

ASSAY FOR DETECTION OF TELOMERASE ACTIVITY

BACKGROUND OF THE INVENTION

5 FIELD OF THE INVENTION

The present invention relates generally to the field of diagnostic and prognostic assays such as diagnostic assays for conditions associated with telomerase activity. More particularly, the present invention provides an assay for measuring telomerase activity as
10 an indicator of cancer, an inflammatory disorder and/or a condition involving embryogenesis and/or requiring stem cell proliferation and agents and kits useful for same. Automated and partially automated assays permitting high throughput screening also form part of the present invention. The subject invention further contemplates methods of treatment using agents identified by the subject assay or where treatment protocols are
15 monitored by the assay.

DESCRIPTION OF THE PRIOR ART

Reference to any prior art in this specification is not, and should not be taken as, an
20 acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in any country.

Telomeres are repeating DNA sequences consisting of tandem GT-rich repeats, represented as (TTAGGG)_n located at the 3' end of chromosomal DNA. Gradual telomere
25 erosion occurs during normal mitotic processes due to the loss of from about 50 to 200 nucleotides of telomeric sequence per cell division ultimately resulting in cellular senescence. Telomeres protect chromosomes from fusion and degradation through the action of telomerase which is a unique reverse transcriptase that elongates teleomeric DNA (Shay *et al*, *Hum. Mole. Gen.* 10:667-685, 2001). Telomerase is relatively abundant in
30 germline and embryonic tissues, inflammatory cells, proliferative cells of renewal tissues, as well as cancer cells. In contrast, telomerase activity is difficult to detect in normal

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somatic human tissues. The correlation of telomerase activity and cellular replication has prompted the association of telomerase and cancer. Telomerase activity has been found in almost all human tumors but not in adjacent normal cells (Kim *et al*, *Science* 266:2011-2015, 1994). In fact, telomerase is activated in approximately 85% of human cancers
5 (Hiyama *et al*, *Cancer Lett.* 194:221-223, 2003). Thus, it has been proposed that upregulation or re-expression of telomerase may be a critical event responsible for continuous tumor cell growth.

Given the association of telomerase activity with diseases of cellular proliferation,
10 including cancer, the detection of telomerase activity is of diagnostic value. Several analytical procedures for the quantification of telomerase activity have been reported. The most frequently utilized assay is Telomeric Repeat Amplification Protocol (TRAP) which is a two stage PCR-based assay. In the first stage, telomerase adds 5'-TTAGGG-3' repeats to the end of a synthetic primer. In the second stage, the extended oligonucleotide
15 products are amplified using a reverse primer. When visualized by autoradiography, a positive test by TRAP shows a ladder of bands. The band volume can then be quantified (Hess *et al*, *Clin. Chem.* 48:18-24, 2002). TRAP is time consuming, labor intensive, PCR-dependent and susceptible to inhibition by extracts of clinical samples. Furthermore, it is difficult to quantify telomerase activity because of logarithmic amplification of telomerase
20 products in the PCR amplification step. The susceptibility of the TRAP assay to *Taq*-polymerase inhibitors often results in the production of false positive and false negative results (Weizmann *et al*, *Chem. Bio.* 5:943-948, 2004).

A similar telomerase assay that replaced the electrophoretic step of the TRAP assay with
25 an ELISA detection system has been developed. This system is also PCR-dependent although the ELISA detection method appears to offer no clear advantage over the traditional TRAP. In an effort to eliminate technical issues associated with TRAP, *in situ* hybridization assays for the quantification of human Telomerase (hTR) RNA and human Telomerase Reverse Transcriptase (hTERT) mRNA were developed. However, hTR and
30 hTERT expression does not necessarily equate to telomerase activity (Hess *et al*, 2002 *supra*).

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Another telomerase assay is disclosed in PCT/IL01/00808 (WO 02/20838). This assay uses rotating quinone-functionalized magnetic beads to generate H_2O_2 within the assay. The endogenous production of H_2O_2 putatively overcomes the problem of luminol being sparingly soluble in aqueous buffer solutions. However, the rotating magnetic beads reduces the ability to develop high through put screening protocols and may impact on the sensitivity depending on the length of oligonucleotide primer employed.

Accordingly, there is a need for a reliable, sensitive and cost effective assay for the detection of telomerase activity in clinical samples which would have diagnostic, prognostic and therapeutic value for cancer, inflammatory disorders and conditions involving embryogenesis and/or in monitoring the potential for stem cells to proliferate. The assay of the present invention is applicable to human and mammalian vertebrates in non-mammalian vertebrates and plants.

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SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the
5 inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide sequences are referred to by a sequence identifier number [SEQ ID NO]. The SEQ ID NOs correspond numerically to the sequence identifiers <400>1 [SEQ ID NO:1],
10 <400>2 [SEQ ID NO:2], etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

The present invention contemplates an assay for telomerase activity in cells which provides a diagnostic and prognostic indicator of the presence of cancer cells as well as
15 inflammatory disorders and conditions involving embryogenesis and/or for monitoring the potential for stem cells to proliferate. The assay is also useful for assessing medical treatment protocols for humans and for screening for agents which modulate telomerase activity or levels. Telomerase activity in non-mammalian vertebrates and plants may also have diagnostic value or as a research tool. In relation to vertebrates, the level of
20 telomerase activity correlates with the presence of certain types of cells such as cancer cells as well as changes in cell physiology or proliferative potential with age and/or in response to a treatment protocol. Similarly, the levels of, or changes in, telomerase activity provides information on inflammation including proliferation as well as conditions involving embryogenesis. The assay may be automated or semi-automated to permit high
25 throughput screening. It is based on epithelial cell capture and lysis to detect telomerase activity. The readout is luminescence. Unlike other telomerase assays, it is not a PCR based assay.

The present invention determines, therefore, the level of telomerase activity by
30 incorporation of a label into a telomerase-catalyzed extension nucleotide sequence.

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Accordingly, one aspect of the present invention contemplates a method for detecting cells from a subject exhibiting telomerase activity, said method comprising:

- 5 i) obtaining a sample of cells from said subject, contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
 - 10 ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
 - iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously
15 added H_2O_2 to generate luminescence; and
 - iv) subjecting the resulting mixture to detection means to read the intensity of the luminescence,
- 20 wherein the level of intensity of luminescence compared to a negative control or a known data set provides the level of telomerase activity and the number of putative cells producing telomerase.

In relation to automation, step (iii) and in particular the addition of luminol, an enhancer
25 and/or H_2O_2 can be added automatically by the luminescence reader.

Another aspect of the present invention provides a method for detecting cells from a subject exhibiting telomerase activity, said method comprising:

- 30 i) obtaining a sample of cells from said subject, contacting magnetic particles carrying an oligonucleotide primer comprising the sequence $(X_n TTAGGY_m)_o$ wherein:

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X is a nucleotide selected from A, T, G and C;

Y is a nucleotide selected from A, T, G and C;

n is 0 or 1;

m is 0 or 1; and

5 o is from about 1 to about 400;

with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated
10 primer;

ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;

15 iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence; and

iv) subjecting the resulting mixture to detection means to read the intensity of the luminescence,

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wherein the level of intensity of luminescence compared to a negative control or a known data set provides the level of telomerase activity and the number of putative cells producing telomerase.

25 Generally, in vertebrates, n is 0, Y is G and o is from about 5 to about 30. In arthropods, n is 0, m is 0 and o is from about 1 to about 30. In plants, X is T, n is 1, Y is G, m is 1 and o is from about 1 to about 30.

30 The presence of telomerase activity or the level of telomerase activity compared to negative or a known data set is indicative of the number of cells which possess telomerase activity. Such cells include cancer cells, inflammatory or proliferative cells or cells

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involved in embryogenesis including stem cells. A "negative control" may exhibit basal levels of telomerase activity. The assay is sensitive permitting the detection of telomerase activity in as few as about 1 cell to greater than 10^6 cells to such as from 1 to 10^6 cells.

5 The present invention provides, therefore, a method for detecting cells selected from cancer cells, inflammatory or proliferative cells and embryogenic cells including stem cells in a sample from a subject, said method comprising:

- 10 i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- 15 ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously
20 added H_2O_2 to generate luminescence; and
- iv) subjecting the resulting mixture to detection means to read the intensity of the luminescence,

25 wherein the level of intensity of luminescence compared to a control such as a control not containing cancer, inflammatory or embryogenic cells or compared to a known data set provides the level of telomerase activity and thereby the number of cells.

The "subject" may be a human or other mammal, a non-mammalian vertebrate or a plant or
30 other entity comprising a telomerase.

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As indicated above, the assay of the present invention can be automated or employed as a single assay or a batch of assays. The step of adding luminol, an enhancer and/or H₂O₂ is conveniently automated. The present invention provides, therefore, kits comprising the reagents required to perform the assay as well as instructions for use. In addition, the
5 assay may be conducted under multiplex conditions with multiple labels. Still further, the assay may be part of a number of assays (i.e. two or more assays) to assist in cell identification or to monitor a therapeutic protocol.

The present invention enables the quantitative detection of telomerase activity in cells by
10 the measurement of the extent of a signal. The present invention extends, however, to the use of the subject assay to provide a qualitative detection of the presence or absence or relative level of telomerase activity. Terms such as "determination", "determining", "detection", "diagnosis", "prognosis" and "identification" are used interchangeably to refer to qualitative, semi-qualitative and qualitative detection of telomerase activity in a cell or
15 sample of cells.

In a particular embodiment, the telomerase assay is used to detect the presence of cancer cells or to monitor the progression of cancer in a subject including monitoring cancer in the presence of a chemotherapeutic agent. A "chemotherapeutic agent" in this context
20 includes a chemical agent as well as an immunological or antibiotic agent. A "cancer" is regarded the same as a tumor as far as the present invention is concerned.

Accordingly, the present invention contemplates a method for detecting cancer cells in a sample from a subject, said method comprising:

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- i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated
30 elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;

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ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;

5 iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H₂O₂ to generate luminescence; and

10 (iv) subjecting the resulting mixture detection means to read the intensity of the luminescence,

wherein the level of intensity of luminescence compared to a control such as not containing cancerous cells or a known data set provides the level of telomerase activity and the presence of or number of putative cancer cells.

15 The present invention further extends to use of the assay to assess the efficacy of a cytotoxic agent such as an anti-cancer chemotherapeutic agent. It can also be used for risk stratification of cancer patients such as leukemia patients.

20 In one embodiment, "obtaining a sample of cells" includes collecting and partially purifying the cells or at least removing unnecessary components in the samples. An aspect of the present invention provides a method for selective purification of the tumor cells and removal of those cells from potentially interfering substances. Purification of the tumor cells is achieved by incubation of the body fluid containing the cells with magnetic beads, which are coated with tumor cell-specific antibody. The tumor cells of interest are washed
25 extensively and therefore separated from other cell types, the body fluid matrix (eg; urine, blood), and interfering substances. This lessens the possibility of false negatives due to interference with the assay and also false positives caused by non-tumor cells such as activated T-lymphocytes which may be present in an infection. The sample workup procedure is thus considered useful in obtaining high clinical sensitivity and specificity
30 values.

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Accordingly, another aspect of the present invention is directed to a method for assessing the activity of a cytotoxic agent, said method comprising:

- 5 i) adding a putative cytotoxic agent to a culture of cancer cells;
- ii) contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from the cancer cells and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the
10 presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- iii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- 15 iv) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer and exogenous H_2O_2 to generate luminescence; and
- v) subjecting the resulting mixture to detection means to read the intensity of
20 luminescence,

wherein the level of intensity of luminescence in the presence of the cytotoxic agent compared to a control such as not containing a cytotoxic agent provides the level of cytotoxicity of the agent.

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The present invention further contemplates a method of treatment using a cytotoxic agent identified using the method defined above. The method of treatment may also involve assessing a clinical protocol using the subject assay. The protocol may be varied depending on how the telomerase levels vary over time with the protocol.

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The TBT can also be used to assess aging and to monitor deterioration or degree of health

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in elderly subjects.

The oligonucleotide primer may be immobilized to the beads by any coupling chemistry including *via* thiol, amine and aldehyde coupling chemistries.

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In one embodiment, the oligonucleotide primer which is the substrate of telomerase is immobilized to the beads *via* a thiol linkage. For example, a suitable linker is represented in SEQ ID NO:5.

- 10 The telomerase assay of the present invention is referred to herein as the "TBT" or "telomerase biosensor technology".

The method of the present invention includes the proviso that elongation of the telomerase substrate oligonucleotide primer is not *via* PCR.

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A summary of sequence identifiers used throughout the subject specification is provided in Table 1.

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TABLE 1
Summary of sequence identifiers

SEQUENCE ID NO:	DESCRIPTION
1	Human Telomerase recognition nucleotide sequence
2	Magnetic bead surface-linked synthetic spacer nucleotide sequence
3	Combined surface linked spacer sequence and telomerase recognition sequence
4	Repeating nucleotide sequence added by telomerase to telomerase recognition sequence
5	Target sequence for telomerase, with a 5' cysteine for thiol coupling
6	Short human telomerase recognition nucleotide sequence
7	Medium human telomerase recognition nucleotide sequence
8	Long human telomerase recognition nucleotide sequence

- 5 A summary of the abbreviations used throughout the subject specification are provided in Table 2.

TABLE 2
Abbreviations

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ABBREVIATION	DESCRIPTION
CPG	Calcium Pectinate Gel
HRP	Horseradish peroxidase
hTERT	Telomerase Reverse Transcriptase
hTR	human Telomerase
TBT	Telomerase biosensor technology
TRAP	Telomeric Repeat Amplification Protocol

BRIEF DESCRIPTION OF THE FIGURES

Some figures contain color representations or entities. Color photographs are available from the Patentee upon request or from an appropriate Patent Office. A fee may be imposed if obtained from a Patent Office.

Figure 1 is a graphical representation showing the method of monitoring the conjugation of target sequence for telomerase to beads. A spectrophotometric method for monitoring the conjugation of an oligonucleotide suitable for extension by telomerase activity to a bead that may be subjected to collection by a magnet or other means. Free oligonucleotide has a peak absorbance around the wavelength of 260 nm while conjugated oligonucleotide has a peak absorbance around 343nm.

Figure 2 is a graphical representation showing the sensitivity of the telomerase assay for LIM1215 cells. This shows that telomerase activity released from lysed LIM 1215 human colon cancer cells can be measured by fluorescence emitted by incorporated fluorescein bound nucleotide. A linear range of detection is apparent as determined by using 100 to 1000 lysed cells.

Figure 3 is a graphical representation showing results of telomerase assay on a superficial bladder cancer sample. This shows telomerase activity released from 1000 lysed LIM 1215 cells and cells collected from a patient with pathologically confirmed superficial bladder cancer. The bladder cancer cells were captured using EpCAM beads. Matched reactions were performed using lysates pretreated with heat to inactivate telomerase enzyme activity (HI). These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with luminol to generate luminescence. The low background signal generated by the streptavidin alone is also shown.

Figure 4 is a graphical representation showing results of telomerase assay on an invasive bladder cancer sample. This shows telomerase activity released from 1000 lysed LIM 1215 cells and cells collected from a patient with pathologically confirmed invasive bladder

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cancer. The bladder cancer cells were captured using EpCAM beads. Matched reactions were performed using lysates pretreated with heat to inactivate telomerase enzyme activity (HI). These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with luminol to generate luminescence. The low background signal
5 generated by the streptavidin alone is also shown.

Figure 5 is a graphical representation showing telomerase activity in cells isolated from fecal samples. These data demonstrate the capacity to isolate known and predetermined colon cancer cells from a faecal sample using EpCAM beads and subsequent release of
10 telomerase activity and measurement using luminescence.

Figure 6 is a graphical representation showing the sensitivity of the assay for HEK293T cells according to an embodiment of the present invention. These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with
15 luminol to generate luminescence. Averaged data are from 10 experiments performed using the manual assay format on different days within three different lysate preparations. This indicates that telomerase activity of stock cell lysates of HEK293T cells can be measured with reproducibility. A linear range of detection is apparent as determined by using the equivalent of 20 to 1250 lysed cells.

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Figure 7 is a graphical representation showing the sensitivity of the assay for low levels of HEK293T cells according to an embodiment of the present invention. Averaged data are from 10 experiments performed using the manual assay format on different days with 3 different lysate preparations. This indicates that telomerase activity of stock cell lysates of
25 HEK293T cells can be measured with reproducibility. A linear range of detection is apparent over the range of 1 to 500 lysed cells. These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with luminol to generate luminescence. These data demonstrate that the TBT assay is sensitive at low cell concentrations.

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Figure 8 is a graphical representation showing results of a statistical evaluation of the lower limit of detection of the TBT assay according to an embodiment of the present invention. Telomerase activity of stock cell lysates of HEK293T was measured with the TBT assay on 10 separate occasions on different days with 3 different lysate preparations. Dashed lines indicate one (1xSD) and 2 (2xSD) standard deviations (SD) above the mean background level (0 CE) which was determined 20 times. The top numbers illustrated within each bar on the histogram are the actual number of SD above background. "n" is the number of individual determinations used to generate the mean. These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with luminol to generate luminescence.

Figure 9 is a graphical representation showing the intra-assay reproducibility of the TBT assay according to an embodiment of the present invention. The telomerase activity of two different concentrations of HEK293T tumor cells was measured. The level of variability, between six separate determinations at each concentration, 100 CE and 1000 CE, within the TBT assay was 5.7% and 4.9% of the total signal, respectively. These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with luminol to generate luminescence. Averaged data (mean $\pm$ standard deviation) are from six replicate samples at each concentration.

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Figure 10 is a graphical representation showing the inter-assay reproducibility of the TBT assay according to an embodiment of the present invention. The telomerase activity of two different concentrations of HEK293T tumor cells was measured on 10 separate occasions. The level of between assay variability at each concentration, 50 CE and 5000 CE, was 6.4% and 8.8% of the total signal respectively. These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with luminol to generate luminescence. Averaged data (mean $\pm$ standard error) are from four separate determinations for each concentration performed on different days.

Figure 11 is a graphical representation showing the specificity of the enzyme specificity of the TBT assay according to an embodiment of the present invention. The telomerase

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activity was determined in the human leukemia cell line TF-1 cells and TF-1 cells over expressing hTERT (human telomerase reverse transcriptase) over a broad range of concentrations. These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin reacted with luminol to generate luminescence. The specificity of the assay is demonstrated by the greater telomerase activity found in the TF-1 cells overexpressing hTERT.

Figure 12 is a graphical representation showing the sensitivity of the TBT assay for the detection of telomerase activity in urine samples according to an embodiment of the present invention. The TBT test was used to measure telomerase activity in cells, isolated from the urine of patients, in cell lysate concentrations ranging from 0 μ l to 2.5 μ l of lysate. Telomerase activity was measured in urine cell lysates from three patients previously showing a positive TBT result, two of which had a high TBT result (Patients #3 and #12) and one patient having a low TBT result (Patient #31). These data indicate that less than 1 μ l of cell lysate, representing less than one hundredth the total number of tumor epithelial cells in each patient sample, was sufficient to give a positive signal.

Figure 13 is a graphical representation showing the sensitivity and specificity of the TBT assay for the detection of telomerase activity in urine samples according to an embodiment of the present invention. The TBT test was used to measure telomerase activity in cells, isolated from the urine of bladder cancer patients (n=29) and normal subjects (n=12). When a 'cut-off' value of 1.5 (fold-change compared to no telomerase control) is used the assay has 96.6% sensitivity and 100% specificity. When the 'cut-off' threshold is 1.2 (dashed line) the sensitivity of the assay is 100% and there is a small increase in the false-positives.

Figure 14 is a graphical representation showing the sensitivity of the TBT assay for the detection of telomerase activity in K562 human leukemia cells according to an embodiment of the present invention. The TBT test was used to measure telomerase activity over a broad range of cell lysate concentrations up to 2500 CE. These data demonstrate telomerase activity using horse radish peroxidase conjugated streptavidin

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reacted with luminol to generate luminescence. The TBT assay shows a high level of sensitivity in analysis of telomerase activity in leukemia cells.

Figure 15 is a graphical representation showing the sensitivity of the TBT assay for the detection of telomerase activity in umbilical cord blood stem cells according to an embodiment of the present invention. CD34-positive cells from the cord blood of three patients were isolated and the TBT assay was performed on 1000 CD34-positive cells. Telomerase activity was detected in all three cord blood samples.

10 **Figure 16** is a graphical representation showing the effect of TBT oligonucleotide length in HEK293T cell lysates.

Figure 17 is a graphical representation of a receiver operating characteristic (ROC) curve showing the diagnostic power of the TBT test in detecting bladder cancer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a sensitive assay for telomerase in a cell or sample of cells. The test is referred to as "TBT" or "telomerase biosensor technology". Reagents useful for
5 conducting the assays also form part of the present invention. The reagents may be part of a kit packaged with instructions for performing the assay or may be separately provided. Detection of telomerase may be quantitative, semi-quantitative or qualitative which are all encompassed by the terms "determination", "determining", "detection", "diagnosis", "prognosis" and "identification". The assay may be automated or semi-automated to
10 permit rapid, high throughput screening. The elucidation of the presence of telomerase activity including the level of telomerase activity is useful for determining the presence or relative levels of cancer cells or cells associated with inflammation, proliferation and/or embryogenesis. Whilst the principle focus of the invention is in humans, the assay may be conducted in all vertebrates, plants and arthropods.

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Having regard to the method and reagents employed in accordance with the present invention, it is apparent that the assay has a range of research and diagnostic applications. The assay is fast, accurate and amenable to single-tube reactions, multiplex protocols, automation and *in situ* detection. The use of magnetic beads enables routine clinical use at
20 a low cost whilst maintaining high sensitivity and clinical sustainability. Other telomerase assays are expensive, cannot be modified for high throughput screening and cannot be routinely used in clinical laboratories. Applications of TBT include, but are not limited to:

i) detection of immortal cells in cancer biopsies for the identification of potential
25 cancer cells;

ii) identification in a cell-based or cell-free screen of agents capable of activating, derepressing, inhibiting or repressing telomerase, including immortalizing agents (e.g. oncogenes) or compounds that might activate telomerase and extend telomeres
30 and replicative lifespan of cells;

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- iii) identification in culture systems or *in vivo* of stem cells or early progenitor cells that possess telomerase activity;
- iv) examination of telomerase regulation during differentiation and development;
- 5 v) identification of telomerase-positive fractions generated during purification of telomerase;
- vi) identification of protozoal or fungal infections; and
- 10 vii) diagnosis of certain types of infertility characterized by an absence of telomerase activity.

The TBT is high throughput, very sensitive inexpensive, and can be routinely employed in
15 a clinical laboratory.

Accordingly, one aspect of the present invention contemplates a method for detecting cells from a subject exhibiting telomerase activity, said method comprising:

- 20 i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and
25 biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting
30 the washed beads with luminol and an enhancer in the presence of exogenously added H₂O₂ to generate luminescence; and

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iv) subjecting the resulting mixture to detection means to read the intensity of the luminescence,

5 wherein the level of intensity of luminescence compared to a negative control or a known data set provides the level of telomerase activity and the number of putative cells producing telomerase.

In a related embodiment, the present invention contemplates a method for detecting cells
10 from a subject exhibiting telomerase activity, said method comprising:

i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract
15 together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;

ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
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iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence wherein said enhancer and H_2O_2 are added automatically in a machine which measures luminescence intensity; and
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iv) subjecting the resulting mixture to detection means to read the intensity of the luminescence,

wherein the level of intensity of luminescence compared to a negative control or a known
30 data set provides the level of telomerase activity and the number of putative cells producing telomerase.

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In one embodiment, "obtaining a sample of cells" includes collecting and partially purifying the cells or at least removing unnecessary components in the samples. An aspect of the present invention provides a method for selective purification of the tumor cells and
5 removal of those cells from potentially interfering substances. Purification of the tumor cells is achieved by incubation of the body fluid containing the cells with magnetic beads, which are coated with tumor cell-specific antibody. The tumor cells of interest are washed extensively and therefore separated from other cell types, the body fluid matrix (eg; urine, blood), and interfering substances. This lessens the possibility of false negatives due to
10 interference with the assay and also false positives caused by non-tumor cells such as activated T-lymphocytes which may be present in an infection. The sample workup procedure is thus considered useful in obtaining high clinical sensitivity and specificity values.

15 By way of example, urine is incubated with magnetic beads coupled with monoclonal antibody, Ber-EP4 (CELLection [Trade Mark] Epithelial Enrich Dynabeads), which selectively captures the epithelial cells. The beads with tumor cells attached are washed several times and lysis of the epithelial cells achieved by addition of CHAPS-based lysis buffer. The advantage of this method is that it separates the tumor cells from potentially
20 interfering substances and also activated lymphocytes, which may contain elevated telomerase activity.

The sample workup procedure removes the cells of interest from many chemicals that may commonly interfere with clinical assays. Isolation of the epithelial cells from blood
25 removes any possibility of interference from hemoglobin, degradative enzymes in urine, or therapeutic compounds such as those used for chemotherapy or other treatments.

The presence of activated lymphocytes has proven problematic for other assays of telomerase activity as these cells can express detectable levels of telomerase activity. The
30 sample workup procedure in the TBT test removes the tumor epithelial cells from activated lymphocytes by selective capture on antibody-attached magnetic beads. Removal of tumor

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cells from activated lymphocytes leads to greater sensitivity and a lower probability of false positives. Whilst useful, this should not be regarded as an essential feature of the present invention.

- 5 The term "subject" includes a vertebrate such as a human or non-human mammal, non-mammalian vertebrate, a plant or other entity comprising a telomerase.

As indicated above, in relation to vertebrates, the cells may be cancer cells or cells associated with inflammation, proliferation or embryogenesis. Accordingly, another
10 aspect of the present invention provides a method for detecting cells selected from cancer cells, inflammatory or proliferative cells and embryogenic cells including stem cells in a sample from a subject, said method comprising:

- 15 i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
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 - ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
 - iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously
25 added H_2O_2 to generate luminescence; and
 - iv) subjecting the resulting mixture to detection means to read the intensity of the luminescence,
- 30 wherein the level of intensity of luminescence compared to a control such as a control not containing cancer, inflammatory or embryogenic cells provides the level of telomerase

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activity and thereby the number of cells.

In a related embodiment, the present invention provides a method for detecting cells selected from cancer cells, inflammatory or proliferative cells and embryogenic cells
5 including stem cells in a sample from a subject, said method comprising:

- i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract
10 together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
15
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence wherein said enhancer and H_2O_2 are added automatically in a machine which measures luminescence intensity; and
20
- iv) subjecting the resulting mixture to detection means to read the intensity of the luminescence,

wherein the level of intensity of luminescence compared to a control such as a control not
25 containing cancer, inflammatory or embryogenic cells provides the level of telomerase activity and thereby the number of cells.

As indicated above, the terms "cancer", "tumor" and "cancerous" may be used interchangeably throughout the subject specification and denotes any cancerous or
30 malignant condition, pre-cancerous condition, myeloma, or any lymphoma or malignant condition, or any other proliferative disorder involving neoplastic cells. The term "cancer"

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- or "tumor" includes breast tumors, colorectal tumors, adenocarcinomas, mesothelioma, bladder tumors, prostate tumors, germ cell tumor, hepatoma/cholangio, carcinoma, neuroendocrine tumors, pituitary neoplasm, small round cell tumor, squamous cell cancer, melanoma, atypical fibroxanthoma, seminomas, nonseminomas, stromal leydig cell
- 5 tumors, sertoli cell tumors, skin tumors, kidney tumors, testicular tumors, brain tumors, ovarian tumors, stomach tumors, oral tumors, bladder tumors, bone tumors, cervical tumors, esophageal tumors, laryngeal tumors, liver tumors, lung tumors, vaginal tumors and Wilm's tumor.
- 10 Examples of particular cancers include but are not limited to adenocarcinoma, adenoma, adenofibroma, adenolymphoma, adontoma, AIDS related cancers, acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, angioma
- 15 sclerosing, angiomatosis, apudoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, branchioma, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma,
- 20 choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-cell lymphoma, carcinoma (e.g. Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhus, bronchiolar, bronchogenic, squamous cell, and transitional cell), carcinosarcoma, cervical dysplasia, cystosarcoma phyllodies, cementoma, chordoma,
- 25 choristoma, chondrosarcoma, chondroblastoma, craniopharyngioma, cholangioma, cholesteatoma, cylindroma, cystadenocarcinoma, cystadenoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, dysgerminoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma,
- 30 fallopian tube cancer, fanconi anaemia, fibroma, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers,

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germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, giant
 cell tumors, ganglioneuroma, glioma, glomangioma, granulosa cell tumor,
 gynandroblastoma, haematological malignancies, hairy cell leukemia, head and neck
 cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease,
 5 human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer,
 hamartoma, hemangioendothelioma, hemangioma, hemangiopericytoma,
 hemangiosarcoma, hemangiosarcoma, histiocytic disorders, histiocytosis malignant,
 histiocytoma, hepatoma, hidradenoma, hondrosarcoma, immunoproliferative small,
 opoma, ontraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer,
 10 langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, li-fraumeni
 syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma,
 Hodgkin's lymphoma, non-Hodgkin's lymphoma, leigomyosarcoma, leukemia (e.g. b-cell,
 mixed-cell, null-cell, t-cell, t-cell chronic, htlv-ii-associated, lymphangiosarcoma,
 lymphocytic acute, lymphocytic chronic, mast-cell and myeloid), leukosarcoma, leydig cell
 15 tumor, liposarcoma, leiomyoma, leiomyosarcoma, lymphangioma, lymphangiocyoma,
 lymphagioma, lymphagiomyoma, lymphangiosarcoma, male breast cancer, malignant-
 rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer,
 mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis
 fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, malignant
 20 carcinoid syndrome carcinoid heart disease, medulloblastoma, meningioma, melanoma,
 mesenchymoma, mesonephroma, mesothelioma, myoblastoma, myoma, myosarcoma,
 myxoma, myxosarcoma, nasal cancer, nasopharyngeal cancer, nephroblastoma,
 neuroblastoma, neurofibromatosis, Nijmegen breakage syndrome, non-melanoma skin
 cancer, non-small-cell-lung-cancer-(nsccl), neurilemmoma, neuroblastoma,
 25 neuroepithelioma, neurofibromatosis, neurofibroma, neuroma, neoplasms (e.g. bone,
 breast, digestive system, colorectal, liver), ocular cancers, oesophageal cancer, oral cavity
 cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer,
 paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-
 neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, osteoma,
 30 osteosarcoma, ovarian carcinoma, papilloma, paraganglioma, paraganglioma
 nonchromaffin, pinealoma, plasmacytoma, protooncogene, rare-cancers-and-associated-

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disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, reticuloendotheliosis, rhabdomyoma, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach
5 cancer, synovial sarcoma, sarcoma (e.g. Ewing's experimental, Kaposi's and mast-cell sarcomas), sertoli cell tumor, synovioma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis/-ureter), trophoblastic cancer, teratoma, theca cell tumor, thymoma, trophoblastic tumor, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer,
10 vulva cancer, Waldenstrom's-macroglobulinemia and Wilms' tumor.

The TBT is a useful assay for risk stratification of cancer patients, such as for risk of remission or cancer spread.

15 An inflammatory or proliferative condition includes cells associated with acne, angina, arthritis, aspiration pneumonia, disease, empyema, gastroenteritis, inflammation, intestinal flu, nec, necrotizing enterocolitis, pelvic inflammatory disease, pharyngitis, pid, pleurisy, raw throat, redness, rubor, sore throat, stomach flu and urinary tract infections, chronic inflammatory demyelinating polyneuropathy, chronic inflammatory demyelinating
20 polyradiculoneuropathy, chronic inflammatory demyelinating polyneuropathy or chronic inflammatory demyelinating polyradiculoneuropathy.

In a preferred embodiment, the telomerase activity is used to quantitate, semi-quantitate or qualitate the presence or level of cancer cells. Reference to "cancer" includes a tumor and
25 a leukemia as well as carcinoma and a sarcoma.

Accordingly, another aspect of the present invention provides a method for detecting cancer cells in a sample from a subject, said method comprising:

30 i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular

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extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;

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ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;

iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence; and

10

(iv) subjecting the resulting mixture detection means to read the intensity of the luminescence,

15 wherein the level of intensity of luminescence compared to a control such as not containing cancerous cells and/or a known data set provides the level of telomerase activity and the number of putative cancer cells.

In a particular embodiment, the present invention provides a method for detecting cancer cells in a sample from a subject, said method comprising:

20

i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;

25

ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;

30

iii) collecting the beads using a non-rotating magnet, washing the beads and contacting

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the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence wherein said enhancer and H_2O_2 are added automatically in a machine which measures luminescence intensity; and

- 5 (iv) subjecting the resulting mixture detection means to read the intensity of the luminescence,

wherein the level of intensity of luminescence compared to a control such as not containing cancerous cells and/or a known data set provides the level of telomerase activity
10 and the number of putative cancer cells.

Cell extracts may be generated by any number of means including sonication, lysis and freeze-thaw methods. In one embodiment, cells are collected by biopsy or in a blood or tissue sample, and lysed using a non-ionic and/or zwitterionic detergent. Cell debris is
15 generally removed by centrifugation or filtration. The supernatant is then collected and used in the assay. Examples of suitable detergents include Tween 20, Triton X-100, Triton X-114, Thesit, NP-40, n-octylglucoside, n-dodecylglucoside, n-dodecyl-beta-D-maltoside, octanoyl-N-methylglucamide (MEGA-8), decanoyl-N-methylglucamide (MEGA-10), and isotridecylpoly(ethyleneglycolether)_n, and preferred zwitterionic detergents include
20 CHAPS (3-((3-cholamidopropyl)dimethylammonio)-1-propane-sulfonate), CHAPSO (3-((3-cholamidopropyl)dimethyl-ammonio)-2-hydroxy-1-propane-sulfonate), N-dodecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate, and digitonin, with CHAPS.

In a preferred embodiment, the cells are lysed with CHAPS buffer [0.5% v/v CHAPS,
25 10mM Tris, 1mM MgCl₂, 1mM EGTA and 10% v/v glycerol with 1 protease inhibitor tablet (Complete Mini, Roche) per 10ml].

Cell collection may be by any means and numbers of cells in a sample to be assayed may vary. Generally from about 1 cell to about 10^6 or greater cells may be assayed at a time.
30 Hence, the present invention is capable of assaying from 1 to 10^{10} cells including 5 to 10^6 cells, 10 to 10^5 cells and so on. Particularly useful cell numbers include 1, 2, 3, 4, 5, 6, 7,

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986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999 or 1000 cells or 5×10^3 , 10^4 , 5×10^4 , 10^5 , 5×10^5 , 10^6 , 5×10^6 and 10^7 cells. The sensitivity of the assay can
25 be seen from Figure 2. In a particularly convenient aspect of the method, cell samples are
not diluted prior to the assay but rather volumes of cells are removed to provide from about
1 cell to 10^6 cells or greater.

Hence, a sensitivity of from 1 to 1000 cells is particularly preferred such as measuring 1, 2,
30 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28,
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947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964,
20 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982,
983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999 or
1000 cells in a sample.

Any type of magnetic particle may be employed in the practice of the assay of the present
25 invention. Typically, the particles are made from Fe_3O_4 , Fe, Co, Ni, their alloys as well as
other ferromagnetic materials. Although not wishing to limit the present invention to any
type of bead, Dynal (trademark - Dynal Invitrogen Corporation, 9099 North Deerbrook
Trail, Brown Deer, WI, USA 53223) or Bioclone (San Diego, CA 92126, USA) beads or
CPG calcium pectinate gel magnetic beads (CPG Inc, Lincoln Pk, NJ 07035, USA) may be
30 employed. Lode Star, polymer-based beads (Polymer Labs, UK and USA) may also be
employed.

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The telomerase substrate, i.e. the oligonucleotide primer, comprises the sequence:



5

wherein:

X is selected from A, T, G and C;

Y is selected from A, T, G and C;

n is 0 or 1;

10 m is 0 or 1; and

o is from 1 to about 400.

Generally, in vertebrates, n is 0, Y is G and o is from about 5 to about 30. In arthropods, n is 0, m is 0 and o is from about 1 to 30. In plants, X is T, n is 1, Y is G, m is 1 and o is

15

In one particular embodiment, the magnetic beads comprise a human telomerase target nucleotide sequence [SEQ ID NO:1] immobilized to their surface. The human telomeric target sequence is 5'-AGGGTTAGGGTTAGGGTTAGGGTTAG-3' [SEQ ID NO:1] which

20

incorporates the repeating (TTAGGG) [SEQ ID NO:4].

Conveniently, the telomerase target sequence [SEQ ID NO:1] is fused at its 5' end to a surface-linked spacer (or anchor) sequence [SEQ ID NO:2] comprising

25

5'-AATCCGTCGAGCAGAGTT-3' [SEQ ID NO:2].

The combined telomerase recognition sequence [SEQ ID NO:1] and the surface-linked spacer sequence [SEQ ID NO:2] is referred to as the spacer-telomerase recognition sequence [SEQ ID NO:3]:

30

5'-AATCCGTCGAGCAGAGTTAGGGTTAGGGTTAGGGTTAGGGTTAG-3'

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[SEQ ID NO:3].

Conveniently, the telomerase recognition sequence is immobilized *via* a thiol linkage. For example, a suitable linker is represented in SEQ ID NO:5:

5

5'SH(SCH₂)₆-TTTTTTAATCCGTCGAGCAGAGTTAGGGTTAG [SEQ ID NO:5].

Whilst the human telomerase recognition sequence is the most preferred to be immobilized to the magnetic beads, the present invention extends to any non-human telomerase recognition sequence which is a substrate for human telomerase. Examples of non-human telomerase sequences include those from non-human primates, livestock animals and laboratory test animals such as from mice, rats, guinea pigs, hamsters, pigs or monkeys.

The TBT may also employ other solid supports including micropatterned surfaces, glass surfaces and supports, quartz crystal microbalance supports, microarrays, porous alumina supports, silica surface supports, nanoparticles, patterned polymer brushes, poly(ethylene glycol) brushes, membranes. The TBT may also be conducted on alternative systems such as nanoparticle amplified surface plasmon resonance (SPR) and BIAcore systems.

20

The present invention is particularly exemplified with respect to the use of biotin labeling of DNA. The biotin moiety on a dUTP is incorporated into the telomerase extended sequence. The biotin serves as a specific binding site to a reagent such as streptavidin-horseradish peroxidase (HRP), avidin-HRP or neutravidin-HRP that acts as a biocatalytic label in the presence of H₂O₂.

25

However, other labels may also be employed as long as an exogenous agent is added to visualize the label or in order to get a detectable signal. Hence, for example, a fluorescent, phosphorescent, chemiluminescent or radioactive label may be incorporated into the extended telomerase recognition sequence provided in order to maximize the resulting signal, an exogenous enhancer and/or signalling-facilitating agent is added. Alternative

30

- 35 -

labels include but are not limited to biotin-dUTP, phycoerythrin-dUTP, fluorescein-dUTP and [α -³²P]-dUTP including all possible isomers thereof. The dNTPs include dATP and dGTP. Enzyme based and chemical detection assays may also be employed.

5 Accordingly, this aspect contemplates a method of detecting cells from a subject exhibiting telomerase activity said method comprising:

10 i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the present of dNTP, including labeled dNTPs to thereby incorporate the label within the elongated primer;

15 ii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with a signal-facilitating agent in order to maximize the signal produced from the label; and

20 iii) subjecting the resulting mixture to detection means to read the intensity of the signal, wherein the level of intensity of the signal compares to a control such as not containing telomerase-containing cells or a known data set provides the level of telomerase activity and the number of putative telomerase-exhibiting cells.

25 Conveniently, step (ii) or part thereof is conducted automatically or semi-automatically, such as in the machine which reads the luminescence intensity.

Although the control is generally a sample not containing a particular cell extract, it may equally be a sample not containing telomerase activity or labeled, dNTPs or other
30 component required for operation of the assay. The control may also be a known data set of values which correlate to cell numbers.

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It is important to note that the aspect of obtaining the cells and contacting an extract these with magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase and incubating the particles in the presence of dNTPs to enable telomerase-mediated primer elongation occurs in the absence of any rotation of the beads. It is
5 considered that the rotation of the beads is not required for acceleration of the kinetics of the reaction or would not accelerate the kinetics of the reaction. Hence, sensitivity of the TBT assay is not dependent on the rate of transport of anolytes or other substances that participate in the assay.

10 The assay of the present invention is also applicable to the detection of telomerase in a cell for research purposes, to determine the health status of the cell or to assess the ability for compounds to inhibit or enhance telomerase activity. This is applicable in all vertebrates, non-vertebrates and plants. In relation to vertebrates and in the case of cancer or an inflammatory condition, the condition may be diagnosed by removing tissue from a subject
15 such as a human in order to screen for the presence of cancer cells or inflammatory cells. In addition, the presence of telomeres of a particular length may be required for proliferation of stem cells such as haematopoietic stem cells. This is important for blood transfusion such as in leukemia subjects. Blood samples may be screened for stem cells having particular telomerase activity which indicates a capacity for the stem cells to
20 proliferate and differentiate into leukocytes and other cells of a hemopoietic lineage. For example, this method may be employed to monitor the success of stem cell mobilization by cytokines such as G-CSF, GM-CSF or other drugs. Thus this method may be used to augment and/or replace other methodologies used to monitor stem cells in peripheral blood or bone marrow.

25 Alternatively, tissue samples may be taken during treatment of a known cancer or inflammatory condition in order to evaluate the success or progress or otherwise of a treatment protocol or therapeutic regime. Such a regime may then be adjusted as necessary.

30

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Accordingly, another aspect of the present invention provides a method for monitoring a treatment protocol such as for cancer or inflammation from a subject undergoing a treatment, said method comprising:

- 5 i) obtaining a sample of cells from said subject, contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs
10 to thereby incorporate biotin within the elongated primer;
- ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting
15 the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence; and
- iv) subjecting the resulting mixture detection means to read the intensity of the luminescence,

20

wherein the level of intensity of luminescence compared to a negative or positive control provides the level of telomerase activity wherein an increase in telomerase activity or a stabilization of telomerase activity is an indicator that the treatment protocol is not adversely affecting the subject.

25

Again, any of the steps but in particular step (iii) above may be conducted automatically.

The assay may also be used to screen for chemotherapeutic agents which reduce telomerase activity. Reference to a "chemotherapeutic agent" includes a chemical
30 compound, immunological compound, natural product or sRNAi complex or a product of an introduced viral vector.

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Accordingly, another aspect of the present invention contemplates a method for assessing the activity of a cytotoxic agent, said method comprising:

- 5 i) adding a putative cytotoxic agent to a culture of cancer cells;
- ii) contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from the cancer cells and incubating the magnetic particles and cell extract together for a time and under conditions sufficient
10 for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- iii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
15
- iv) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer and exogenous H_2O_2 to generate luminescence; and
- 20 v) subjecting the resulting mixture to detection means to read the intensity of luminescence,

wherein the level of intensity of luminescence in the presence of the cytotoxic agent compared to a control such as not containing a cytotoxic agent provides the level of
25 cytotoxicity of the agent.

The present invention further provides chemotherapeutic agents identified by the subject method as well as pharmaceutical compositions comprising same.

30 Generally, the subject being tested is a human. However, the present invention extends to any animal subject, and in particular a mammalian subject including primates (e.g. gorillas,

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marmosets, chimpanzees, monkeys), livestock animals (e.g. sheep, cattle, pigs, horses, goats), laboratory test animals (e.g. mice, rats, rabbits, guinea pigs, hamsters), companion animals (e.g. cats, dogs) and wild animals. Non-vertebrate mammals and plants are also contemplated by the present invention.

5

In the method of the present invention, the presence of the incorporated label is determined by the information of a signal, e.g. electrical signal, color signal or light emission. The sensing member is such that it can sense the signal, generally following a chemical or electronic signal. When the signal is emission of light the detector is a light detector.

10

When the signal is electrical, it results from the transfer of electrons between an electrode and an electron transfer chain, where the label is a member of that electron transfer chain.

Electrodes suitable for use in the method of the subject invention are made of or coated with conducting or semi-conducting materials, for example, gold, platinum, palladium, silver, carbon, copper and indium tin oxide.

15

Reference herein to "luminescence" includes chemiluminescence, bioluminescence, crystalloluminescence, electroluminescence, cathodoluminescence, photoluminescence, phosphorescence, fluorescence, sonoluminescence, thermoluminescence or triboluminescence.

20

The assay of the present invention is also applicable to the simultaneous or sequential detection of more than one label such as occurs during a multiplexing assay. In one example, multiple labels may be employed for different patient samples or from the same patient at different times or after different treatments. In such a case, the magnetic particles carry more than one label (either on the same magnetic particle or on different magnetic particles). In order for simultaneous detection to take place, the assay conditions are those that would allow the simultaneous formation of reaction signals that are distinguishable for each label. Accordingly, the presence of one label leads to a reaction signal of one type (e.g. light emission) while the presence of another label leads to a

25

30

- 40 -

reaction signal of another type (e.g. emission of light in a different spectrum). Alternatively, the detection of the more than one label is achieved in sequence, such that after one assay is performed, the magnetic particles are collected, washed and provided with different assay conditions for the detection of another label. In such case, the reaction
5 signal may be the same, provided that in each assay the reaction signal would be obtained solely in connection with the presence of a single label.

In the diagnostic methods of the invention, the assay is conducted to determine whether an elevated level of telomerase is present. The phrase "elevated level" means that the
10 absolute level of telomerase activity in the particular cell is elevated compared to normal somatic cells in that individual or compared to normal somatic cells in other individuals not suffering from a disease condition. Generally, any detectable level of telomerase activity is considered elevated in cells from normal, post-natal human somatic tissue. Although telomerase activity is present in germline cells and low levels of telomerase
15 activity can be detected in stem cells and certain hematopoietic stem cells, such cells do not present problems for the practitioner of the present method unless these cells are part of blood or tissue being transplanted. In that case (e.g. during a blood transfusion), stem cells with telomerase activity is desirable to ensure an ability to differentiate and proliferate. Germline cells can be readily distinguished and/or separated from human somatic tissue
20 samples, and the telomerase activity present in stem cells and certain hematopoietic cells is present at such low levels that the few such cells present in somatic tissue samples will not create false positive signals from a telomerase activity assay. The detection of telomerase activity in somatic cells is indicative of the presence of immortal cells, such as certain types of cancer cells or inflammatory cells and can be used to make that determination
25 even when the cells would be classified as non-cancerous or non-inflammatory pathology. Thus, the method of the present invention allows cancerous conditions to be detected with increased confidence before cells become visibly cancerous.

The diagnostic tests of the present invention can also be carried out in conjunction with
30 other diagnostic tests. In some instances, such combination tests can provide useful information regarding the progression of a disease, although the present method for testing

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for telomerase activity provides much useful information in this regard. When the present method is used, for example, to detect the presence of cancer cells in a patient sample, the presence of telomerase activity can be used to determine where a patient is at in the course of progression of the disease, whether a particular tumor is likely to invade adjoining tissue
5 or metastasize to a distant location and whether an occurrence of cancer is likely to recur. Tests that may provide additional information in conjunction with the present method include diagnostic tests for the estrogen receptor, progesterone receptor, DNA ploidy, fraction of cells in S-phase, nodal status, Her-2/neu gene products, p53, p16, p21, ras; EGF receptor, A33 (colon specific antigen) [Catimel *et al*, *J. Biol. Chem* 271(41):25664-25670,
10 1996], NY-ESO-1 (cancer testes antigen) [Chen *et al*, *Proc. Natl. Acad. Sci. USA* 94(5):1914-1918, 1997] or other oncogenes.

As indicated above, the TBT of the present invention is also useful for assaying for stem cells such as embryonic stem cells. In particular, TBT can be used to assess the
15 therapeutic involvement of stem cells in disease conditions such as Parkinson's disease, heart disease, diabetes, arthritis, blood disease, osteoporosis, organ transplantation and spinal cord injury. The TBT is useful for monitoring the engraftment of stem cells or stem cell-derived tissue and to monitor the lifespan or state of differentiation of stem cells.

20 The present invention also provides kits for performing the diagnostic method of the present invention. Such kits can be prepared from readily available materials and reagents and can come in a variety of embodiments. For example, such kits can comprise any one or more of the following materials: reaction tubes, buffers, detergent, oligonucleotide telomerase substrates, control reagents, hydrogen peroxide and instructions. An especially
25 preferred kit of the subject invention comprises a reaction tube in which is placed a telomerase substrate and dNTPs and biotylated dUTPs. A wide variety of kits and components can be prepared according to the present invention, depending upon the intended user of the kit and the particular needs of the user.

30 The present invention further contemplates the use of an assay which comprises:

- 42 -

- i) obtaining a sample of cells from a subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads, optionally automatically, with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence; and
- iv) subjecting the resulting mixture detection means to read the intensity of the luminescence;

in the generation of a diagnostic protocol to detect cancer in a subject.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1***Telomerase luminescence assay***

- This example describes the experimental protocols for a highly sensitive and selective biosensor assay, using luminescence as readout, to measure quantitatively telomerase in exfoliated tumor cells in the urine of bladder cancer patients or the stools from patients with colon cancer. Briefly, this assay uses superparamagnetic beads functionalized using thiol coupling to a nucleotide primer that contains the recognition sequence for telomerase. These beads (Biobeads) are incubated with tumor cell extracts, containing telomerase, in the presence of a nucleotide mixture that includes biotinylated-dUTP. Telomerase-induced elongation of the primers proceeds, with the incorporation of biotin-labeling. A number of biotin molecules are incorporated resulting in signal amplification. Avidin-Horseradish Peroxidase (HRP) is added which binds with high affinity (10^{-15}M) to the incorporated biotin. The Biobeads are then well washed, which minimizes contamination by other potentially interfering substances in the bulk biological sample (the magnetic particles can be efficiently trapped using a magnet) and transferred to a 96 well plate in a BMG Luminometer. Hydrogen peroxide, luminol and a chemical enhancer are added, optionally automatically and the luminescence signal detected.
- This protocol conveniently uses a Kingfisher Magnetic Particle Processor to aid assay automation. A BMG Luminometer (BMG, Lattech, Germany) is also used. The chemiluminescence reader may also be modified to allow for automation such as the addition of enhancer, luminol and/or H_2O_2 . It can be set up in multiple plate format.
- The use of the magnetic particles is designed to facilitate automation. The particles themselves can be picked up and manipulated using a magnet: the Kingfisher Magnetic Particle Processor (Thermo Corporation, USA), for example, mixes and moves magnetic particles with electromagnetic magnetic rods covered by disposable tips which prevent cross contamination. The Kingfisher Software allows custom-made protocols to be designed for specific applications. During the initial steps, beads are collected, buffer, reaction mixture and samples added and mixed. Manual intervention is required to transfer

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the plates to a Labnet Shaking Incubator to elevate the temperature (37C, 30 min) to drive the telomerase extension reaction. Use of the Kingfisher 96, which has built in temperature control, obviates this requirement. After incubation the plate is then transferred back to the Kingfisher for the final addition of the streptavidin HRP followed by the rigorous washing steps, which are essential to maintain the constant low background observed in the assay. Finally, the magnetic beads carrying the extended oligonucleotide are transferred into a Nunc 96 well LumiNunc plate: these plates have minimum autoluminescence. The plate is then transferred manually to a BMG Fluorostar Luminometer (BMG Labtech, Germany): this fully automated microplate based multi-detection reader, which is equipped with injectors that deliver reagent at the point of measurement, can be programmed for the addition of luminol (Pierce SuperSignal ELISA Femto Substrate) and peroxide. The same instrument can be used for fluorescence detection. Transfer between workstations (Kingfisher and BMG) can be further automated using robotic transfer (e.g. Zymark Twister, Beckman Sagian). Likewise, alternative technologies can be substituted for the Kingfisher (e.g. Beckman Biomek, Bruker Daltronics ClinProt Robot) or the BMG Fluorostar (e.g. Molecular Devices LMax II).

Experimental Protocol for Kingfisher Magnetic Particle Processor

Add the following to Plate A1 (and B1 if using two plates):

20

- Row A - 100µl Elongation Buffer
- Row B - specified volume of Reaction Mix (total volume once telomerase added is 50µl)
- Row B - add telomerase enzyme extract
- 25 • Row A -finally add 10µl of oligo coupled dynal beads (bead stock of 30mg/ml) to the 100µl of elongation buffer in each well - ensure sufficient mixing.

Place plate(s) into Kingfisher instrument and slide in a comb(s) coverslip to protect the magnets.

30

Select program - "Telomerase Assay"

- 45 -

Hit "Start" twice.

When Kingfisher pauses - it will instruct 'Incubate at 37C'

- 5
 - Take plate(s) out, cover Rows A & B with Nescofilm (Registered Trade Mark) to ensure plate sealed well.
 - Place plate(s) into Labnet shaking incubator (settings: Temp = 37C; Time = 30 min; RPM = 12)
- 10 Following 30 minutes incubation, remove Nescofilm (Registered Trade Mark) and fill the remaining rows as follows:
 - Row C - 100µl 1% w/v SDS/10mM HEPES
 - Row D - 100µl 1% w/v SDS/10mM HEPES
 - 15
 - Row E - 100µl Working Buffer/Tween
 - Row F - 100µl Working Buffer/Tween
 - Row G - 100µl Working Buffer/Tween
 - Row H - 100µl Working Buffer/Tween
- 20 Place plate(s) back into Kingfisher and press "Start" (method will continue).

Set up plate A2 (and B2 if using 2 plates)

Add the following to Plate A2 (B2):

25

- Row A - 100µl Working Buffer/Tween
- Row B - 500µl of 0.5ug/ml Strept HRP (KPL) in working buffer/tween
- Row C - 100µl Working Buffer
- Row D - 100µl Working Buffer
- 30
 - Row E - 100µl Working Buffer
 - Row F - 100µl Working Buffer

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- Row G - 100µl Working Buffer
- Row H - 50µl Working Buffer

When Kingfisher pauses and instructs 'Change plates'

5

- Swap plates over

Press "Start" and the method will resume.

- 10 Prime BMG FluoroStar Luminometer with Luminol in pump A and peroxide in pump B – set up plate template (APL assay – well mode) and volumes (50ul of each).
Luminometer gain to be set to 2000.

At end of method, machine will beep continually, press end.

15

Take plate(s) from Kingfisher and transfer Row H into a NUNC white 96 well plate and place in BMG FluoroStar Luminometer for reading.

BUFFERS

20

Elongation Buffer

20mM Tris-HCl

1.5mM MgCl₂

63mM KCl

25 1mM EGTA

1mM EDTA

150mM NaCl

0.05% Tween20

30 **SDS Buffer**

0.1% w/w SDS

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10mm HEPES

Working Buffer

0.1M Tris, pH 7.4

5 0.1M KCl

(0.05% v/v Tween 20)

Reaction Mix

1 x Elongation buffer

10 0.25% w/v BSA

12.5uM B-dUTP

18.75uM dAdG

Telomerase (or extract containing telomerase activity)

MilliQ H₂O

15

- Remove two aliquots of sample to be assayed (typically 1 - 5µl).
- Heat one aliquot at 95°C for 20 minutes to heat inactivate the sample then place on ice.
- Aliquot reagents into 96 well Kingfisher plate and place in Kingfisher particle processor (see protocol in separate document).

20

- Wash Dynal magnetic beads coupled with the specific telomerase oligonucleotide sequence (Oligo-bead) in 100µL elongation buffer (1x) for 2min.
- Transfer beads into 50µL of the reaction mix (elongation buffer, 12.5µM Biotin-dUTP, 0.25% w/v BSA, 18.75µM dAdG) and sample.

25

- Manually transfer plate to the heater/shaker instrument.
- Incubate oligo-beads with reaction mix for 30 minutes at 37°C to enable elongation of the oligo by the enzyme in the sample.
- Manually transfer plate back to Kingfisher instrument.
- Wash oligo-beads x 2 with 100µL 1% w/v SDS/1mM HEPES for 2 minutes at RT.

30

- Wash oligo-beads x5 with 100µL elongation buffer (1x) for 2 minutes at RT.
- Incubate oligo-beads with 50µl of 1ug/ml Streptavidin-HRP for 30 minutes at RT.

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- Wash oligo-beads x5 with 100µL of working buffer (1M Tris-HCl, 1M KCl, pH 7.4) for 2 minutes at RT.
- Resuspend oligo-beads in a final volume of 50µL working buffer and transfer sample into a white luminescence plate.
- 5 • Place white plate into luminometer for luminescence results (50µL of luminol and 50uL of peroxide are added automatically by the instrument).

EXAMPLE 2

Thiol coupling of target sequence to beads

10

A target sequence for telomerase, with a 5' cysteine for thiol coupling (5'SH(CH₂)₆-TTTTTTAATCCGTCGAGCAGAGTTAGGGTTAGGGTTAG [SEQ ID NO:5]) was conjugated to magnetic beads using the heterobifunctional crosslinker Sulfo-LC-SPDP (Pierce). The oligo is reduced using 50mM trialkylphosphine (tris(2-carboxyethyl) phosphine) (TCEP) for 2 hr at RT. The reduced oligo is purified from the TCEP by size exclusion chromatography on a Superpose 12 HPLC column (Amersham). The reduced oligo is then incubated with Sulfo-LC-SPDP modified magnetic beads overnight at 4°C. The conjugation is monitored via an increase in the 343nm absorbance reading (see Figure 1).

20

EXAMPLE 3

Telomerase Biosensor Test (TBT)

A telomerase assay was conducted as follows:

25

The telomerase target sequence [SEQ ID NO:1] was synthesized using a bead surface-binding oligonucleotide [SEQ ID NO:2] and the combined sequence [SEQ ID NO:3] immobilized to a Dynal (Dynal Invitrogen Corporation, 9099 North Deerbrook Trail, Brown Deer, WI, USA 53223). Immobilization was *via* a cysteine residue binding to the 5' end of SEQ ID NO:3.

30

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Cells were obtained containing putative cancer cells and lysed with CHAPS buffer [0.5% v/v CHAPS, 10mM Tris, 1mM MgCl₂, 1mM EGTA and 10% v/v glycerol with 1 protease inhibitor tablet (Complete Mini, Roche) per 10ml]. The lysed cell extract was then added to the magnetic beads with dNTPs and biotinylated dUTP. Streptavidin-HRP was then added. After incubation, the beads were collected using a magnet without rotation and washed. The beads were then transformed to a 96 well plate. Luminol and an enhancer were added together with hydrogen peroxide. Luminescence was then read.

EXAMPLE 4

10

Sensitivity of assay

LIM1215 carcinoma cells (Whitehead *et al*, *J Natl Cancer Inst* 74(4):749-765, 1985) were counted and aliquots removed containing from 100 to 1000 cells and assayed for telomerase. The results are shown in Figure 2. The graph shows that the sensitivity is as low as one cell. Samples comprising 10⁶ cells or greater were also assayed with good detection of telomerase activity.

EXAMPLE 5

20

Sample preparation

A list of cancers and the sampling technique is provided below. The list is only exemplary of the types of cancers which can be detected.

Sample workups for some of these are included under Item 11. Examples of the type of clinical sample on which the TBT would be used are indicated for each cancer.

bladder cancer: sedimented cells in urine, bladder washings;

urogenital tract cancer: renal pelvic washings, bladder washings;

renal cancer: renal pelvic washings, bladder washings;

30 *colon cancer*: exfoliated faecal epithelial cells, endoscopic biopsy specimens;

- 50 -

leukemia: bone marrow and peripheral blood;

melanoma: peripheral blood, fine needle aspirates;

skin cancer: biopsy, peripheral blood, fine needle aspirates;

5 *lung cancer*: Bronchial alveolar lavage, bronchial brushings and washings, sputum, scrapings and smears, fine needle aspirates, biopsies and tissue sections;

prostate cancer: fine needle aspirates, sedimented cells in urine;

head and neck cancer: scrapings and smears;

lymph nodes: fine needle aspirates;

pancreas: fine needle aspirates;

10 *salivary gland*: fine needle aspirates;

breast: fine needle aspirates, nipple discharge;

liver: fine needle aspirates;

thyroid: fine needle aspirates;

brain cancer: cerebrospinal fluid; and

15 *cervical, vaginal and ovarian cancer*: smears, peritoneal washings.

EXAMPLE 6

Superficial bladder cancer sample

20 Samples of 5µl comprising cells were assayed.

The LIM1215 colon cancer cell line (Whitehead *et al*, 1985 *supra*) is a positive control. Note reduction is signal following heat inactivation (HI). Ep-CAM beads were used to separate the cancer cells from activated lymphocytes during the sample workup. The results are shown in Figure 3.

25

EXAMPLE 7***Invasive bladder Cancer sample***

Samples of 1µl comprising cells were assayed. The results are shown in Figure 4.

5

EXAMPLE 8***Comparison of telomerase assay with the TRAP method***

Using telomerase assay as escribed in Example 3, a comparison was made with the TRAP
 10 assay (Hess *et al*, 2002 *supra*). The results are shown in Table 3. The telomerase assay is
 clearly more sensitive than the TRAP assay. See also Example 18.

TABLE 3***Clinical Samples assayed using the TBT assay***

15

Cancer Type	Telomerase assay	TRAP Results
Superficial	Positive	Negative
Superficial	Positive	Negative
Superficial (CIS)	Positive	NT
Invasive	Positive	Negative
Invasive	Positive	NT
Normal	Negative	NT
Normal	Negative	NT

NT = Not Tested

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EXAMPLE 9

Isolation of colonocytes from fecal samples

- Determine weight of fecal sample.
- 5 • Vigorously vortex sample in 50mL/g PUCK's dispersing buffer with additives (See Puck's buffer recipe).
- Filter slurry through 100µm membrane and collect flow through.
- Filter collect liquid through 60µm membrane and collect flow through.
- Centrifuge flow through for 10 minutes at 1000g at 4°C.
- 10 • Resuspend cell pellet in 2.5mL of PUCK's dispersing buffer with antibiotics.
- Layer over a discontinuous gradient of 7.5mL Percoll and centrifuge at 20 000 x g for 20 minutes @ 4°C (fixed angle rotor).
- Collect the cell fraction and make up to 10mL with PBS containing 1% v/v FCS and 0.6% w/v sodium citrate.
- 15 • Centrifuge for 10 minutes at 1000g at 4°C.
- Resuspend in PBS containing 1% v/v FCS and 0.6% w/v sodium citrate (2ml).
- Aliquot 40µl (1×10^7) Dynal EpCAM beads into 2 tubes and wash x2 in PBS/0.1% w/v BSA.
- Aliquot 2mL sample into the 2 tubes (1mL each) containing 40µl EpCAM beads
- 20 and incubate for 30 at 4°C with rotation.
- Place tubes in magnet and remove S/N (Keep for cytospin).
- Resuspend in PBS/0.1% w/v BSA (200µL) – Pool both tubes into one and put into magnet to remove the 400µl of supernatant.
- Repeat 200uL PBS/0.1% w/v BSA wash x 2.
- 25 • Add 200uL of CHAPS lysis buffer.
- Lyse cells by passing through a fine needle.
- Incubate lysates on ice for 30 minutes.
- Spin at 10,000g for 20 minutes at 4°C.
- Aliquot S/N and snap freeze in LN2.
- 30 • Snap freeze remaining cell pellet.

The assay results are shown in Figure 5.

EXAMPLE 10

5

Sensitivity of TBT

HEK293T (Graham *et al*, *J Gen Virol* 36:59-74, 1977) tumor cell lysate was assayed for telomerase over a broad range of lysate concentrations, from 10-1250 cell equivalents (CE). The relationship between the TBT result (luminescence signal) and the lysate
10 concentration is linear up to approximately 1250 CE, after which the TBT signal begins to plateau. The TBT response relationship is linear at concentrations of cells expected in the urine of bladder cancer patients. The results are shown in Figures 6 and 7.

The TBT assay performs at very low cell concentrations. Statistical evaluation of the lower
15 limit of detection revealed that the minimum number of cells detectable with the TBT assay is as few as 20 CE. The results are shown in Figure 8. The TBT test can detect positive signals from very few numbers of telomerase-expressing cells. It, therefore, has the capability of detecting very small numbers of exfoliated tumor cells in urine.

20

EXAMPLE 11

Intra- assay reproducibility

The within assay reproducibility of the TBT test was assessed by measuring the telomerase activity of two different concentrations, 100 CE and 1000 CE, of HEK293T tumor cells.
25 The level of variability, between six replicate samples at each concentration, within the TBT assay was approximately 5%. The results are shown in Figure 9.

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EXAMPLE 12

Inter- assay reproducibility

The between assay reproducibility of the TBT test was assessed by measuring the telomerase activity of two different concentrations of HEK293T tumor cells, 50 CE and 5000 CE. The assay was performed on four separate occasions on different days. The level of between assay variability for each concentration ranged from 6-9%. The results are shown in Figure 10.

EXAMPLE 13

Specificity of assay

The specificity of the TBT test was determined by measuring the telomerase activity of tumor cells overexpressing the human telomerase reverse transcriptase (hTERT). Telomerase activity was measured in the TF-1 human erythroleukaemia cell line (Kitamura *et al*, *Blood* 73(2):375-380, 1989) containing retroviral vectors expressing the human telomerase reverse transcriptase [hTERT] (Li *et al*, *Leukemia* 20:1270-1278, 2006). The results are shown in Figure 11.

EXAMPLE 14

Measurement of telomerase activity in urine samples

The TBT test was used to measure telomerase activity in cells, isolated from the urine of bladder cancer patients, in cell lysate concentrations ranging from 0 μ l to 2.5 μ l of lysate. Telomerase activity was measured in urine cell lysates from three patients previously showing a positive TBT result, two of which having a high TBT result (Patient #3, TBT ratio 6.70 and Patient #12, TBT ratio 6.38) and one patient having a low TBT result (Patient #31, TBT ratio 1.59). The results are shown in Figure 12.

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EXAMPLE 15***Bladder cancer monitoring***

The TBT test was used to measure telomerase activity in cells, isolated from the urine of
5 bladder cancer patients and normal subjects. A positive TBT test signal is defined as a
signal >1.5-fold higher in magnitude than the background signal. The results are shown in
Figure 13 and Table 4.

TABLE 4***Clinical data – summary***

Ratio (Test/HI – heat inactivated)		
Patient Group	Mean $\pm$ SEM	N
Normal	1.20 $\pm$ 0.05	12
Cancer	3.02 $\pm$ 0.27	29

TBT data

Mean data given above represents averaged TBT results. Aside from the relation to the
15 "Cut-Off" value, there appears to be little correlation between the magnitude of the TBT
result and the stage and severity of bladder cancer.

EXAMPLE 16***Leukemia***

20

The TBT test was used to measure telomerase activity in the human leukemia cell line
K562 (Lozzio and Lozzio, *Blood* 45:321-334, 1975). The TBT assay is sensitive for the
detection of telomerase activity in leukemia cells, is quantitative and relatively simple to
perform compared to existing methods for measuring telomerase in leukemia cells. The
25 results are shown in Figure 14.

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EXAMPLE 17

Umbilical cord blood stem cells

- The TBT test was used to measure telomerase activity in umbilical cord blood stem cells.
- 5 The TBT assay was sensitive for the detection of telomerase activity in umbilical cord blood stem cells in all three cord blood samples, using 1000 CE. The results are shown in Figure 15.

EXAMPLE 18

Comparative assays

10 The assay features of the present invention and the standard TRAP assay are compared. A summary of the comparative features is provided in Table 5. The comparison highlights the improved efficacy of the TBT compared to the TRAP assay.

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TABLE 5
Comparison with standard TRAP

Assay Feature	TBT	Reasons	TRAP	Reasons
Time frame	<2 ½ hours	Employs test tubes and plates, simple pipetting steps, and magnetic beads	1 day	Employs PCR thermocycling, running of gels, and complicated image analysis
Operating throughput	High	When fully automated, can run 96 wells (or more) every 2 ½ hours.	Low	Limited by gel running and complicated analysis
Routine Use	High	Uses standard laboratory equipment. Highly amenable to automation on typical pathology lab robotic systems	Not possible	The need to run PCR products on gels and the sophisticated image analysis prevents routine clinical utility
Automation	High	Magnetic bead-based method suitable for liquid handling robotics readily available in routine pathology laboratories.	Not possible	Non-standard assay methodology (gels, image analysis) prevents automation
Sensitivity - research use	High (<10 cells)	Positive signals are detected below 10 HEK293T or LIM1215 cells	High (variable)	Optimal conditions for PCR difficult to attain
Sensitivity - clinical samples	High (93-97%)	Positive signals detected from clinical samples close to cut-off	Low	Very poor sensitivity in our hands, possibly due to PCR contamination issues.
Interference	Low	Sample workup purifies cells of interest and removes them from interfering substances.	High	PCR reaction easily "poisoned" by interfering substances in clinical samples; eg; blood components (haemoglobin).

Assay Feature	TBT	Reasons	TRAP	Reasons
Robustness	High	Magnetic beads are very stable. Telomerase reaction robust. Chemiluminescence widely used in clinical labs.	Low	Typical PCR issues such as cross-contamination, and interference of Taq polymerase by endogenous substances. PCR introduces errors in the quantity and ratio of extension products.
Quantitation	Quantitative	Luminescence measurement can be defined per cell number (equivalent lysate)	Semi-quantitative at best	Logarithmic laddering of the product necessitates complicated image analysis of DNA on gels.
False results	Rare, if any	.	Common	Both false negative and false positive results are common due to PCR inhibitors, over-sensitivity or PCR contamination
Ease of operation	Good	Easy pipetting procedures, can be fully automated.	No. Time consuming and requires skilled operator	Need to run gels. Difficult analysis. Some TRAP assays use radioactivity for detection.
Costs	Low	Instrumentation simple, reagents relatively inexpensive.	High	Expensive kits

EXAMPLE 19***Monitoring telomerase activity in conjunction with telomerase therapeutics***

The TBT is of benefit in selecting and monitoring patients who are subject to therapies that
5 target telomerase activity and components of the telomerase complex. Such applications
include vaccines against telomerase components as may used in the treatment of cancers or
autoimmune or hyper-proliferative disorders.

Similarly, the TBT can be used to monitor the reactivation of telomerase activity as part of
10 therapies such as stem cell activation in tissue regeneration, replacement, repair and
restoration such as skin or other organs. Other contexts include the activation of stem cell
activity in bone marrow, neurogenic zone of the adult brain, and the reactivation of
T lymphocytes in HIV patients. Other contexts include gastrointestinal and respiratory
tract recovery following damage such as that produced by chemotherapies or
15 radiotherapies.

Similarly, the TBT is applicable to monitor the efficacy of telomerase inhibitors in the
context of drug development in the laboratory setting, in animal models and in patients.

20 Similarly, the TBT is useful to monitor the maintenance of stem and progenitor cell
activity in tissues reconstituted with embryonic stem cell-derived cells and tissues where
there is a need to achieve short or long term tissue replacement.

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EXAMPLE 20

Repetitions of the telomerase repeat sequence

Design of the telomerase-specific oligonucleotide template attached to the magnetic bead
5 is critical for maximizing sensitivity of the TBT assay. The minimal recognition DNA
sequence for base-pairing between the RNA component of telomerase and the telomere
end is 9 bases –TAGGGTTAG, however, multiple repeats of this sequence more
accurately depict the nature of chromosome telomere ends and the scanning nature of
enzymes used to achieve accurate base-pair recognition. Telomerase templates include
10 those ranging from 1.5-3 hexameric repeats.

Three forms of the oligonucleotide were tested. A short version containing a partial (0.5)
repeat, a slightly longer version containing 2.5 repeats, and a longer version with 3.5
repeats. The longer version provided better absolute signal relative to the background
15 signal (no telomerase extract). This increased dynamic range is likely to translate into
increased sensitivity of the assay. The results are shown in Figure 16.

The oligonucleotides tested were as follows:

20 **Short:** 5'SH-(CH₂)₆-TTTTTTAATCCGTCGAGCAGAGTT (SEQ ID NO:6)
Medium: 5'SH-(CH₂)₆-TTTTTTAATCCGTCGAGCAGAGTTAGGGTTAGGG
TTAG (SEQ ID NO:7)
Long: 5'SH-(CH₂)₆-TTTTTTAATCCGTCGAGCAGAGTTAGGGTTAGG
GTTAGG GTTAGGGTTAG (SEQ ID NO:8)

25

The length of oligonucleotide may also be important for shelf-life stability. Reaction beads
are stored in the presence of EDTA and EGTA which bind/sequester free metal ions. Metal
ions are essential co-factors for enzymes that degrade nucleic acids and therefore their
removal protects the oligonucleotides from degradation.

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EXAMPLE 21

Automation

The TBT assay is highly amenable to automation because it uses standard magnetic bead
5 technology. Magnetic bead-based liquid handling robotic systems are used commonly in
routine pathology laboratories for a variety of applications. The TBT assay can be easily
adapted to a variety of such systems and is not machine-dependent.

Automation of the TBT assay puts it at a distinct advantage compared to other techniques
10 such as TRAP and the assay described in PCT/IL01/00808 (WO 02/20838). The latter
employs a rotating electromagnet and cannot be readily automated. It is not suitable for
routine pathology lab use. TRAP in its original form requires that PCR products are run on
electrophoresis gel and subsequently analysed by imaging, hence it is not suitable for high
throughput automation.

15

EXAMPLE 22

Cell capture beads

Any cell-specific antibodies, receptors or mimetics can be used for purification of cells of
20 interest for telomerase activity measurement. These include for example:

- Anti-EGF receptor (for tumour cells);
- Anti-CD34 (stem cells);
- Anti-CD45 (common leukocyte antigen);
- 25 Anti-CD19 (pan-B-cell antigen) CD4 and CD8 (lymphocytes);
- Anti-BerEP4 (pan-epithelial cell surface antigen); and
- Anti-A33 (Colonic epithelial antigen)

Cells can also be purified or isolated by other methods such as continuous or non-
30 continuous ficoll gradients for isolation of peripheral blood mononuclear cells (PBMC).

EXAMPLE 23***Sample preparation method and workups***

The TBT test can be used for the detection of malignant cells in relation to many different
5 cancers. Typical clinical samples that may be analysed using the TBT test include, but are
not restricted to, the following:

10 Bronchial alveolar lavage, bronchial brushings and washings, sputum, scrapings, smears
for the detection of neoplasms in the bronchial tree, lung cancer, head and neck cancer.

Fine needle aspirates, biopsies and tissue sections for the detection of malignant cells in the
lung, lymph nodes, pancreas, salivary gland, breast, liver, thyroid, and in prostate cancer.

15 Sedimented cells in urine, renal pelvic washings, bladder washings for the detection of
prostate cancer, bladder cancer, urogenital tract cancer, and renal cancer.

Blood for the detection of melanoma and cancers of the haematopoietic system.

20 Body cavity fluids (pleural fluid, peritoneal fluid, pericardial fluid, peritoneal washings,
gutter washings) for the detection of malignant neoplasms.

Cerebrospinal fluid for the detection of malignant cells in the CSF.

Endoscopic biopsy specimens for the detection of cancer of the gastrointestinal tract.

25 Faecal specimens for the detection of malignant cells in colon cancer and other cancers of
the gastrointestinal tract.

Nipple Discharge: for the detection of breast cancer and cancers causing nipple discharge.

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PAP Test™ / PAP smears (Cervical/Vaginal Screening) for the detection of cervical, vaginal and ovarian cancer. May also be used for the detection of certain infectious and inflammatory conditions.

- 5 Skin (TZanck Smear) for vesicular diseases secondary to herpes virus infections (Herpes Simplex virus and Varicella-Zoster virus).

In the case of bladder cancer, tumor epithelial cells are isolated by selective capture from urine using epithelial cell-specific antibodies attached to magnetic beads.

10

EXAMPLE 24

Urine processing procedure – sample workup

15 All steps are performed on ice to prevent the non-specific attachment of cells to the Dynal beads.

- 20
1. Urine is collected (at least 50mls) and kept on ice. The urine is transferred to a 50ml tube. If there is more than 50mls, the urine is divided into 2 equal volumes in the 50ml tubes and each processed as below.
 2. Sample is centrifuged at 750g for 5 minutes at 4°C. Supernatant is discarded into a beaker containing a HazTab.
 - 25 3. Pellet is resuspended in 10ml PBS (pH 7.4), supplemented with 0.1% w/v BSA and a protease inhibitor tablet (thereafter referred to as wash buffer).
 4. Sample is centrifuged at 750g for 5 minutes at 4°C. Supernatant is discarded into the beaker with the HazTab.
 - 30 5. Washing step is repeated (steps 4-5).

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6. During step 5, the Epithelial Enrich Collection Dynal beads are washed once with 100µl wash buffer (using the Dynal magnetic trap).
7. Following centrifugation, the pellet is re-suspended in 1ml of wash buffer and transferred to a 1.5ml eppendorf tube.
8. Washed beads are added to the washed urine cells from Step 5. For pellets that are less than 1mm in diameter, 25µl of beads are used. For pellets between 1-2mm, 30µl of beads are used. For anything larger than 2mm, 40µl of beads are used.
9. The beads and urine cells are mixed gently for 30 minutes at 4°C with rotation (60 r.p.m.).
10. Samples are centrifuged (Capsule Tomy HF120) for 30 sec to ensure that no beads or buffer is left in the lid of the eppendorf tube.
11. Tubes are placed in the Dynal Magnetic Trap (Dynal MPC-S), and the supernatant carefully transferred to a fresh 1.5ml Eppendorf tube using a Gilson P1000 pipette: Supernatant is centrifuged at 13,000 r.p.m. in a Hereaus Biofuge for 5 minutes at 4°C. Supernatant is removed and cells in the pellet lysed (this contains cells that have not bound to the Epithelial Enrich Collection Dynal beads). This fraction may contain activated lymphocytes and should be stored separately as a frozen cell pellet (-70°C) for subsequent analysis, if required.
12. Beads from Step 11 are washed by re-suspending in 1ml wash buffer and then the supernatant is removed using the Dynal magnetic trap as described above. This supernatant is discarded.
13. CHAPS lysis buffer (100µl) is added to the Dynal beads bound to the epithelial cancer cells.

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14. Cells are lysed by pipetting up and down at least 10 times using a Gilson P200 pipette.
15. Lysates are incubated on ice for 30 minutes.
- 5 16. Lysates are centrifuged at 13,000 r.p.m. in a Hereaus Biofuge for 5 minutes at 4°C.
17. Beads are removed by place tubes in the magnetic trap.
- 10 18. Supernatants (~30µl) are aliquotted into each of 3 tubes and the pellet discarded.
19. Lysates are snap-frozen on dry ice for 5 minutes and transferred to -70°C refrigerator.

15

EXAMPLE 25

Sample workup for exfoliated colonocytes from faecal samples of colon cancer patients

- Faecal samples are collected under informed consent from patents with clinically proven colorectal cancer. Samples are collected at home and transported immediately to the
- 20 laboratory (less than 2 hours) where aliquots (2g) are dispersed in Puck's saline with antibiotics (500 U/L penicillin, 500 mg/L Streptomycin-sulphate, 1.25 mg/L amphotericin B and 50 mg/L gentamicin). The faecal slurry is filtered sequentially through 100 µm and 60 µm membranes (Nylon/Net membrane filters, Millipore, Australia) to remove large debris before being centrifuged at 400 g for 10 minutes at 4°C. The pellet is washed twice
 - 25 with PBS containing 1% v/v FCS and 0.6% w/v sodium citrate, followed by recovery of epithelial cells using 40 µl Epithelial Enrich CELlection (Trade Mark) Dynabeads. The cells are incubated with the Dynabeads for 30 min at 4 °C and the supernatant then removed using the Dynal Magnetic Particle Processor. The cells attached to the magnetic beads are washed 3 times with PBS containing 0.1% w/v BSA before lysis with 200 µl
 - 30 CHAPS lysis buffer. The resulting supernatant is snap frozen in liquid nitrogen and stored at -70°C.

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EXAMPLE 26***Sample workup for umbilical cord stem cells: (enrichment of lineage-negative cells)***

Human umbilical cord blood (UCB) is collected in sterile bottles containing an
5 anticoagulant citrate buffer and processed within 18 hours of collection. To deplete red
blood cells, UCB is diluted 1:2 with Dulbecco's phosphate-buffered saline, and red blood
cells agglutinated at room temperature using 1% w/v Hespan (DuPont Pharma,
Wilmington, DE). Residual red blood cells are lysed with 0.17 mM NH₄Cl, 10 mM Tris-
Cl at pH 7.2, 0.25 mM EDTA. Lineage-negative (Lin-) cells are isolated by depletion of
10 cells expressing glycophorin A, CD3, CD2, CD56, CD24, CD19, CD66b, CD14, and
CD16 using the StemSep kit (Stem Cell Technologies, Vancouver, British Columbia,
Canada) according to kit instructions. The percentage of CD34+ cells in the resulting Lin-
fraction ranges from 63% to 82%.

15

EXAMPLE 27***Sample workup for leukemia cells***

Cells for diagnosis and analysis of leukemia patients are isolated from bone marrow or
peripheral blood. Ten-ml human bone marrow aspirates, taken from the iliac crest of
20 normal donors, are diluted 1:1 with phosphate-buffered saline and centrifuged at 900 g for
10 minutes at room temperature. The washed cells are resuspended in PBS to a final
volume of 10 ml and layered over an equal volume of 1.073 g/ml Percoll solution. After
centrifugation at 900 g for 30 minutes, the mononuclear cells (MNCs) are recovered from
the gradient interface and washed with PBS. Percoll-fractionated MNCs or
25 non-fractionated bone marrow cells are suspended in PBS for analysis. MNCs are isolated
from buffy coats of peripheral blood by Ficoll-Paque density gradient centrifugation and
washed in PBS.

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EXAMPLE 28

Receiver operating characteristic curve

Figure 17 shows a "Receiver Operating Characteristic" curve (ROC curve) evidencing the
5 sensitivity of the TBT test in detecting bladder cancer.

The ROC curve depicts the pattern of sensitivities and specificities observed in the clinical study when the performance of the TBT test is evaluated at different diagnostic thresholds. The overall diagnostic performance of the TBT test is judged by the position of the ROC
10 line. Poor tests have lines close to the rising diagonal, whereas lines for perfect tests rise steeply and pass close to the top left hand corner, where both the sensitivity and specificity are 1. The ROC line for the TBT closely approaches the line for a perfect diagnostic test.

15 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or
20 more of said steps or features.

BIBLIOGRAPHY

Catimel *et al*, *J. Biol. Chem* 271(41):25664-25670, 1996

Chen *et al*, *Proc. Natl. Acad. Sci. USA* 94(5):1914-1918, 1997

Graham *et al*, *J Gen Virol* 36:59-74, 1977

Hess *et al*, *Clin. Chem.* 48:18-24, 2002

Hiyama *et al*, *Cancer Lett.* 194:221-223, 2003

Kim *et al*, *Science* 266:2011-2015, 1994

Kitamura *et al*, *Blood* 73(2):375-380, 1989

Li *et al*, *Leukemia* 20:1270-1278, 2006

Lozzio and Lozzio, *Blood* 45:321-334, 1975

Shay *et al*, *Hum. Mole. Gen.* 10:667-685, 2001

Weizmann *et al*, *Chem. Bio.* 5:943-948, 2004

Whitehead *et al*, *J Natl Cancer Inst* 74(4):749-765, 1985

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CLAIMS:

1. A method for detecting cells from a subject exhibiting telomerase activity, said method comprising:

- i) obtaining a sample of cells from said subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence; and
- iv) subjecting the resulting mixture detection means to read the intensity of the luminescence,

wherein the level of intensity of luminescence compared to a control not containing the cells to be detected or to a known data set provides the level of telomerase activity and the number of cells.

2. The method of Claim 1 wherein the oligonucleotide primer comprises the sequence $(X_n TTAGGY_m)_o$ wherein:

X is a nucleotide selected from A, T, G and C;

Y is a nucleotide selected from A, T, G and C;

n is 0 or 1;

m is 0 or 1; and

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o is from about 1 to about 400.

3. The method of Claim 2 wherein the oligonucleotide primer comprises the nucleotide sequence as set forth in SEQ ID NO:1.
4. The method of Claim 2 wherein the oligonucleotide primer comprises the nucleotide sequence as set forth in SEQ ID NO:3.
5. The method of Claim 1 or 2 or 3 or 4 wherein the subject is a human.
6. The method of Claim 1 or 2 or 3 or 4 wherein the subject is non-human or vertebrate.
7. The method of Claim 1 or 2 or 3 or 4 wherein the subject is a plant.
8. The method of Claim 5 or 6 wherein the cells from the subject comprise cancer cells.
9. The method of Claim 5 or 6 wherein the cells from the subject comprise inflammatory cells.
10. The method of Claim 5 or 6 wherein the cells from the subject comprise stem cells.
11. The method of Claim 1 wherein the oligonucleotide primer is immobilized to the magnetic particles *via* a thiol linkage.
12. The method of Claim 1 wherein the addition of luminol, enhancer and/or H₂O₂ is automatic or semi-automatic.

13. The method of Claim 1 wherein the cancer is any cancerous or malignant condition, pre-cancerous condition, myeloma, or any lymphoma or malignant condition, or any other proliferative disorder involving neoplastic cells.

14. The method of Claim 13 wherein the cancer includes breast tumors, colorectal tumors, adenocarcinomas, mesothelioma, bladder tumors, prostate tumors, germ cell tumor, hepatoma/cholangio, carcinoma, neuroendocrine tumors, pituitary neoplasm, small round cell tumor, squamous cell cancer, melanoma, atypical fibroxanthoma, seminomas, nonseminomas, stromal leydig cell tumors, sertoli cell tumors, skin tumors, kidney tumors, testicular tumors, brain tumors, ovarian tumors, stomach tumors, oral tumors, bladder tumors, bone tumors, cervical tumors, esophageal tumors, laryngeal tumors, liver tumors, lung tumors, vaginal tumors and Wilm's tumor.

15. The method of Claim 13 wherein the cancer is adenocarcinoma, adenoma, adenofibroma, adenolymphoma, adontoma, AIDS related cancers, acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, angioma sclerosing, angiomatosis, apudoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, branchioma, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-cell lymphoma, carcinoma (e.g. Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell), carcinosarcoma, cervical dysplasia, cystosarcoma phyllodies, cementoma, chordoma, choristoma, chondrosarcoma, chondroblastoma, craniopharyngioma, cholangioma, cholesteatoma, cylindroma, cystadenocarcinoma, cystadenoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, dysgerminoma,

endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibroma, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, giant cell tumors, ganglioneuroma, glioma, glomangioma, granulosa cell tumor, gynandroblastoma, haematological malignancies, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, hamartoma, hemangioendothelioma, hemangioma, hemangiopericytoma, hemangiosarcoma, histiocytic disorders, histiocytosis malignant, histiocytoma, hepatoma, hidradenoma, hondrosarcoma, immunoproliferative small, opoma, ontraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leiomyosarcoma, leukemia (e.g. b-cell, mixed-cell, null-cell, t-cell, t-cell chronic, htlv-ii-associated, lymphangiosarcoma, lymphocytic acute, lymphocytic chronic, mast-cell and myeloid), leukosarcoma, leydig cell tumor, liposarcoma, leiomyoma, leiomyosarcoma, lymphangioma, lymphangiocyoma, lymphagioma, lymphagiomyoma, lymphangiosarcoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, malignant carcinoid syndrome carcinoid heart disease, medulloblastoma, meningioma, melanoma, mesenchymoma, mesonephroma, mesothelioma, myoblastoma, myoma, myosarcoma, myxoma, myxosarcoma, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, Nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsccl), neurilemmoma, neuroblastoma, neuroepithelioma, neurofibromatosis, neurofibroma, neuroma, neoplasms (e.g. bone, breast, digestive system, colorectal, liver), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer,

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paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, osteoma, osteosarcoma, ovarian carcinoma, papilloma, paraganglioma, paraganglioma nonchromaffin, pinealoma, plasmacytoma, protooncogene, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, reticuloendotheliosis, rhabdomyoma, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, sarcoma (e.g. Ewing's experimental, Kaposi's and mast-cell sarcomas), sertoli cell tumor, synovioma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis/-ureter), trophoblastic cancer, teratoma, theca cell tumor, thymoma, trophoblastic tumor, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia and Wilms' tumor.

16. The method of Claim 9 wherein the inflammatory cells are involved in acne, angina, arthritis, aspiration pneumonia, disease, empyema, gastroenteritis, inflammation, intestinal flu, nec, necrotizing enterocolitis, pelvic inflammatory disease, pharyngitis, pid, pleurisy, raw throat, redness, rubor, sore throat, stomach flu and urinary tract infections, chronic inflammatory demyelinating polyneuropathy, chronic inflammatory demyelinating polyradiculoneuropathy, chronic inflammatory demyelinating polyneuropathy or chronic inflammatory demyelinating polyradiculoneuropathy.

17. The method of any one of Claims 1 to 16 wherein the cells are selectively purified or enriched prior to detecting the level of telomerase activity.

18. The method of Claim 17 wherein the cells were purified or enriched by incubating the cells in magnetic beads located with cell-specific antibodies followed by washing to remove potentially interfering substances.

19. A method for assessing the activity of a cytotoxic agent, said method comprising:

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- i) adding a putative cytotoxic agent to a culture of cancer cells;
- ii) contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from the cancer cells and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;
- iii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iv) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer and exogenous H_2O_2 to generate luminescence; and
- v) subjecting the resulting mixture to detection means to read the intensity of luminescence,

wherein the level of intensity of luminescence in the presence of the cytotoxic agent compared to a control such as not containing a cytotoxic agent provides the level of cytotoxicity of the agent.

20. Use of an assay which comprises:

- i) obtaining a sample of cells from a subject and contacting magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;

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- ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence; and
- iv) subjecting the resulting mixture detection means to read the intensity of the luminescence,

in the generation of a diagnostic protocol to detect cancer in a subject.

21. The method of Claim 19 or the use of Claim 20 wherein the oligonucleotide primer is immobilized to the magnetic particles *via* a thiol linkage.

22. The method of Claim 19 or the use of Claim 20 wherein the addition of luminol, enhancer and/or H_2O_2 is automatic or semi-automatic.

23. The method of Claim 19 or use of Claim 20 wherein the cells are selectively purified or enriched prior to detecting the level of telomerase activity.

24. The method or use of Claim 23 wherein the cells were purified or enriched by incubating the cells in magnetic beads located with cell-specific antibodies followed by washing to remove potentially interfering substances.

25. A kit for detecting cancer or for assessing the cytotoxic potential of anti-cancer agent said kit comprising in compartment form a first compartment comprising magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase; a second compartment comprising reagents including hydrogen peroxide; and a third compartment comprising dNTPs and/or biotinylated dNTPs said kit further comprising instructions for use comprising i) obtaining a sample of cells from said subject and contacting

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magnetic particles carrying an oligonucleotide primer which is a substrate for telomerase with a cellular extract from said cell sample and incubating the magnetic particles and cell extract together for a time and under conditions sufficient for telomerase-mediated elongation of the oligonucleotide primer to occur in the presence of the NTPs and biotinylated UTPs to thereby incorporate biotin within the elongated primer;

- ii) contacting the magnetic particles with streptavidin-horseradish peroxidase;
- iii) collecting the beads using a non-rotating magnet, washing the beads and contacting the washed beads with luminol and an enhancer in the presence of exogenously added H_2O_2 to generate luminescence; and
- iv) subjecting the resulting mixture detection means to read the intensity of the luminescence,

wherein the level of intensity of luminescence compared to a control not containing the cells to be detected or to a known data set provides the level of telomerase activity and the number of cells.

26. The kit of Claim 25 wherein the oligonucleotide primer comprises the sequence $(X_n TTAGGY_m)_o$ wherein:

X is a nucleotide selected from A, T, G and C;

Y is a nucleotide selected from A, T, G and C;

n is 0 or 1;

m is 0 or 1; and

o is from about 1 to about 400.

27. The kit of Claim 26 wherein the oligonucleotide primer comprises the nucleotide sequence as set forth in SEQ ID NO:1.

28. The kit of Claim 26 wherein the oligonucleotide primer comprises the nucleotide

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sequence as set forth in SEQ ID NO:3.

29. The kit of Claim 25 or 26 wherein the oligonucleotide primer is immobilized to the magnetic particles *via* a thiol linkage.

30. The kit of Claim 25 wherein the addition of luminol, enhancer and/or H₂O₂ is automatic or semi-automatic.

31. The kit of Claim 25 wherein the cancer is any cancerous or malignant condition, pre-cancerous condition, myeloma, or any lymphoma or malignant condition, or any other proliferative disorder involving neoplastic cells.

32. The kit of Claim 31 wherein the cancer includes breast tumors, colorectal tumors, adenocarcinomas, mesothelioma, bladder tumors, prostate tumors, germ cell tumor, hepatoma/cholangio, carcinoma, neuroendocrine tumors, pituitary neoplasm, small round cell tumor, squamous cell cancer, melanoma, atypical fibroxanthoma, seminomas, nonseminomas, stromal leydig cell tumors, sertoli cell tumors, skin tumors, kidney tumors, testicular tumors, brain tumors, ovarian tumors, stomach tumors, oral tumors, bladder tumors, bone tumors, cervical tumors, esophageal tumors, laryngeal tumors, liver tumors, lung tumors, vaginal tumors and Wilm's tumor.

33. The kit of Claim 31 wherein the cancer is adenocarcinoma, adenoma, adenofibroma, adenolymphoma, adontoma, AIDS related cancers, acoustic neuroma, acute lymphocytic leukemia, acute myeloid leukemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, angioma sclerosing, angiomatosis, apudoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, branchioma, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukemia, childhood soft tissue sarcoma, chondrosarcoma,

choriocarcinoma, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancers, cutaneous T-cell lymphoma, carcinoma (e.g. Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell), carcinosarcoma, cervical dysplasia, cystosarcoma phyllodies, cementoma, chordoma, choristoma, chondrosarcoma, chondroblastoma, craniopharyngioma, cholangioma, cholesteatoma, cylindroma, cystadenocarcinoma, cystadenoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, dysgerminoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibroma, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, giant cell tumors, ganglioneuroma, glioma, glomangioma, granulosa cell tumor, gynandroblastoma, haematological malignancies, hairy cell leukemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, hamartoma, hemangioendothelioma, hemangioma, hemangiopericytoma, hemangiosarcoma, hemangiosarcoma, histiocytic disorders, histiocytosis malignant, histiocytoma, hepatoma, hidradenoma, hondrosarcoma, immunoproliferative small, opoma, ontraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leigomyosarcoma, leukemia (e.g. b-cell, mixed-cell, null-cell, t-cell, t-cell chronic, htlv-ii-associated, lymphangiosarcoma, lymphocytic acute, lymphocytic chronic, mast-cell and myeloid), leukosarcoma, leydig cell tumor, liposarcoma, leiomyoma, leiomyosarcoma, lymphangioma, lymphangiocyoma, lymphagioma, lymphagiomyoma, lymphangiosarcoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, malignant

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carcinoid syndrome, carcinoid heart disease, medulloblastoma, meningioma, melanoma, mesenchymoma, mesonephroma, mesothelioma, myoblastoma, myoma, myosarcoma, myxoma, myxosarcoma, nasal cancer, nasopharyngeal cancer, neuroblastoma, neurofibromatosis, Nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsc), neurilemmoma, neuroblastoma, neuroepithelioma, neurofibromatosis, neurofibroma, neuroma, neoplasms (e.g. bone, breast, digestive system, colorectal, liver), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, osteoma, osteosarcoma, ovarian carcinoma, papilloma, paraganglioma, paraganglioma nonchromaffin, pinealoma, plasmacytoma, protooncogene, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, reticuloendotheliosis, rhabdomyoma, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (scl), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, sarcoma (e.g. Ewing's experimental, Kaposi's and mast-cell sarcomas), sertoli cell tumor, synovioma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis/-ureter), trophoblastic cancer, teratoma, theca cell tumor, thymoma, trophoblastic tumor, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia and Wilms' tumor.

34. The kit of Claim 25 further comprising magnetic particles having antibodies immobilized thereon to cancer or leukemia or inflammatory or stem cells.