EWOG-MDS
EWOG-SAA

Coordinating Study Center
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Instructions for acquisition of non-hematopoietic cells ("germline material")

Pathogenic mutations in certain genes, including *PTPN11, NRAS, KRAS, CBL*, *NF1*, *GATA2, RUNX1*, or *TP53,* can be acquired (somatic) or constitutional (germline). Establishing the germline status of such mutations can be of critical relevance for the clinical management and prognostication.

The following non-myeloid material can be used to establish germline or somatic origin of pathogenic mutations in patients with SAA, MDS, JMML, and other types of myeloid neoplasia:

- **Hair follicles** (at least 15 visible follicles, larger amounts might be needed in infants)

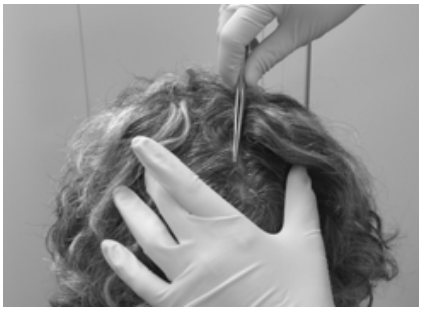
- **Buccal swabs** (two buccal swabs from different sites of mouth cavity). This DNA source allows for discrimination of somatic mutations (e.g. JMML-type mutations), however germline status cannot be reliably confirmed (due leukocyte contamination).

- **Skin fibroblasts** cultured from a punch or incision biopsy ("gold standard")

  Bone marrow derived mesenchymal fibroblasts are not yet used in routine diagnostics.

Instructions for sample acquisition are given on the following page.

Hair follicles

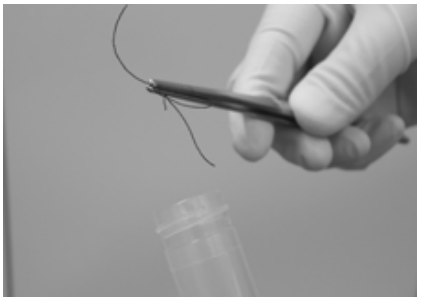


1. For sampling please use scalp hair.

2. Please wear a mouth guard, gloves and sterile forceps for tearing out the hairs. Alternatively you can pluck hair using sterile gloves only.

3. Make sure to collect at least **15 hairs including follicles**.

4. Please use a sterile tube (such as those for microbiological analyses). Label the sample with the following information:
   - Patient´s name and date of birth
   - Date of sampling



5. Shipment: at room temperature using regular mail, addressed to the diagnostic laboratory of the EWOG-MDS Coordinating Study Center:
   Pediatric Hematology and Oncology
   Mathildenstr. 1, 79106 Freiburg, Germany
   Phone: +49761-270-45150
   Email: zkj-onklab@uniklinik-freiburg.de
   The lab can receive samples on Saturdays

Skin biopsy

In case the mutation analysis from hair follicles is returned with no or ambiguous results (e.g. due to insufficient material), or when a fibroblast culture is necessary for diagnostics (e.g. in case of exome sequencing where larger amounts of DNA are required), a skin biopsy is indicated.
A skin biopsy can be taken using 2 mm disposable punch, performed along with other procedure that already requires anesthesia, e.g. BM collection or implantation of a central venous catheter.
Shipment: in culture medium or sterile 0,9% NaCl, at room temperature using express mail.