

Ewing Sarcoma Family of Tumors

USED TO CONFIRM THE HISTOLOGICAL DIAGNOSIS OF EWING SARCOMA BY DETECTION OF EITHER THE TYPE 1 OR TYPE 2 EWS-FLI1 TRANSLOCATION

Test Highlights

- Detects the most common types of *EWS-FLI1* translocations that occur in the Ewing sarcoma family of tumors.
- Distinguishes between the *EWS-FLI1* type 1 and type 2 fusions.
- Uses real-time RT-PCR with dual-labeled probes specific for *EWS-FLI1* translocations.

Disease Overview

- Tumors classified in the Ewing family (Ewing sarcoma, PNET, and Askin sarcoma) are the most common malignant bone and soft-tissue tumors occurring in childhood and young adulthood.
- When using light microscopy, it is sometimes difficult to differentiate tumors within the Ewing family from each other and from other small, round-cell tumors. Accurate diagnosis of the tumor type is essential for prognosis and determining therapy. Real-time RT-PCR can be used to identify specific tumor types within the Ewing family by the detection of characteristic translocations.^{1,2}

Genetics

- The two most common types of translocations in the Ewing family of tumors are the *EWS-FLI1* gene fusion [t(11;22)(q24;q12)] and the *EWS-ERG* gene fusion [t(21;22)(q22;q12)]. Both translocations are diagnostic for Ewing sarcoma.
- Other chimeric genes, including *EWS-ETV1* [t(7;22)], *EWS-ELAF* [t(17;22)], and *EWS-FEV* [t(2;22)], have been observed on a rare basis in Ewing sarcoma.
- The *EWS-FLI1* fusion transcripts occur in several forms. The type 1 transcript is the most common (65 percent of cases) and is created by the fusion of the *EWS* exons 1–7 to *FLI1* exons 6–9. The type 2 translocation results from *EWS* exons 1–7 joining to exons 5–9 of *FLI1* and is seen in approximately 25 percent of *EWS-FLI1* cases. Some studies suggest that there is a better outcome with the type 1 translocation.³

Indication for Ordering

This assay should be used to confirm the histological diagnosis of Ewing sarcoma by detection of either the type 1 or type 2 *EWS-FLI1* translocations.

Additional Ordering Notes

- It is suggested that approximately 50 percent of the submitted sample contain tumor.
- Specimen type should be fresh frozen.

Interpretation

- A positive *EWS-FLI1* gene fusion is reported when an amplification curve is present in the *EWS-FLI1* assay (testing for the presence of type 1 and type 2 fusions) and the *MRPL19* control assay.
- A Not Detected *EWS-FLI1* result is reported when there is amplification of the control gene (*MRPL19*) but no transcript specific amplification for either the type 1 or type 2 *EWS-FLI1* fusions.
- A Not Detected result does not exclude the diagnosis of Ewing sarcoma or other tumor types in the Ewing family since other transcripts (e.g., *EWS-ERG*) can also define the disease.

Limitations

- The *EWS-FLI1* transcript can be detected within a wild-type background of one in 5,000.
- The assay is limited to detecting the two most common translocations observed in the Ewing family of tumors.

Methodology

- RNA from patient samples and controls is extracted and reverse transcribed using gene-specific primers for the *EWS-FLI1* fusion and the *MRPL19* control gene. The cDNA is then PCR amplified for the *EWS-FLI1* fusion and *MRPL19* gene in the presence of fluorescently labeled sequence-specific probes.
- Amplification of the control gene and each fusion type is done in separate reactions (i.e., not multiplexed).

References

1. Athale UH, et al. Use of reverse transcriptase polymerase chain reaction for diagnosis and staging of alveolar rhabdomyosarcoma, Ewing's sarcoma family of tumors, and desmoplastic small round cell tumor. *J Ped Hematol* 2001;23:99–104.
2. Kojima T, et al. Detection of chimeric genes in Ewing's sarcoma and its clinical applications. *Biol Pharm Bull* 2002;25:991–4.
3. Lin PP, et al. Differential transactivation by alternative *EWS-FLI1* fusion proteins correlates with clinical heterogeneity in Ewing's sarcoma. *Cancer Research* 1999;59:1428–32.

Test Information

0051220 **Ewing Sarcoma by RT-PCR**

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.