Abstract: The present invention relates to methods for producing a number of products. Those products include vaccines, clinical laboratory kits and treatment components. The vaccine products cover: vaccines for bacteria, viruses and parasites in humans and animals; and vaccines for the prevention of malignant tumors in patients showing malignant transformation or as a supportive treatment of patients already having a tumor. The clinical laboratory kits covers: diagnostic kits for efficient diagnosis of diseases caused by microorganisms in humans and animals; diagnostic kits for malignant tumors; tissue typing kits for organ transplantation; and monitoring kits for organ rejection and hypersensitivity. The treatment components will help in treatment of difficult disorders including, among others, hypersensitivity disorders, chronic graft rejection, and autoimmune disorders. In effect, the products that can be produced based on this discovery are innumerable. The invention methods are based on our discovery of a new function for RBCs which is their ability to selectively transport self and foreign antigens.
Description

METHODS FOR PREPARATION OF VACCINES, LABORATORY KITS, AND TREATMENT COMPONENTS

Technical Field

[I] Human and animal vaccines
[2] Human and animal diagnostic kits
[3] Human tissue typing kits
[4] Human treatment components

Background Art

[5] Diagnosis of diseases caused by microorganisms
[6] Diagnosis of malignant transformation and tumors
[7] Diagnosis and monitoring of chronic rejection syndrome for different organs transplantations
[8] Diagnosis of autoimmune disorders
[10] Typing of donor and recipient for organ transplantation
[III] Vaccination of humans and animals against different microorganisms
[12] Vaccination of humans against malignant tumors
[13] Treatment of autoimmune disorders
[14] Treatment of chronic rejection syndrome for different organs transplantations
[15] Treatment of hypersensitivity to particular antigens
[16] Treatment of some fertility problems
[17] Diagnosis and treatment of disorders caused by abnormal proteins, e.g., Alzheimer syndrome

Disclosure of Invention

Disclosure

We found that the critical issue in the process of tolerance is the transport mechanism of antigens. This mechanism should be capable of hiding those antigens from various immune system cells and/or antibodies during their journey in the blood circulation until they reach their target immune system organs. We have proved that Red Blood Cells (RBCs) do this job.

RBCs’ absorbed antigens will reach the central organ of the immune system (e.g., thymus and bone marrow) and induce central tolerance. The degree of induced tolerance is dependant on the size of the RBCs antigens store. In effect, the larger the size of the store, the higher is the probability these antigens meet their corresponding premature lymphocytes in thymus and bone marrow with consequent induction of their
apoptosis. Antigens are tolerated as far as there are enough stores of those antigens in RBCs. In this way, the degree of immune response to a particular antigen may vary.

[20] We proved that during pregnancy, male spouse antigens are transported to placenta where they neutralize any harmful antibodies. In prenatal life, we expect that if antigens are introduced to a fetus while the immune system is still incapable to respond, there are good chances for those antigens to be processed by phagocytic cells and absorbed by RBCs. When mature lymphocytes production starts, later in life, antigens stores of RBCs are used to induce tolerance.

[21] In addition to proving that RBCs transport function is beneficial for body integrity and health, we also found that it is a security hole that gets exploited in a harmful way, for instance, by microorganisms, and malignant tumors.

**Background Knowledge**

[22] Normally, the immune system distinguishes between what is self and what is non-self (foreign body): tolerates self antigens and responds to non-self antigens. The phenomenon of tolerating antigens is called immune tolerance or simply tolerance. It can be defined as "a specific unresponsive state induced by prior exposure to an antigen which is due to either a programmed failure of the immune system or active suppression." Tolerance is a basic function of the immune system that is important to maintain body integrity and health.

[23] Exposure to antigens during intrauterine life induces tolerance. During postnatal life, tolerance to a particular antigen depends on dose, antigen-nature, and route of administration. Generally artificial induced tolerance is of finite duration. Basically, there is no absolute tolerance. Low-affinity auto-antibodies exist normally against self antigens.

[24] Consequently, tolerance to self antigens is a process that continues throughout life but begins during fetal development. There are a number of mechanisms that describe how tolerance occurs, which can be classified into: central, peripheral, and anergy. In central tolerance immature self-reactive T lymphocytes recognize antigens in the thymus and undergo negative selection (deletion). In peripheral tolerance mature self-reactive T lymphocytes that escape central tolerance and recognize self antigens in peripheral tissues can be inactivated ( anergy), killed (deletion) or regulated (suppressed). In anergy mature self-reactive lymphocytes do not respond to antigens in non-inflamed environment. Those mechanisms are explained through the following theories: clonal deletion, clonal anergy, clonal ignorance, receptor editing, and suppressor cells.

[25] RBCs are specialized for transport. Besides, RBCs have pinocytic activity which is well documented. Their cell membrane antigens can function as receptors, e.g., Duffy antigen has been proved to act as a receptor for Plasmodium vivax malaria to invade
RBCs. Consequently, RBCs easily absorb soluble antigens through pinocytosis while particulate antigen need receptor sites on RBCs in order to be absorbed.

It is important that the discovered function of RBCs fill a gap in understanding of tolerance. Notice that there is a general agreement about this gap. Part of this gap can be expressed in the following questions:

- Why soluble antigens administered intravenously favor tolerance while particulate antigens injected into the skin favor immunity.
- Why ingested large doses of soluble proteins induce systemic T lymphocyte tolerance, whereas the components of vaccines such as the Sabin polio vaccine induce an effective local immune response.
- Why tolerance is easier to induce in prenatal than postnatal life.

Further, the discovered function of RBCs answers some other questions or research puzzles including:

- How circulating IgG antibodies against blood groups 'A' and 'B' in a pregnant woman with blood group 'O' do not harm the fetus whose blood group is not 'O'.
- How microorganisms and malignant tumors overcome the immune system's arsenal.
- Why some hosts are susceptible to particular microorganisms while others are resistant to them.

It should be remarked that there is a lot of research on RBCs or erythrocytes proteome. This research is directed towards the identification of proteins from both erythrocyte membrane and cytoplasm. Besides studying the identified proteins, the clinical goal is to make a relation between proteins and diseases to discover a biomarker. This approach is not based on RBCs transport function so it is not targeted towards the identification of antigens related to tissues or microorganisms. Consequently, the relation between, for instance, tumors or microorganisms antigens and the identified protein, is not recognized. In autoimmune disorders, our approach is completely in another direction which is to identify protein(s) that is/ are missing in erythrocytes.

Experiments

We have proved that RBCs transport self and foreign antigens through the following experiments.

Experiment I: We have demonstrated that pregnant women store the 'A' and/or 'B' blood group antigens in their RBCs, if and only if their male spouse is not blood group 'O'. The technique is based on competitive inhibition of RBCs agglutination. If the hemolysate contains ABO specific antigens, then those antigens will compete with RBCs and prevent their agglutination. The experiment is done as follows:
• Blood samples were taken on heparin. RBCs and plasma were separated in two tubes.
• Females spouse, RBCs were washed in normal saline several times, frozen until they are ruptured, and then centrifuged.
• Males spouse RBCs were washed several times in saline then some of the RBCs were used to prepare a 5% suspension.
• Some of the washed male spouse RBCs were frozen until they are ruptured, and then centrifuged.
• As a positive control, serial dilutions of female spouse plasma were made using normal saline. A drop of the male spouse's hemolysate is added before adding his RBCs suspension.
• In test tubes, serial dilutions of the female spouse's plasma were made using normal saline. A drop of the female spouse's hemolysate was added before adding a drop of the male spouse's RBCs suspension.
• It was observed that agglutination was inhibited by female spouse hemolysate and was not inhibited by male spouse hemolysate. In most cases agglutination was inhibited in the first tube. However, agglutination was never observed in subsequent tubes.

Experiment II: RBCs store self HLA antigens and the male spouse HLA antigens. In effect, a pregnant woman tolerates her fetus and placenta using the same mechanism by which her body tolerates herself antigens, i.e., a fetus is part of self. The experiment was done using commercial HLA Typing Trays for the identification and definition of HLA Class I Antigens using the microlymphocytotoxicity assay. It is, also, based on competitive inhibition. Consequently, if typing wells that show positive reaction were inhibited in corresponding testing wells by adding hemolysate, this proves the existence of specific competing antigens. The experiment is done as follows:
• Blood samples were taken on heparin. The lymphocytes were separated using the Ficoll hypaque technique. The RBCs were then separated to prepare the hemolysate. The hemolysate is prepared by washing RBCs in phosphate buffer saline several times, and then frozen until RBCs are ruptured.
• For each couple, four typing trays were used:
  • The first is used for ordinary typing of the female spouse
  • The second is used for ordinary typing of male spouse
  • The third is used to detect self HLA antigens in male hemolysate
  • The fourth is used to detect male spouse HLA antigens in female spouse hemolysate
• Positive controls: In the first and second trays, positive controls are as usual.
In the third and fourth trays a hemolysate from a third person is added to control wells. Notice that the whole experiment is better to be done in two steps; first do the typing then test for the antigen existence in a next step. This gives a chance to select a hemolysate of a person that has a different HLA typing. Notice that hemolysate can be kept for a long time in deep freeze.

- The first and second trays are used for typing of HLA Class I for the couple.
- In the third tray, we added male hemolysate (diluted 1/16) in typing wells before adding male lymphocytes, and then examine wells that gave positive reaction in the second tray. It was observed that male spouse hemolysate inhibited the typing reaction indicating the existence of self HLA antigens.
- In the fourth tray, we added female hemolysate (diluted 1/16) before adding male lymphocytes, and then examine wells that gave positive reaction in the second tray, too. It was observed that female spouse hemolysate inhibited the typing reaction indicating the existence of male spouse HLA antigens.

Experiment III: Because RBCs transport antigens to central organs of the immune system, then RBCs will transport Tissue Specific Antigens (TSAs). The objective of this experiment is to prepare antibodies against TSAs through injecting white mice RBCs into rabbits. The experiment is done as follows:

- A number of white mice were slaughtered to collect their blood on sodium citrate and their organs (liver, kidney and spleen) were preserved on 10% formalin.
- The separated RBCs which were washed many times with sodium citrate.
- The washed RBCs were diluted with 3% formol-saline to kill any bacterial contamination.
- RBCs were injected subcutaneously into a number of rabbits for four times on weekly intervals.
- Blood was collected from those rabbits ear-vein after 28 days from the first injection.
- Sera were separated and examined for antibodies against mice RBCs using direct agglutination slide test.
- Then, sera were examined for antibodies against TSAs of liver, kidney and spleen using sandwich technique in histopathology sections.
- All sections show florescence which means that sera have antibodies against TSAs

Experiment IV: If a host is susceptible to a particular microorganism, then host RBCs will transport its antigens. The experiment is done as follows:

- A number of white mice were infected by Escherichia coli 0157 through subcutaneous injection.
Mice were slaughtered after 5 days to collect their blood on sodium citrate.

The separated RBCs were washed many times with sodium citrate.

The washed RBCs were diluted with 3% formol-saline to kill any bacterial contamination.

Rabbits were vaccinated by those RBCs. Rabbits were injected subcutaneously with one ml on weekly basis for three weeks.

Blood was collected from the ear-vein after 21 days from the first injection.

Rabbits sera were separated and examined for antibodies against E. coli 0157 using direct bacteria slide agglutination test.

All rabbits vaccinated by RBCs of mice infected by E. coli show high titer.

Experiment V: Tolerance is part of the dynamics of immune response. The Brucella melitensis Rev. I vaccine was used to vaccinate white mice. The RBCs of white mice were used to vaccinate rabbits similar to Experiment IV. Antibodies against B. melitensis were detected using Rose Bengal agglutination test. All rabbits vaccinated by RBCs of mice infected by B. melitensis gave high titer.

Description of Invention

The present invention exploits the following technologies and resources:

- Techniques from genomics and proteomics: these are used for separation, identification and synthesis of proteins
- Public databases of already identified proteins: those databases are provided by organizations like: European Bioinformatics Institute (EBI), which provides the universal protein database (UniProt), the American National Institute of Health (NIH), and Human Proteome Organization (HUPO)
- Data Mining (DM) in locally built database: DM can be targeted towards the identification of RBCs' proteins related to a particular disease or towards the identification of RBCs' proteins that are missing in a particular disease. Notice that in disorders that are caused by microorganisms, DM may not be essential.

The steps done in all the methods of the invention involves the following procedures:

1. Collect blood samples from either patients or normal individuals
2. Separate and wash the RBCs many times with some isotonic solution
3. Hemolyse RBCs
4. Separate proteins from RBCs hemolysate
5. Identify the separated proteins through sequencing and then query public databases for acceptable match
6. Store results in a local database
7. Prepare monoclonal antibodies against those proteins

The invention of new generation of microorganisms' vaccines depends on identifying...
antigen(s) in RBCs of patients or animals, which belong(s) to a particular microorganism. Antibodies against those antigens will be used in the diagnosis of their corresponding diseases' conditions. Those antibodies can be prepared to be used as passive vaccines, too. In this regard, new technologies, such as Affibody, can be efficiently used to replace natural antibodies.

Separation of malignant tumors' antigens from patients' RBCs will enable the preparation of antibodies against those antigens. Those antibodies can be used as passive vaccines and/or in the detection of tumors' antigens in the RBCs of patients. This detection test will be very sensitive as it can detect malignant transformation even before the tumor exists. Also, the separated antigens can be used to vaccinate those patients to prevent the development of the tumor or as a supportive treatment.

The separation of self and non-self antigens from the RBCs of normal individuals will help in many directions that cannot be enumerated. Among those directions:

- Preparation of antibodies against tissues specific antigens; this will enable better typing of donor and recipient
- Preparation of tissues specific antigens for desensitization treatment of autoimmune diseases or graft rejection syndrome
- Building of databases that enable data mining for discovering general health important information
- Preparation of antigens related to environment and food; this will help to desensitize patients that are sensitive to those antigens while preparing antibodies against those antigens will help to monitor the level of RBCs store for those antigens.

Example 1: Preparing a vaccine and diagnostic kits for Tuberculosis

Mycobacterium tuberculosis is the causative microorganism of tuberculosis disease. Collecting blood samples from patients suffering from tuberculosis will enable the identifications of protein(s) related to M. tuberculosis in the samples' RBCs. This is done through the following steps:

1. Store erythrocytes proteome of patients' TB cases in the local database.
2. Assuming that there exists a database containing cases of erythrocytes proteome for normal individuals. Select protein(s) that exist(s) in proteome of patients' TB cases and do / does not exit in proteome of normal individuals.
3. Use protein(s) identified in step 2 to query public databases.
4. Use protein(s) related to M. tuberculosis to prepare the vaccine and their corresponding monoclonal antibodies to prepare the diagnostic kit.

The dose and the number of injections that can make an individual immune are to be determined. Also, the interpretation of the titer of the antigen(s) that indicates active TB, immunity, or exposure is to be determined.
The monoclonal antibodies against this active vaccine can be packaged as kits suitable for different laboratory equipment (e.g., ELISA, Flowcytometry, etc.).

**Example 2: Preparing vaccines and diagnostic kits for a malignant tumor**

Every type of malignancy has its specific tissue antigens. A group of tumors may share (a) specific tissue antigen(s). Consequently, collecting blood samples from patients suffering from the same type of tumor will enable the identification of malignancy antigen(s) of that tumor. The use of data mining will be very helpful. It will enable the identification of malignant tissue antigens that are shared between a group of tumors. If the proteome of the malignant tumor is known, then steps similar to "Example 1" can be applied. Consequently, the steps done are:

1. Store erythrocytes proteome of patients' cases in the local database.
2. Select protein(s) that exist(s) in proteome of patient cases and do / does not exit in proteome of normal individuals records.
3. If the proteome of the malignant tumor is known, then use protein(s) identified in step 2 to query the database of the tumor proteome.
4. Use protein(s) related to the tumor to prepare the active vaccine. Otherwise, use protein(s) identified in step 2 to prepare the active vaccine.
5. Accordingly, prepare monoclonal antibodies.

The dose and the number of injections that can make an individual immune are to be determined. Also, the interpretation of tumor antigen titer is to be standardized.

**Example 3: Preparing diagnostic kits and treatment components for autoimmune disorders and chronic rejection syndrome**

The invention of new generation of diagnostic kits that help in the treatment of autoimmune disorders and chronic rejection syndrome depends on identifying tissue specific antigen(s) that patients have antibodies against it / them. Consequently, such kits will consist of tissue specific antigens separated from normal individuals and packaged for different laboratory equipment. The antigens that react with patients' antibodies can be used to desensitize those patients. This is done through the following steps:

1. Store erythrocytes proteome of patients' cases, for a particular clinically diagnosed disease condition, in the local database.
2. Assuming that there exists a database containing cases of erythrocytes proteome for normal individuals. Select protein(s) that exist(s) in proteome of normal individuals and do / does not exit in proteome of patients.
3. Use proteins identified in step 2 to query public databases so that tissues or
organs of these proteins may be determined.
4. Screen those patients for antibodies against the proteins identified in step 2.
5. Manufacture the proteins that react with patients' antibodies to be used in the diagnosis and treatment of this particular disease condition.

Important Remarks

Although in proteomics the separation of proteins depends on either two dimensional gel electrophoresis, or chromatography, e.g., HPLC, there are still other methods that can be used to separate selectively target proteins. A simple and direct example is Western blot, either using one or two dimensional gel electrophoresis. Another example which involves indirect way of separation can be done through the following steps:
1. Collect a blood sample from a tuberculosis patient, separate RBCs, wash RBCs, and then divide the RBCs sample in two portions.
2. Use the first portion of RBCs to immunize rabbit to obtain antibodies against all the RBCs antigens.
3. Prepare a suspension of Mycobacterium tuberculosis from culture.
4. Separate plasma from the immunized rabbit, add the prepared suspension of M. tuberculosis, and then add hemolysate of the second RBCs portion.
5. Use ultrafiltration membrane to get Mycobacterium tuberculosis antigens.
6. Prepare antiserum against the filtered antigens and test against bacteria suspension as an insurance measure.
7. Identify the separated proteins through sequencing.

The disadvantage of this method is that it takes a long period of time and it does not guarantee the separation of the related antigen because of protein-protein interactions.

In general, the technology of separation, identification, and synthesis of proteins are not the issue in the described methods. Also, the use of monoclonal antibodies, Affibody, or any other similar products are not the issue. The issue is to exploit the existence or absence of proteins in RBCs in diagnosis, treatment, or prevention of a particular disease condition.

Description Of Drawings

Fig. 1. depicts the methods of preparing different products for humans. These methods can be described in the following steps:
1. The main resource is the RBCs samples of patients and normal individuals.
2. For each sample all proteins of the hemolysate are separated and sequenced.
3. The sample proteome is stored in the local database.
4. The proteins that are found in a particular disease are used to query external databases storing human proteomes, microorganism proteomes, and other
proteomes.
5. The disease related proteins are used in methods of antibodies preparation and vaccine preparation.
6. The proteins that are found in normal individuals and identified are used in methods of antibody preparation and component preparation.
7. Diagnostic kits are produced using prepared antibodies, if proteins exist in RBCs of patients. If proteins do not exist in patients and exist in normal individual, diagnostic kits are prepared from prepared components to detect circulating antibodies.
8. The methods: "Prepare Vaccine" and "Prepare Component" are similar. They are separated just to clarify the concept.

[51] Fig. 2, depicts the methods of preparing different products for animals. By animals, we mean the dictionary definition: "A multicellular organism of the kingdom Animalia, differing from plants in certain typical characteristics such as capacity for locomotion, nonphotosynthetic metabolism, pronounced response to stimuli, restricted growth, and fixed bodily structure." These methods can be described using the following steps:
1. RBCs samples from a particular animal kind are collected.
2. For each sample all proteins of the hemolysate are separated and sequenced.
3. The sample proteome is stored in the local database.
4. The proteins that are found in a particular disease are used to query external databases storing microorganism proteomes.
5. The proteins that are found in a particular disease are used in methods depicted in the figure.
Claims

[1] A method for preparing different groups of clinical laboratory tests, a group of vaccines, and a group of treatment component using proteins that are extracted and identified from within RBCs of animals, patients and normal individuals.

[2] A method for developing diagnostic kits that can detect microorganisms' protein(s) in RBCs of patients or animals as a group of clinical laboratory tests, according to claim [1]. The kit is prepared by first extracting the protein(s) from patients or diseased animals, and then antibodies against this / these protein(s) are prepared. Such kit can detect exposure to a microorganism, and differentiate between exposure, immunity, and disease through standardization.

[3] A method for developing malignant tumors diagnostic kits that can detect, generic or specific, tumors' protein(s) in RBCs of patients as a group of clinical laboratory tests, according to claim [1]. Although the kit is prepared similar to claim [2], there are two types in this group. The first is used for scanning protein(s) that is / are shared in a number of tumors. The second is used to detect protein(s) that is / are specific to a particular tumor.

[4] A method for developing organ tissue typing kits that can match donor and recipient for organ transplantation as a group of clinical laboratory tests, according to claim [1]. TSAs are extracted from normal individuals. Antibodies against those proteins can be used in kits for direct typing of donor and recipient.

[5] A method for developing monitoring kits to estimate RBCs antigen store as a group of clinical laboratory tests, according to claim [1]. The kit is prepared by first extracting the protein, then antibodies are prepared against this protein.

[6] A method for developing molecular diagnosis kits for autoimmune disorders using TSAs as a group of clinical laboratory tests, according to claim [1]. It is based on identifying TSAs that react with circulating autoimmune antibodies.

[7] A method for developing microorganisms' vaccines as a group of vaccines, according to claim [1]. Each vaccine consists of proteins which are related to a particular microorganism and extracted from RBCs of patients or animals. Microorganisms include bacteria, viruses, and parasites.

[8] A method for developing malignant tumors' vaccines as a group of vaccines, according to claim [1]. Each vaccine consists of protein(s) which are extracted from RBCs of patients. The vaccine can be personalized according to the diagnostic kits of claim [3].

[9] A method for developing passive vaccines for all extracted microorganisms and malignant tumor proteins as a group of vaccines, according to claims [1], [2], and [3].
A method for developing treatment components for hypersensitivity as a group of treatment component, according to claim [I]. The components are prepared from RBCs proteins extracted from normal individuals. The selection of those components depends on acquiring erythrocyte proteome for hypersensitivity patients and then applying data mining. Skin hypersensitivity test can be then used to refine the selection.

A method for developing treatment component to treat chronic rejection syndrome and autoimmune disorders, as a group of treatment component, according to claim [I]. TSAs are prepared from RBCs proteins extracted from normal individuals. The existence of antibodies against TSAs is screened using kits of claim [6]. TSAs that show positive reaction can be used to desensitize patients.

A method for developing a data mining tool that exploit a database containing records of RBCs proteome for normal individuals as well as different disease conditions. Such tool can be used to reveal the existence or absence of proteins in a particular disease condition. This can help either directly or indirectly in diagnosis, prognosis, and monitoring of patients. Also, they will help in identifying an efficient treatment of some conditions.

The methods from previous claims will be applied in any disease condition according to the following rules:

a) If disease related protein(s) exist(s) in RBCs, kits can be prepared similar to claims [2] and [3]; and vaccines may be prepared similar to claims [7] and [8].

b) If protein(s) do/does not exist in a disease condition, a diagnostic kit can be prepared similar to claim [6] and a treatment component may be prepared similar to claim [H].
A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/50 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Category</th>
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[Special categories of cited documents]

'A' document defining the general state of the art which is not considered to be of particular relevance
'E' earlier document but published on or after the international filing date
'L' document which may throw doubts on novelty claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
'O' document referring to an oral disclosure, use, exhibition or other means
'P' document published prior to the international filing date but later than the priority date claimed

[Date of the actual completion of the international search]

13 February 2009

[Date of mailing of the international search report]

03/03/2009

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Moreno de Vega, C
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

This International Searching Authority found multiple inventions in this international application, as follows:

**see additional sheet**

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- [ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- [x] No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1(part), 2, 7, 9 (part), 13(part)
   Methods for developing diagnostic kits for microorganisms and microorganism vaccines

2. claims: 1 (part), 3, 8, 9 (part), 13 (part)
   Methods for developing diagnostic kits and vaccines for malignant tumours

3. claims: 1(part), 4, 11(part), 13(part)
   Methods for developing organ tissue typing kits and treatment components to treat chronic rejection syndrome

4. claims: 1(part), 5, 13 (part)
   Method for developing monitoring kits to estimate RBCs antigen store

5. claims: 1(part), 6, 11(part), 13(part)
   Methods for developing molecular diagnosis kits for autoimmune disorders and treatment components to treat autoimmune disorders

6. claims: 1(part), 10, 13(part)
   Method for developing treatment components for hypersensitivity

7. claim: 12
   Method for developing a data mining tool that exploits a database containing records of RBCs proteome for normal individuals.
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