CLAIMS

1. A method for the preparation and analysis of a purified specimen for obtaining data suitable for the diagnosis of tumoural conditions and/or of the corresponding state of advance, comprising the steps of:

a). enriching a specimen of an organic fluid obtained from a patient in at least one population of cells comprising at least one type of circulating tumor cells or disseminated tumor cells or portions thereof;

b). isolating at least one cell or portion of cell from amongst said at least one type of circulating tumor cells or disseminated tumor cells or portions thereof and obtaining a purified specimen;

c). carrying out a step of molecular analysis on the cells recovered in said purified specimen;

characterized in that said step b) further comprises the step of individually selecting and isolating at least one cell or portion of cell from amongst said at least one type of circulating tumor cells or disseminated tumor cells or portions thereof by manipulating single cells or portions of cells by means of a microfluidic device operating on a specimen which has been previously enriched in at least one population of cells comprising at least one type of circulating tumor cells or disseminated tumor cells or portions thereof by carrying out said step a); wherein

i)- said individual selection of said cells is made on the basis of cell parameters that can be detected by means of a sensor internal or external to the microfluidic device;

ii)- the cell parameters in the individual selection step are evaluated in the absence of flow of said organic fluid,

in order to obtain a purified specimen having a purity of at least 90%.

2. The method according to Claim 1, characterized in that said step of isolating at least one cell or portion of cell from amongst said at least one type of circulating tumor cells or disseminated tumor cells or portions thereof is performed so as to obtain a diagnostic specimen having a purity of at least 95%.

3. The method according to Claim 2, characterized in that said step of isolating at least one cell or portion of cell from amongst said at least one type of circulating tumor cells or disseminated tumor cells or portions thereof is performed so as to obtain a diagnostic specimen having a purity of 100%.

4. The method according to any one of claims 1 to 3, characterized in that said microfluidic system isolates said cells in a non-manual way.

5. The method according to claim 4, characterized in that said microfluidic system isolates said cells in an automatic or semi-automatic way.

6. The method according to any one of claims 1 to 3, characterized in that said individual selection of said cells is made on the basis of the evaluation of images of said cells.

7. The method according to the claim 6, characterized in that said images comprise fluorescence images.

8. The method according to any one of claims 1 to 3, characterized in that said individual selection of said cells is made on the basis of impedentiometric properties.

9. The method according to any one of claims 1 to 3, characterized in that it comprises a step of labeling of the circulating tumor cells or disseminated tumor cells or portions thereof, with at least one type of tracer.

10. The method according to claim 9, characterized in that said at least one type of

tracer is a FISH probe.

11. The method according to Claim 9, characterized in that said individually selecting and isolating step is performed by selecting individually single cells present in said microfluidic device, previously labeled with said at least one tracer.

12. The method according to Claim 11, characterized in that said at least one type of tracer is an antibody conjugated with a marker chosen in the group consisting of:

- fluorophors
- chromogens
- fluorescent or non-fluorescent microbeads.

13. The method according to claim 12, characterized in that said at least one type of antibody recognizes an antigen chosen in the group consisting of:

- EpCAM
- Cytokeratin 8
- Cytokeratin 18
- Cytokeratin 19
- Cytokeratin 20
- CD45
- EGFR
- IGFR
- PSA
- TTF1
- HER2/neu
- MUC-1.

14. The method according to any one of claims 1 to 3, characterized in that said

step of isolating at least one cell or portion of cell from amongst said at least one type of circulating tumor cells or disseminated tumor cells or portions thereof is performed by capturing single cells, preferably each at a specific site of a plurality of sites of said microfluidic device arranged in the microfluidic device according to an array; subsequently single cells from among the captured cells being selected, on the basis of at least one parameter that can be detected by means of a sensor internal or external to the microfluidic device.

15. The method according to any one of claims 1 to 3, characterized in that said step of enriching said specimen of said organic fluid comprises a selection of cells made on the basis of at least one parameter chosen in the group consisting of:

a. mass density;

b. morphology;

c. electrical properties;

d. chemical properties;

e. mechanical properties;

f. expression of surface antigens;

g. expression of intracytosolic antigens;

h. dielectric properties;

i. magnetic properties;

l. optical properties;

m. geometrical properties;

or combinations thereof.

16. The method according to any one of claims 1 to 15, characterized in that said step of enriching said specimen of said organic fluid in at least one population of cells

comprising at least one type of circulating tumour cells or disseminated tumour cells or portions thereof comprises the step of treating said specimen of said organic fluid so as to separate the nucleated cells and enrich it subsequently in nucleated cells.

17. The method according to claim 16, characterized in that said step of enriching said specimen of said organic fluid in at least one population of cells comprising at least one type of circulating tumor cells or disseminated tumor cells or portions thereof comprises at least the step of centrifuging said specimen of said organic fluid in density gradient.

18. The method according to Claim 16, characterized in that said step of enriching said specimen of said organic fluid in at least one population of cells comprising at least one type of circulating tumour cells or disseminated tumor cells or portions thereof comprises at least the step of carrying out a selective lysis of the erythrocytes.

19. The method according to any one of claims 1 to 18, characterized in that said step of enriching said specimen of said organic fluid in at least one population of cells comprising at least one type of circulating tumour cells or disseminated tumour cells or portions thereof comprises at least the step of carrying out a positive and/or negative selection via magnetic beads coupled to antibodies according to at least one of the following modalities:

a) CD45 negative

b) CD45 and GPA negative

c) CD45 and CD14 negative

d) CD45, CD14 and GPA negative

e) EpCAM positive

f) CK positive.

20. The method according to any one of claims 1 to19, characterized in that said at least one enrichment step is performed in combination with said isolation step within the same said microfluidic device used for performing the isolation step.

21. The method according to claim 20, characterized in that a microfluidic device is used equipped with a plurality of different chambers, separated from one another and hydraulically connected, delimited on at least one face by a single chip or by a plurality of separate chips.

22. The method according to any one of claims 1 to 21, characterized in that said step c of molecular analysis is performed by means of a procedure selected from among:

- sequencing
- microsatellite analysis
- comparative genomic hybridization (CGH)
- array CGH
- end-point PCR
- real-time PCR
- methylation analysis:
- quantitative methylation analysis with pyrosequencing
- bisulphite-genomic-sequencing PCR (BSP)
- methylation-specific PCR (MSP)
- Combined Bisulphite Restriction Analysis of DNA (COBRA)
- methylation-sensitive single nucleotide primer extension (MS-SNuPE)
- gene-expression analysis
- RT-PCR
- single-cell gene expression

- digital PCR.

23. The method according to Claim 22, characterized in that said step of molecular analysis comprises assessing the presence of single-nucleotide mutations.

24. The method according to any one of Claims 1 to 22, characterized in that said step of molecular analysis comprises evaluating the presence of hypermethylation of oncosuppressor genes.

25. The method according to any one of Claims 1 to 22, characterized in that said step of molecular analysis comprises evaluating the presence of deletions and/or duplications of genomic DNA.

26. The method according to Claim 25, characterized in that said step of molecular analysis comprises evaluating the presence of deletions in the chromosome region 13q14 in tumour cells in patients with suspected prostate cancer.

27. The method according to any one of Claims 1 to 22, characterized in that said step of molecular analysis comprises evaluating the over-expression of at least one of the genes BCL2, CCND1 and WNT3A in tumour cells in patients with suspected prostate cancer.

28. The method according to claim 9, characterized in that in said individually selecting and isolating step said cells are scanned using three different fluorescence channels, a first one for enabling identification of positive nucleated cells; a second one for enabling identification of said at least one type of circulating tumor cells or disseminated tumor cells or portions thereof, previously labeled with at least one type of tracer; and a third one for enabling identification of lymphocytes.