

THE MAINTRAC APPROACH FOR DETECTION OF CIRCULATING  
TUMOUR CELLS: SENSITIVITY, SPECIFICITY, REPRODUCIBILITY.

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MAINTRAC is a new extremely sensitive and specific approach to quantitate minimal numbers of circulating tumour cells in blood and bone marrow in patients with solid tumours. The MAINTRAC method combines magnetic cell enrichment and Laser Scanning Cytometry combined with visual control. For validation the method was applied to an artificial system, mimicking seeding of tumour cells into the peripheral blood by diluting cells of a tumour cell line into normal peripheral blood. After red blood cell lysis and staining with an FITC conjugated anti-human epithelial antibody (HEA) the MAINTRAC method unequivocally detected 1 positive cell in  $10^4$  white blood cells without previous enrichment and it was possible to reliably recover 50 out of 60 tumour cells added to a 10ml blood volume after magnetic bead enrichment. Most important was the possibility to rescreen the positive events for morphological verification in order to distinguish cell debris and non-cell events from true stained cells. Repeated measurements of the same sample resulted in almost identical numbers of positive cells (CV=1%). Three different runs of the same blood sample over enrichment columns gave comparable values (CV=10%). The specificity of the reaction was controlled by staining the cells with an unrelated antibody, and by testing ten normal healthy controls. No positive cells were detectable before or after enrichment with HEA-magnetic beads. We, also, were able to compare our results for 10 patients to those obtained by immunochemistry showing at least a tenfold increased sensitivity over the conventional approach. This was due, among others, to reducing purification and washing steps and thus minimum cells loss. In principle all sorts of tumour cells can be recovered, irrespective of their behaviour in density gradients. Calculation of the numbers of positive cells is performed per blood volume and per WBC, the latter, however, being a relative number dependent on patient status (with or without chemotherapy, with or without growth factors) and recovery of cells. Additional analyses of defined tumour cells are possible, such as restaining for Her2/neu, FISH analysis or other verification methods. The MAINTRAC method is applicable for quantitating circulating tumour cells of all sorts of tumours for which specific markers are available, it can be performed in a timely fashion scale for therapeutic decisions and investigation of the seeding behaviour of different tumours and allow correlation of this parameter with metastasising properties and prognosis.