

Peripheral blood processing

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Basic procedure of peripheral blood processing

For a good result of cytogenetic examination it is necessary to maintain the following procedure:

1. **Blood Collection.** Peripheral blood is collected in a heparinized syringe (heparin is an effective anticoagulant - a substance that reduces blood clotting) in an amount of about 3 ml. We use alcohol as a disinfectant to clean the injection site. Other substances are toxic to the cells and their use would make it impossible to perform the test.
2. **Cultivation.** The blood is further cultured for three days in a medium containing phytohemagglutinin. This highly immunogenic substance agglutinates blood cells and stimulates lymphocytes to divide.
3. **Use of colcemid.** Colcemid must be added to the culture approximately two hours before the end of the cultivation. Colcemid is a spindle venom that disrupts the dividing spindle and thus stops the ongoing division in the metaphase of mitosis.
4. **Hypotonization.** Subsequently, the cell suspension is poured into a test tube, centrifuged and the supernatant is discarded. We add a hypotonic solution of potassium chloride. Water gradually begins to penetrate into the cell, the cell increases in volume and the cell membrane gradually thins. Chromosomes separate from each other due to the large volume of the cell.
5. **Fixation.** Cells are fixed with methanol - acetic acid (3: 1). We repeat this procedure three times.
6. **Preparation of chromosome preparations.** Drip the suspension onto a damp, clean and cooled glass. We drip the suspension from a sufficient height to disrupt the cell membrane and release the chromosomes. After adhesion, chromosomes are able to stain.

Peripheral blood lymphocytes are the most commonly used material when performing postnatal examinations. Another possible material is fibroblasts.

Links

References

- KOČÁREK, Eduard and Martin PÁNEK. Clinical cytogenetics I: Introduction to clinical cytogenetics. 2nd edition. Prague: Karolinum, 2010. 134 pp. ISBN 978-80-246-1880-7.

Source

- ws:Zpracování periferní krve

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