of clarity associated with such studies with respect to the tissue to be studied *vis-à-vis* the condition of a subject, there is a knowledge gap which exists in this area of work.

[006] Stem cells, particularly of embryonic origin, possess pluripotency markers viz. Oct4, Nanog, Sox2 and their isoforms are indicative of varied differentiation potentials into multiple tissues forming organs in development, homeostasis and aging. Since stem cells contribute to tissue development, they act as molecular biosensors implicative of tissue damage and injury, a hallmark of medical conditions.

[007] Thus, there is a dire need in the art to deploy non-invasive methods based on analyzing the expression of stem cell markers, as stem cell marker are prominent biomarkers for determining severity of medical conditions and identification of embryonic-like stem cell markers in body fluids can detect medical condition non-invasively.

SUMMARY OF INVENTION

[007a] In an aspect of the present disclosure, there is provided an *in-vitro* method for detecting a medical condition in a subject, said method comprising: (a) layering a blood sample, wherein the blood sample is obtained from the subject and a salt solution is added to said blood sample, over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900 g to obtain a first pellet comprising red blood cells (RBC); (b) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (c) centrifuging the RBC-lysed solution at a speed in the range of 400–4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells; (d) subjecting the second pellet to a lysis for obtaining nucleic acid from the enriched very small embryonic like stem cells; (e) performing an assay with the nucleic acid of step (e) for analyzing expression level of Oct 4A in the very small embryonic like stem cells; and (f) comparing the expression level of Oct 4A in the very small embryonic like stem cells from the blood sample with an expression level of Oct 4A in a control sample for determining an increase or

decrease in the expression level, wherein an increase in the range of 1.1-3 folds in the expression level of Oct 4A in the very small embryonic like stem cells from the blood sample as compared to the expression level of Oct 4A in a control sample detects the presence of a medical condition in the subject.

[007b] In an aspect of the present disclosure, there is provided an *in-vitro* method for predicting onset of cancer or predicting the presence of tumor or cancer in a subject, said method comprising: (a) layering a blood sample, wherein the blood sample is obtained from a subject and a salt solution is added to the blood sample, over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900 g, to obtain a first pellet comprising red blood cells (RBC); (b) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (c) centrifuging the RBC-lysed solution at a speed in the range of 400-4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells; (d) subjecting the second pellet to a lysis for obtaining nucleic acid from the enriched very small embryonic like stem cells; (e) performing an assay with the nucleic acid of step (e) for analyzing expression level of Oct 4A in the very small embryonic like stem cells; and (f) comparing the expression level of Oct 4A in the very small embryonic like stem cells from the blood sample with an expression level of Oct 4A in a control sample for detecting an increase or decrease in the expression level, wherein an increase in the range of 3-5 folds in the expression level of Oct 4A in the very small embryonic like stem cells from the blood sample as compared to the expression level of Oct 4A in the control sample predicts the onset of cancer in the subject, wherein an increase of at least 5 folds in the expression level of Oct 4A in the very small embryonic like stem cells from the blood sample as compared to the expression level of Oct 4A in the control sample detects the presence of tumor or cancer in the subject.

[007c] In an aspect of the present disclosure, there is provided a method for detecting presence of a medical condition in a subject, said method comprising: (a) layering a

blood sample, wherein the blood sample is obtained from a subject and a salt solution is added to the blood sample, over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900g, to obtain a first pellet comprising red blood cells (RBC); (b) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (c) centrifuging the RBC-lysed solution at a speed in the range of 400-4000g to obtain a second pellet comprising enriched very small embryonic like stem cells; (d) enumerating the number of very small embryonic like stem cells in the second pellet; and (e) comparing the number of very small embryonic like stem cells in the blood sample with the number of very small embryonic like stem cells in a control blood sample for detecting an increase or decrease in the number of very small embryonic like stem cells, wherein an increase in the number of very small embryonic like stem cells in the blood sample by at least two folds as compared to the number of very small embryonic like stem cells in a control blood sample detects the presence of a medical condition in the subject.

[007d] In an aspect of the present disclosure, there is provided a method for detecting presence of tumor or cancer or onset of cancer in a subject, said method comprising: (a) layering a blood sample, wherein the blood sample is obtained from a subject and a salt solution is added to the blood sample, over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900g, to obtain a first pellet comprising red blood cells (RBC); (b) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (c) centrifuging the RBC-lysed solution at a speed in the range of 200-4000g to obtain a second pellet comprising enriched very small embryonic like stem cells; (d) enumerating the number of very small embryonic like stem cells in the second pellet; and (e) comparing the number of very small embryonic like stem cells in the blood sample with the number of very small embryonic like stem cells in a control blood sample, wherein an increase in the number of very small embryonic like stem cells in the blood sample in the range of 2-5 folds as compared to the number of very small embryonic like stem cells in a

control blood sample detects the onset of cancer in the subject; and wherein an increase in the number of very small embryonic like stem cells in the blood sample by at least 5 folds as compared to the number of very small embryonic like stem cells in a control blood sample detects the presence of tumor or cancer in the subject.

[007e] In an aspect of the present disclosure, there is provided an *in-vitro* method for detecting a positive response to anti-cancer therapy, said method comprising: (a) enriching very small embryonic like stem cells from a blood sample-I obtained before administration of an anti-cancer therapy by a process comprising: (bi) adding a salt solution to the blood sample I (bii) layering the blood sample-I of step (bi) over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900g, to obtain a first pellet comprising red blood cells (RBC); (biii) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (biv) centrifuging the RBC-lysed solution at a speed in the range of 400-4000g to obtain a second pellet comprising enriched very small embryonic like stem cells from the blood sample-I; (b) enriching very small embryonic like stem cells from a blood sample-II obtained after at least 2 weeks of administration of the anti-cancer therapy by a process comprising: (di) adding a salt solution to the blood sample II, (dii) layering the blood sample-II of step (di) over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900g, to obtain a first pellet comprising red blood cells (RBC); (diii) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (div) centrifuging the RBC-lysed solution at a speed in the range of 400-4000g to obtain a second pellet comprising enriched very small embryonic like stem cells from the blood sample-II; (e) obtaining nucleic acid-I from the enriched very small embryonic like stem cells from the blood sample-I; (f) obtaining nucleic acid-II from the enriched very small embryonic like stem cells from the blood sample-II; (g) independently performing an assay with the nucleic acid-I and the nucleic acid-II for analysing expression level of Oct 4A; and (h) comparing the expression levels of Oct 4A from the nucleic acid-II with the

expression level of Oct 4A from the nucleic acid-I for detecting an increase or decrease in the expression level, wherein a decrease in the expression level of Oct 4Afrom the nucleic acid-II as compared to the expression level of Oct 4A from the nucleic acid-I detects a positive response to the anti-cancer therapy.

[007f] In an aspect of the present disclosure, there is provided an *in-vitro* method for detecting cancer, said method comprising: (a) layering a blood sample, wherein the blood sample is obtained and a salt solution is added to the blood sample, over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900g, to obtain a first pellet comprising red blood cells (RBC); (b) lysing the RBC in the first pellet to obtain a RBC-lysed solution; (c) centrifuging the RBC-lysed solution at a speed in the range of 400-4000g to obtain a second pellet comprising enriched very small embryonic like stem cells; (d) subjecting the second pellet to a lysis for obtaining nucleic acid from the enriched very small embryonic like stem cells; (e) performing an assay with the nucleic acid for analysing expression level of Oct 4A in very small embryonic like stem cells; (f) comparing the expression level of Oct 4A in very small embryonic like stem cells in the sample with an expression level of Oct 4A in very small embryonic like stem cells in a control sample for determining an increase or decrease in the expression level, wherein an increase in the expression level of Oct 4A in very small embryonic like stem cells in the sample by at least 5 folds as compared to the expression level of Oct 4A in very small embryonic like stem cells in the control sample indicates presence of cancer; and (g) performing sequence-based assays on the nucleic acid and analyzing for mutation in at least one cancer-related marker, wherein presence of mutation in the at least one cancer-related marker indicates presence of a specific type of cancer based on the cancer-related marker analysed.

[007g] In an aspect of the present disclosure, there is provided a method for detecting the presence of a medical condition in a subject, said method comprising: (a) enumerating the number of very small embryonic like stem cells in a blood sample

obtained from a subject, wherein the sample is diluted with a salt solution; and (b) comparing the number of very small embryonic like stem cells in the blood sample with the number of very small embryonic like stem cells in a control blood sample for determining an increase or decrease in the number of very small embryonic like stem cells, wherein an increase in the number of very small embryonic like stem cells in the blood sample by at least 2 folds as compared to the number of very small embryonic like stem cells in a control blood sample detects the presence of a medical condition in the subject.

[007h] In an aspect of the present disclosure, there is provided a method for predicting the onset of cancer or detecting presence of tumor or cancer in a subject, said method comprising: (a) enumerating the number of very small embryonic like stem cells in a blood sample obtained from a subject, wherein the sample is diluted with a salt solution; and (b) comparing the number of very small embryonic like stem cells in the blood sample with the number of very small embryonic like stem cells in a control blood sample for determining an increase or decrease in the number of very small embryonic like stem cells, wherein an increase in the number of very small embryonic like stem cells in the blood sample in the range of 2-5 folds as compared to the number of very small embryonic like stem cells in a control blood sample predicts the onset of cancer in the subject, and wherein an increase in the number of very small embryonic like stem cells in the blood sample by at least 5 folds as compared to the number of very small embryonic like stem cells in a control blood sample detects the presence of tumor or cancer in the subject.

[008] In an aspect of the present disclosure, there is provided an *in-vitro* method for detecting a medical condition in a subject, said method comprising: (a) obtaining a blood sample and adding a salt solution to the blood sample; (b) layering the blood sample of step (a) over a neutral buffer and subjecting the blood sample to a density gradient centrifugation at a speed in the range of 200-900 g to obtain a first pellet comprising red blood cells (RBC); (c) lysing the RBC in the first pellet to obtain a

RBC-lysed solution; (d) centrifuging the RBC-lysed solution at a speed in the range of 400–4000 g to obtain a second pellet comprising enriched very small embryonic like stem cells; (e) subjecting the second pellet to a lysis for obtaining nucleic acid from the enriched very small embryonic like stem cells; (f) performing an assay with the nucleic acid of step (e) for analysing expression level of Oct 4A in the very small embryonic like stem cells ; and (g) comparing the expression level of Oct 4A in the very small embryonic like stem cells from the blood sample with an expression level of Oct 4A in a control sample for determining an increase or decrease in the