

Reference center – cerebrospinal fluid diagnostics of the
 Research and Treatment network HIT
 Institute of Neuropathology
 Prof. Dr. med. U. Schüller
 Building O38
 Martinstraße 52
 20246 Hamburg

FAX: 040-7410-54929
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Submitting clinic (stamp)

Physician (block letters with extension):

PLEASE FILL IN ALL FIELDS (also for repeated puncture)!

_____._____._____ _____ _____
 First Name Last Name (Molecular) tumor diagnosis
 Or (if preoperative) suspected diagnosis

_____._____._____ _____ _____
 Date of birth Study/ Register Date of tumor surgery

Clinical Data:

Primary Diagnostics/Staging

preoperative intraoperative postoperative

lumbar ventricular

Puncture Date: ____ . ____ . _____

Follow-up Diagnostics

Justification: During therapy before achieving CR Suspicion of recurrence
 Examination for R+ and/or Metastases after Treatment Element

Details of therapy branch and/or current therapy:

After cycle/ Block No. ____ After Radiation After HDCT
 Other time: _____ Follow-Up

Lumbar-CSF Ventricular-CSF Puncture Date: ____ . ____ . _____

Details of local findings:

positive negative unclear not performed

Please send at least 2 (preferably 5) unstained, unfixed and air-dried cytospin-preparations!

If possible, please send EDTA blood and cell-free CSF supernatant in a separate tube.

(Please also refer to the next page for production)

Instructions for the preparation of cytopsin preparations and preservation of CSF supernatants

(e.g. using the Shandon cytocentrifuge)

Collect as much CSF as possible and ideally transfer it directly into DNA LoBind® Tubes (Eppendorf, #0030122208)

**Continue processing the CSF immediately after collection.
Concurrently, if possible collect 1 tube of EDTA blood (note the date!)**

1. Centrifuge the CSF at 700 rpm for 5 minutes, avoiding higher speeds and longer durations to prevent cytolytic damage to the cell nuclei.
2. Transfer **supernatant** to a new DNA LoBind® Tube (Eppendorf, #0030122208) (for shipment to Hamburg)
3. Resuspend the **sediment** with NaCl using the same volume as originally collected from the patient.
4. Label uncoated slides with patient name, collection date, and collection type (e.g., lumbar, ventricular, etc.)
5. Place a filter card on the slide (important: ensure that the smooth paper side is in contact with the slide)
6. If necessary, mark the exit port on the back of the slides.
7. Place cuvettes onto the prepared slides and secure them with a clip (aligning the cuvette opening with the filter paper opening). Use only dry cuvettes, otherwise cytolysis of the cells will occur
8. Load centrifuge with prepared slides.
9. Add 1-2 drops of serum albumin to the cuvettes (e.g. *Medion Diagnostics: Specific Albumin 22% Ref 050111*)
10. Add 0,5 ml of carefully and well mixed CSF to each cuvette.
11. Centrifuge at 700rpm for 5 minutes
12. To prevent cytolysis: Carefully remove the preparations from the centrifuge immediately after centrifugation
13. Allow preparations to dry well, do not fix.
14. Perform panoptic staining using the Pappenheim method.
15. Perform differentiation.
16. Count cells as in differential blood count: 100% or n = number of cells found
17. Review of entire specimen for tumor cells / tumor cell clusters is required.

**Send a minimum of 2 (preferably 5) untreated, unstained and air-dried preparations to the reference laboratory.
For this purpose, be sure to use a courier with overnight service.**

If possible, send supernatant and EDTA blood with the specimen (overnight shipment without ice)