PML-RARa Suggested Protocol

PML-RARa Clinical Relevance:

Acute Promyelocytic Leukemia (APL) accounts for 10-15% of Acute Myeloid Leukemia (AML) and is one of the most curable forms of leukemia with good sensitivity to all-trans retinoic acid (ATRA). Nearly all APL cases are characterized by the presence of the PML-RARa t(15;17) fusion gene transcript which is required for ATRA treatment response. Researchers have identified the utility of measuring PML-RARa transcripts to aid in the classification of APL, predicting treatment response, and monitoring minimal residual disease (MRD).[1,2]

Scope of Procedure:

The following is a recommended procedure for the detection of the three most common PML-RARa fusions by quantitative PCR (bcr1, bcr2, bcr3). Researchers are advised to utilize this protocol as a guide in the development of their own procedure. This protocol was developed utilizing ancillary reagents and equipment purchased from external vendors. Protocol modifications will be required dependent on the selection of ancillary reagents and equipment utilized in your laboratory.

The following protocol provides instructions for processing samples. Laboratories are encouraged to develop their own guidelines on the selection and use of standards, controls and data analysis.

CytoGenes Reagents (Required):

Cat #	Item
BA-WT	BCR-ABL Wt Primer Mix
PR-B1	PML-RARa bcr1 Primer Mix
PR-B2	PML-RARa bcr2 Primer Mix
PR-B3	PML-RARa bcr3 Primer Mix

CytoGenes Reagents (Optional):

Cat #	Item	Assay Targets Included	Concentration
PR-S1	PML-RARa Standard-1	ABL, PML-RARa (bcr1, bcr2, bcr3)	$5e^5$ copies/ul
PR-S2	PML-RARa Standard-2	ABL, PML-RARa (bcr1, bcr2, bcr3)	$5e^4$ copies/ul
PR-S3	PML-RARa Standard-3	ABL, PML-RARa (bcr1, bcr2, bcr3)	$5e^3$ copies/ul
PR-HC	PML-RARa High Control	ABL, PML-RARa (bcr1, bcr2)	3ng/ul
PR-LC	PML-RARa Low Control	ABL, PML-RARa (bcr1, bcr2)	0.3ng/ul

Ancillary Reagents (Recommended):

- EXPRESS One-Step Superscript® qRT-PCR (ThermoFisher)

Equipment and Supplies (Required):

- Quantitative PCR instrument
- PCR plates, tubes and sealing film.
- Pipettors (range 1-1000ul)
- Aerosol barrier pipette tips.
- Microcentrifuge

Protocol:

Critical Note: Prepare PCR reactions on a cold block and initiate PCR cycling within 2 hours after the addition of reverse transcriptase.

1. Isolate RNA from samples utilizing standard procedures utilized by your laboratory.

2. Prepare separate PCR reactions for each of the PCR primer sets to be tested. (Note: for multiple samples, prepare a master mix and scale the volumes appropriately based on the number of samples to be tested).

Reagent	Volume (ul)
EXPRESS SuperScript® qPCR SuperMix	10
CytoGenes 25X PCR Primer Mix	0.8
EXPRESS SuperScript® Mix for One-Step qPCR	2
Nuclease Free Water	5.2
Sample	2
Total volume	20

3. Prior to initiating the run, program Q-PCR instrument to detect the probes utilizing the appropriate filter sets. The probes in each of the master mixes are labeled with a FAM reporter and BHQ quencher.

4. Initiate PCR protocol appropriate for a one-step reverse transcriptase Q-PCR reaction. The following is a recommended protocol based on our instruments and ancillary reagents utilized.

Cycle	Step	Temp (°C)	Time (Min:Sec)	Repeats
1	1	60	15:00	1
2	1	95	2:00	1
3	1	95	:20	40
	2	60	:30	

5. Analyze results.

References:

1. Cull EH, Altman JK. Contemporary Treatment of APL. *Current hematologic malignancy reports*. 2014;9(2):193-201. doi:10.1007/s11899-014-0205-6.

2. Lo-Coco F, Cicconi L. History of Acute Promyelocytic Leukemia: A Tale of Endless Revolution. *Mediterranean Journal of Hematology and Infectious Diseases*. 2011;3(1):e2011067. doi:10.4084/MJHID.2011.067.