

| | | | | |
|--------------|---------------|------------|-------|----------------|
| Patient NAME | DATE OF BIRTH | DISEASE | STAGE | Physician NAME |
| Mrs John Doe | 1992-Jun-12 | Colorectal | II | Administrator |
| SPECIMEN | VIAL IDs | | | |
| 20ml Blood | 1 | | | |

REPORT SUMMARY

CTCs COUNT: Isolated 3.3 cells/ml , SD +/- 0.3 cells

Information

Laboratory Process

Isolation of malignant cells using flow cytometry with which the circulating tumor cells are enumerated and immunophenotyped

Index of circulating cells number

If over limit: Advanced or progression of disease.

If less than limit: Early disease or disease is responding to a treatment plan

| | |
|-----------------|---------------------------------|
| Breast Cancer | < 5 cells / 7.5 ml |
| Prostate Cancer | < 20 cells / ml |
| Sarcoma | < 15 cells / 6.5 ml |
| Colon Cancer | < 5 cells / ml |
| Lung Cancer | (Lc=0, r=0.99):< 10 cells / ml |

All cancer types other than those listed above should be < 5 cells / ml

Disclaimers

*This test will NOT DETECT cancers of the brain or other cancers that have been "encapsulated" by the body, not releasing circulating tumor or stem cells (CTC, CSC) into the blood stream or if any of these cells are dormant. We still recommend the use of biopsy, blood markers and/or various scans with this test when cancer is suspected or known to exist. No test is 100% accurate

*The methodology has a sensitivity of 86,2% and specificity of 83,9%

Markers

| Category | Name | Results |
|--|----------|---------------------------|
| Significant CD45 positive cells (Hematologic origin cells) | Nanog | Positive |
| | OKT-4 | Negative |
| | Sox-2 | Dim |
| | CD15 | Positive |
| CD45 negative cells (non Hematologic origin) | Nanog | Positive (25% of all CTC) |
| | OKT-4 | Negative |
| | Sox-2 | Dim |
| | EpCam | Positive (50% of all CTC) |
| | CD133 | Negative |
| | c-MET | Dim |
| | CD31 | Positive (75% of all CTC) |
| | PanCK | Negative |
| | CK7 | Dim |
| | EpCAM+ve | 2.7 cells/ml |

Index of markers

| | |
|-------------------------------------|--|
| CD44, CD133, Sox-2*, OKT-4*, Nanog* | Tumor stem cell marker |
| c-MET* | Membrane antigen that regulates the mesenchymal to epithelial transition |
| CD34* | Hematological stem cell and blast cell marker, epithelioid |
| CD45 | Hematologic origin cell |
| BCR-ABL, CD30, CD15 | Hematologic malignancy marker |
| CD19 (CD45 negative cells) | Lung neuroendocrine malignancy |
| CD19 (CD45 positive cells) | Hematological malignancy |
| CD31 | Endothelial cell membrane antigen |
| CD63 | Melanoma cell marker |
| CD99 | Sarcoma marker |
| EpCam | Epithelial origin marker |
| MUC-1 | Breast cancer antigen |
| PSMA | Prostate specific cancer stem cell membrane antigen |
| VHL mut | Renal carcinoma marker |
| panCK | Epithelial origin cell marker |

*Significant markers

Sincerely,



Dr. Ioannis Papatirou MD, PhD, SCym

- a. Ntanovasilis DA, Apostolou P and Papatotiriou I. Flow Cytometric Detection of Circulating Tumor Cells in Breast Cancer Patients: A Blinded Study. *Journal of Cancer Therapy*, 10, 708-715. doi: 10.4236/jct.2019.108058.
- b. Hatzidaki E, Iliopoulos A, Papatotiriou I. A Novel Method for Colorectal Cancer Screening Based on Circulating Tumor Cells and Machine Learning. *Entropy (Basel)*. 2021 Sep 25;23(10):1248. doi: 10.3390/e23101248. PMID: 34681972; PMCID: PMC8534570.
- c. Vidlarova M, Rehulkova A, Stejskal P, Prokopova A, Slavik H, Hajduch M, Srovnal J. Recent Advances in Methods for Circulating Tumor Cell Detection. *Int J Mol Sci*. 2023 Feb 15;24(4):3902. doi: 10.3390/ijms24043902. PMID: 36835311; PMCID: PMC9959336.
- d. Saltos A, Khalil F, Smith M, Li J, Schell M, Antonia SJ, Gray JE. Clinical associations of mucin 1 in human lung cancer and precancerous lesions. *Oncotarget*. 2018 Nov 2;9(86):35666-35675. doi: 10.18632/oncotarget.26278. PMID: 30479696; PMCID: PMC6235019.
- e. Wang YW, Shi DB, Liu YM, Sun YL, Chen X, Xiang S, Fu Q, Wei JM, Gao P. Aberrant expression of CD227 is correlated with tumor characteristics and invasiveness of breast carcinoma. *J Cancer Res Clin Oncol*. 2014 Aug;140(8):1271-81. doi: 10.1007/s00432-014-1676-5. Epub 2014 May 1. PMID: 24788565.
- f. Mejia O, Vazquez T, Alexis J. CD63 expression in metastatic melanoma and melanocytic nevi in lymph nodes. *Pathol Res Pract*. 2021 Jul;223:153464. doi: 10.1016/j.prp.2021.153464. Epub 2021 May 13. PMID: 34051511.
- g. Deng Z, Wu S, Wang Y, Shi D. Circulating tumor cell isolation for cancer diagnosis and prognosis. *EBioMedicine*. 2022 Sep;83:104237. doi: 10.1016/j.ebiom.2022.104237. Epub 2022 Aug 27. PMID: 36041264; PMCID: PMC9440384.
- h. Castro-Giner F, Aceto N. Tracking cancer progression: from circulating tumor cells to metastasis. *Genome Med*. 2020 Mar 19;12(1):31. doi: 10.1186/s13073-020-00728-3. PMID: 32192534; PMCID: PMC7082968.
- i. Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q, Zhu F, Zhou D, Zheng S, Chen Y, Zhou J. Circulating tumor cells: biology and clinical significance. *Signal Transduct Target Ther*. 2021 Nov 22;6(1):404. doi: 10.1038/s41392-021-00817-8. PMID: 34803167; PMCID: PMC8606574.