2X SSC) to slides.
- Incubate slides in a hybridization chamber at 73°C for 5 minutes.

1. Hybridization:

- Apply 10 µL of 1p36 and 19q13 FISH probes to each slide.
- Cover slides with cover slips and seal with rubber cement or similar suitable material.
- Hybridize slides in a hybridization chamber at 37°C overnight.

Day 2:

C. Post-Hybridization Washes:

1. Remove cover slips by soaking slides in 2X SSC for 5 minutes.
2. Wash slides in 0.4X SSC/0.3% NP-40 at 73°C for 2 minutes.

3. Wash slides in 2X SSC/0.1% NP-40 at room temperature for 1 minute.
4. Dehydrate slides in an ethanol series (70%, 85%, 100%) for 2 minutes each.
5. Air-dry slides.

D. Counterstaining and Visualization:

1. Apply 10 µL of DAPI counterstain to each slide.
2. Cover with cover slips and incubate at room temperature for 10 minutes in the dark.
3. Examine slides under a fluorescent microscope with appropriate filters.
4. INTERPRETATION OF RESULTS

- Count the number of fluorescent signals (red and green) for each probe per nucleus in at least 100 non-overlapping, intact tumor nuclei.
- Calculate the ratio of signals for 1p36 (red) relative to the control (green). Do the same for 19q13.
- 1p/19q deletion is identified if there is a significant loss of the red signal relative to the green control signal.

1. QUALITY CONTROL

- Run positive control tissue with known 1p/19q status alongside each batch of patient samples.
- Document and review control results to ensure the assay is performing within accepted parameters.

1. REPORTING RESULTS

- Results should be reported as either "1p/19q co-deletion detected," "1p/19q co-deletion not detected," or "inconclusive."
- Include the percentage of tumor cells showing the deletion and note any technical issues encountered.

1. LIMITATIONS

- Interpret results only in the context of other clinical and pathological information.
- Poor tissue quality or inadequate hybridization may lead to inconclusive results.

1. SAFETY CONSIDERATIONS

- Handle all reagents and samples following standard biosafety regulations.

- Wear appropriate personal protective equipment (PPE) including lab coat, gloves, and eye protection.

1. REFERENCES

- Provide references to relevant scientific literature, vendor instructions, and clinical guidelines.

By following this SOP, laboratory personnel will ensure accurate and reliable detection of 1p/19q deletions in gliomas using FISH methodology. Regular review and updates to the SOP will ensure adherence to best practices and advances in the field.