A novel method for direct detection and characterization of circulating tumor cells combining the use of EpiSep XRC (Xenographic Retention Chromathography) and fluorescent microsphere technology.

#5612

Hanne K.Høifødt [1)], Chris Feistel [2)], Hang Le[1)] and Øystein Fodstad [1,3)].
1) Dept. of Tumor Biology, The Norwegian Radium Hospital, Oslo, Norway. 2) WaveSense, Irvine, CA 92618, US.
3) Cancer Research Institute, Univ. of South Alabama, Mobile, US.

Abstract.

Several methods to study the presence of circulating, micrometastatic cells in bone marrow (BM) and peripheral blood (PB) and its relationship to clinical status of patients with solid cancer have been described. However, all reported methods have limitations regarding specificity, sensitivity and standardization. We have developed a new approach by combining the advantages of EpiSep XRC technology with our previously described fluorescent microsphere method. The method is based on the use of immunomagnetic beads coated with specific antibodies for enrichment of the target cells present in a mononuclear cell fraction, applying the enriched cell suspension onto a magnetic target cell capture area on the EpiSep slide. Non-target cells and unbound fluorescent microspheres are absorbed by adsorbent pads contained in the EpiSep slide. In this system we used simultaneously 2–4 types of non-magnetic small microspheres, containing different fluorophores, each type coated with an antibody known to bind cell surface markers relevant for the cancer type studied. The detection of target cells is greatly faciliated by the strong fluorescense obtained with the microspheres. This approach allowed confirmation of the nature of each, individual target cell and also visualizing the expression of tumor specific and prognostically important cell membrane markers, such as EGFR and erbB2. In addition, we were able to demonstrate the expression of specific intracellulare antigens, either by immunofluorescense or by the binding of fluorescent microspheres. The method has been used in model experiments on cell lines with convincing sensitivity, reproducibility and ease of use, demonstrating the validity of the approach. We are at present applying the technique to PB and BM samples from patients with ocular melanoma with several years of clinical follow up.

Material.

Mononuclear cells (MNC) from aspirates of bone marrow (BM) and peripheral blood (PB) from patients with ocular melanoma were analyzed for circulating tumor cells using the EpiSep XRC technology. The melanoma cell line FEMX, and the breast cancer cell lines MCF-7, SKBR3 and MA-11 were used in model experiments.

Antibodies.

9.2.27 - reacts with a 250kD proteoglycan (HMW) melanoma-associated antigen.

376.96 - reacts with a 100kD glycoprotein expressed by human melanoma and carcinoma cells

EP-1 - reacts with a HMW melanoma –associated antigen

HMB45 - reacts with an intracytoplasmic antigen expressed in melanomas

MOC31 - reacts with a 42kD carcinoma antigen

BM-7 - reacts with MUC 1, carbohydrate epitope.

c-erbB2- reacts with the extracellular domain of Her2.

425.3 - reacts with the EGF receptor

A45-B/B3- a pan-cytokeratin antibody

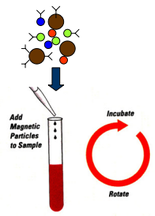
Beads/microspheres.

Immunomagnetic:SAM M450 (4.5 um) Dynabeads

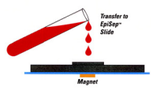
Molecular Probes Fluorescent Microspheres (1.0– 2.0 um)

EpiSep XRC immunomagnetic and fluorescent bead technology.

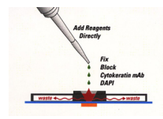
Immunomagnetic beads and fluorescent microspheres are added directly to the cell suspension in a tube and incubated under constant rotation.



After incubation, the sample is transferred to the well of an EpiSep slide. The slide rests on a NeoMag Dock with a magnetic field. Epithelial cells bound by immunomagnetic beads are arrested upon contact with the magnetic field area. Excess liquid and non-targeted cells are absorbed in the pads.



Extracellular or intracellular staining of the captured cells is possible on the slides.



The EpiSep slide is removed from the NeoMag Dock for direct evaluation in an inverted fluorescent microscope. The slides can be handled and stored similar to conventional immuno-fluorescent slide preparations.

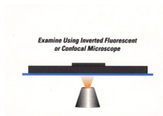
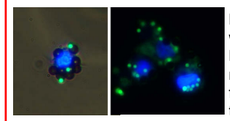
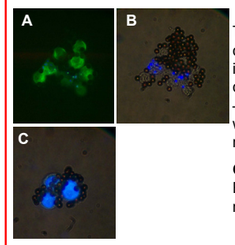


Figure 1. Model experiments with breast cancer cell lines MCF-7, MA-11 and SKBR3.

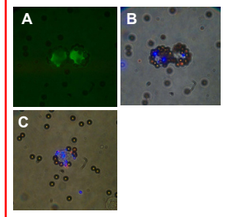


MCF-7 breast cancer cells rosetted with BM-7 immuno-magnetic beads, MOC31 green fluorescent microspheres and 376.96 blue fluorescent microspheres as seen in the microscope after they are captured in the EpiSep slide. Nuclear detection with DAPI.



The same cluster of SKBR3 breast cancer cells rosetted with ErbB2 immunomagnetic beads; **A:** in combination with FITC labelled Pan –cytokeratin and **B:** in combination with MOC31 blue fluorescent microspheres.

C:The same SKBR3 cells with ErbB2 immunomagnetic beads and nuclear detection with DAPI.



MA-11 breast cancer cell with MOC31 immunomagnetic beads **A:** in combination with FITC labelled Pan-cytokeratin and **B:** the same cells with 376.96 blue fluorescent microspheres, **C:** in combination with 425.3 red fluorescent microspheres and 376.96 blue fluorescent microspheres.

Summary.

•We present a simple, rapid and reproducible method for detection and characterization of circulating cancer cells. EpiSep XRC is simply a cell capture platform that works like a magnetic test strip.

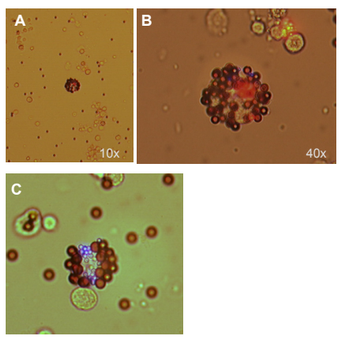
•The method may easily be standardized for more wide-spread use in prognostication and for monitoring effect of therapy.

•The use of several antibody-bead conjugates simultaneously allowes confirmation of the nature of each, individual target cell.

•Captured cells can be further characterized by intracellular staining and nuclear detection is possible with DAPI. The EpiSep slides can be stored similar to conventional immunofluorescent slide preparations.

•This direct EpiSep XRC detection method enables quantitative analysis of the circulating tumor cells.

Figure 3. Immunomagnetic detection of tumor cells in bone marrow (BM) of patients with ocular melanoma.

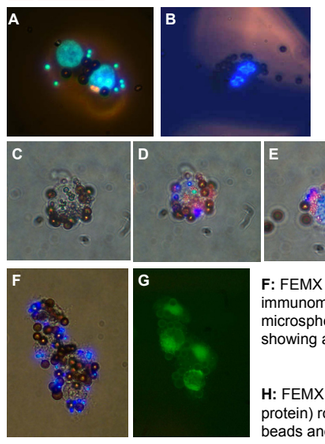


Samples of 20 million mononuclear cells isolated from BM aspirates of ocular melanoma patients were analyzed for micrometastatic tumor cells by immunomagnetic enrichment, using SAM M-450 Dynabeads coated with antibodies against tumor-associated antigens. Simultaneously, smaller fluorescent microspheres coated with other antibodies were incubated in the cell suspension. The rosetted cells were trapped by the magnet, and non-target cells and unbound fluorescent microspheres were washed away. The pictures show positive rosetted target cells as viewed in the microscope.

A,B: The same target cell, visualized with 10x and 40x magnification, rosetted with 9.2.27 immunomagnetic beads and 376.96 red fluorescent microspheres.

C: Positive target cell in the same patient sample rosetted with 376.96 immunomagnetic beads and with 9.2.27 blue fluorescent microspheres.

Figure 2. Model experiments with the FEMX melanoma cell line.



A, B: FEMX melanoma cells with two different cell membrane markers; 9.2.27 immunomagnetic beads and blue fluorescent microspheres coated with the 2g12 antibody.

Nuclear detection with DAPI.

C,D: The same FEMX cells captured with 376.96 immunomagnetic beads, blue 2g12, red 9.2.27 and green EP-1 fluorescent microspheres
E: in addition nuclear detection with DAPI.

F: FEMX melanoma cells with 376.96 immunomagnetic beads, 9.2.27 blue fluorescent microspheres and **G:** the same cell cluster showing also intracellular staining with HMB45.

H: FEMX transfected with RFP (red fluorescent protein) rosetted with 376.96 immunomagnetic beads and 9.2.27 blue fluorescent microspheres.

hhoifodt@labmed.uio.no
ofodstad@usouthal.edu
chris@wavesense.net

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