CLAIMS:

1. An in-vitro process for the quick determination of the infection status of a *Mycobacterium tuberculosis* infection from whole blood in terms of an active or latent tuberculosis, comprising the steps of:

- stimulating an antigen-specific T cell that is present in a first sample of whole blood with purified protein derivative (PPD) in the presence of antibodies against CD28, or CD28 and CD49d;
- processing of PPD by antigen-presenting cells (APC) by incubating for 1.5 h to 2.5 h, especially for 2 h, at 35 °C to 39 °C, especially at 37 °C, optionally with adding CO_2;
- then adding a secretion inhibitor;
- effecting an intensive mixing; and
- another incubation for a period of at least 2.5 h at a temperature of from 35 °C to 39 °C, and
- determining a cytokine profile from both the intracellular INF-γ production and the intracellular IL-2 production of the antigen-specific T cell; wherein
- the presence of an active tuberculosis is indicated by a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive T cells.

2. The process according to claim 1, wherein another stimulation with SEB, Staphylococcus Enterotoxin B (SEB), ESAT-6 and CFP-10 and/or vaccination antigen is effected in addition to said stimulation with PPD.

3. The process according to claim 1 or 2, wherein a negative control is performed by adding a physiological buffer such as PBS 0.9%NaCl or 5%

Glucose to an antigen-specific T cell that is present in the whole blood of a second sample, which is authentic with said first sample.

4. The process according to at least one of claims 1 to 3, wherein a positive control is performed by adding SEB to an antigen-specific T cell that is present in the whole blood of a third sample, which is authentic with said first sample.

5. The process according to at least one of the claims 1 to 4 wherein a vaccination control took place by adding the vaccination antigen to an antigen-specific T-cell that is present in the whole blood of a fourth sample which is authentic with said first sample.

6. The process according to at least one of claims 1 to 5, wherein a BCG-vaccination control is effected by adding ESAT-6 (early secreted antigenic target) and CFP-10 (culture filtrate protein) to an antigen-specific T cell that is present in the whole blood of a fourth sample, which is authentic with said first sample.

7. The process according to at least one of claims 1 to 6 wherein a control whether or not a contact with non-tuberculosis mycobacteria took place in the whole blood donor is effected by adding ESAT-6 (early secreted antigenic target) and CFP-10 (culture filtrate protein) to an antigen-specific T cell that is present in the whole blood of a fifth sample, which is authentic with said first sample.

8. The process according to at least one of claims 1 to 7, wherein said secretion inhibitor is selected from the group consisting of brefeldin A, monensin or the like.

9. The process according to at least one of claims 1 to 8, wherein the determination of the cytokine profile is effected by flow cytometry.

10. The process according to at least one of claims 1 to 9, wherein the whole blood of the samples is derived from humans or animals.

11. The process according to at least one of claims 1 to 10 applied for contact tracing wherein whole blood was obtained from a person with close contact to a patient with active TB or suspect to have had a close contact to a patient with active TB.

12 The process according to claim 11 wherein a treatment decision is made as follows:

a. a prophylaxis, such as INH, or an active therapy, such as quadruple therapy, is indicated in cases, where a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive cells was detected;

b1. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and no information about previous testing is available, no acute therapy is necessary and the patient has to retested within 2 to 4 weeks;

b2. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and if in previous testing for latent infection with *Mycobacterium tuberculosis* did reveal no contact with *Mycobacterium tuberculosis* so far, a prophylaxis, such as INH therapy is indicated;

c1. no acute therapy is indicated if the patient shows no response.

c2. in case the patient reveals any clinical symptoms, retesting is indicated.

13. The process according to claim 1 to 10 wherein whole blood was obtained from a person before receiving immunosuppressive drugs or in an immune-incompetent state like HIV infection.

14. The process of claim 13 wherein a treatment decision is made as follows:

a. a prophylaxis, such as INH or an active treatment, such as quadruple therapy, and optionally delaying of immunosuppressive therapy, is indicated in cases, where a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive cells was detected;

b1. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and no information about previous testing is available, immunosuppressive therapy is possible and the patient has to retested within 2 to 4 weeks;

b2. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and if in previous testing for latent infection with *Mycobacterium tuberculosis* did reveal no contact with *Mycobacterium tuberculosis* so far, a prophylaxis, such as INH therapy, and optionally delaying of immunosuppressive therapy, is indicated as well as retesting of the patient within 2 to 4 weeks;

c1. immunosuppressive therapy is to begin if the patient shows no response;

c2. in case the patient reveals any clinical symptoms, retesting is indicated.

15. The process according to claim 1 to 10, wherein whole blood was obtained from a person suspected for an active TB infection.

16. The process of claim 15 wherein a treatment decision is made as follows:

a. an active treatment, such as quadruple therapy is indicated in cases, where a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive cells was detected;

b1. if diagnostic investigations revealed an latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and no information about previous testing is available, an acute infection is unlikely, an alternative diagnosis is to be considered, and the patient has to retested within 2 to 4 weeks;

b2. If diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and if in previous testing for latent infection with *Mycobacterium tuberculosis* did reveal no contact with *Mycobacterium tuberculosis* so far, a prophylaxis, such as INH or an active treatment, such as quadruple therapy is indicated and the patient has to be retested within 2 to 4 weeks;

c1. no acute therapy is indicated if the patient shows no response;

c2. in case the patient reveals any clinical symptoms, retesting is indicated.

CLAIMS:

1. An in-vitro process for the determination of the infection status of a *Mycobacterium tuberculosis* infection from whole blood in terms of an active or latent tuberculosis, comprising the steps of:

   - stimulating an antigen-specific T cell that is present in a first sample of whole blood with purified protein derivative (PPD) in the presence of antibodies against CD28, or CD28 and CD49d;

   - processing of PPD by antigen-presenting cells (APC) by incubating for 1.5 h to 2.5 h, especially for 2 h, at 35 °C to 39 °C, especially at 37 °C, optionally with adding CO_2;

   - then adding a secretion inhibitor;

   - effecting an intensive mixing; and

   - another incubation for a period of at least 2.5 h at a temperature of from 35 °C to 39 °C, and

   - determining a cytokine profile from both the intracellular IFN-γ production and the intracellular IL-2 production of the antigen-specific T cell; wherein

   - the presence of an active tuberculosis is indicated by a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive T cells.

2. The process according to claim 1, wherein another stimulation with Staphylococcus Enterotoxin B (SEB), ESAT-6 and CFP-10 and/or vaccination antigen is effected in addition to said stimulation with PPD.

3. The process according to claim 1 or claim 2, wherein a negative control is performed by adding a physiological buffer such as PBS 0.9%NaCl or 5%

Glucose to an antigen-specific T cell that is present in the whole blood of a second sample, which is authentic with said first sample.

4. The process according to at least one of claims 1 to 3, wherein a positive control is performed by adding SEB to an antigen-specific T cell that is present in the whole blood of a third sample, which is authentic with said first sample.

5. The process according to at least one of the claims 1 to 4, wherein a vaccination control took place by adding the vaccination antigen to an antigen-specific T-cell that is present in the whole blood of a fourth sample which is authentic with said first sample.

.6. The process according to at least one of claims 1 to 5, wherein a BCG-vaccination control is effected by adding ESAT-6 (early secreted antigenic target) and CFP-10 (culture filtrate protein) to an antigen-specific T cell that is present in the whole blood of a fourth sample, which is authentic with said first sample.

7. The process according to at least one of claims 1 to 6, wherein a control whether or not a contact with non-tuberculosis mycobacteria took place in the whole blood donor is effected by adding ESAT-6 (early secreted antigenic target) and CFP-10 (culture filtrate protein) to an antigen-specific T cell that is present in the whole blood of a fifth sample, which is authentic with said first sample.

8. The process according to at least one of claims 1 to 7, wherein said secretion inhibitor is selected from the group consisting of brefeldin A, monensin or the like.

9. The process according to at least one of claims 1 to 8, wherein the determination of the cytokine profile is effected by flow cytometry.

10. The process according to at least one of claims 1 to 9, wherein the whole blood of the samples is derived from humans or animals.

11. The process according to at least one of claims 1 to 10, applied for contact tracing wherein whole blood was obtained from a person with close contact to a patient with active TB or suspect to have had a close contact to a patient with active TB.

12 The process according to claim 11, wherein a treatment decision is made as follows:

a. a prophylaxis, such as INH, or an active therapy, such as quadruple therapy, is indicated in cases, where a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive cells was detected;

b1. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and no information about previous testing is available, no acute therapy is necessary and the patient has to retested within 2 to 4 weeks;

b2. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and if in previous testing for latent infection with *Mycobacterium tuberculosis* did reveal no contact with *Mycobacterium tuberculosis* so far, a prophylaxis, such as INH therapy is indicated;

c1. no acute therapy is indicated if the patient shows no response.

c2. in case the patient reveals any clinical symptoms, retesting is indicated.

13. The process according to at least one of claims 1 to 10, wherein whole

blood was obtained from a person before receiving immunosuppressive drugs or in an immune-incompetent state like HIV infection.

14. The process according to claim 13, wherein a treatment decision is made as follows:

a. a prophylaxis, such as INH or an active treatment, such as quadruple therapy, and optionally delaying of immunosuppressive therapy, is indicated in cases, where a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive cells was detected;

b1. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and no information about previous testing is available, immunosuppressive therapy is possible and the patient has to retested within 2 to 4 weeks;

b2. if diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and if in previous testing for latent infection with *Mycobacterium tuberculosis* did reveal no contact with *Mycobacterium tuberculosis* so far, a prophylaxis, such as INH therapy, and optionally delaying of immunosuppressive therapy, is indicated as well as retesting of the patient within 2 to 4 weeks;

c1. immunosuppressive therapy is to begin if the patient shows no response;

c2. in case the patient reveals any clinical symptoms, retesting is indicated.

15. The process according to at least one of claims 1 to 10, wherein whole blood was obtained from a person suspected for an active TB infection.

16. The process of according to claim 15, wherein a treatment decision is

made as follows:

a. an active treatment, such as quadruple therapy is indicated in cases, where a shift of the cytokine profile towards IFN-γ single positive cells accompanied by a decrease of IFN-γ/IL-2 double positive cells was detected;

b1. if diagnostic investigations revealed an latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and no information about previous testing is available, an acute infection is unlikely, an alternative diagnosis is to be considered, and the patient has to retested within 2 to 4 weeks;

b2. If diagnostic investigations revealed a latent infection with *Mycobacterium tuberculosis* by IFN-γ/IL-2 double positive cells and if in previous testing for latent infection with *Mycobacterium tuberculosis* did reveal no contact with *Mycobacterium tuberculosis* so far, a prophylaxis, such as INH or an active treatment, such as quadruple therapy is indicated and the patient has to be retested within 2 to 4 weeks;

c1. no acute therapy is indicated if the patient shows no response;

c2. in case the patient reveals any clinical symptoms, retesting is indicated.