high amount of the biological samples to achieve the detection of antibodies being in a low amount.

In view of the foregoing, there exists a need for addressable systems and methods, which can provide additional improvements in high throughput, cost-effectiveness, and accuracy for molecular diagnosis of antibody-generating diseases. The present invention satisfies these and other needs.

Summary

In one aspect, there is provided an *in vitro* assay method for detecting at least two different target antibodies present in a biological sample from a subject, said method comprising the steps of: (a) providing a first fusion protein comprising: a polypeptide comprising a first epitope that is recognized by a first target antibody and a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity said AGT polypeptide being the SNAP mutant of SED ID NO:2, (b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide, (c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody, (d) providing a second fusion protein comprising: a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity, (e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide, (f) obtaining a second solid support covalently coupled with a second epitope that is recognized by the second target antibody, but not by said first target antibody, wherein said first and second solid supports can be specifically identified from each other, (g) contacting said biological sample with the first and second solid supports obtained in steps (c) and (f), (h) detecting the presence of said at least two target antibodies.

In another aspect, there is provided a kit for detecting at least different two target antibodies present in a biological sample from a subject, comprising the at least two solid supports as defined above: - a first solid support as obtained in step (c) as defined above, which is covalently coupled with a first epitope that is recognized by a first target antibody, and a second solid support as obtained in step (f) as defined above, which is

covalently coupled with a second epitope that is recognized by a second target antibody, and not by said first target antibody, wherein the at least two solid supports can be specifically identified from each other and enable to detect two different target antibodies, and further wherein the at least two solid supports are mixed together in at least one single compartment.

In another aspect, there is provided the use of the kit as defined herein, for detecting at least two target antibodies in a biological sample from a subject.

In another aspect, there is provided the use of the kit as defined herein, for diagnosing at least two target diseases in a subject, wherein said target disease is a viral infection caused by a Papillomavirus or a RNA virus from the family of the Flaviviridae (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the Togaviridae (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the Bunyaviridae (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the Caliciviridae (Hepatitis E virus), the Arenaviridae (Lassa) or the Filoviridae (Ebola, Marburg), a bacterial infection caused by Leptospirosa Interrogans, or an infection caused by Plasmodium falciparum.

In another aspect, there is provided an *in vitro* method for diagnosing at least one target disease in a subject, said target disease being known to induce the synthesis of at least one target antibody in said subject, comprising performing the assay method as defined herein, wherein said subject is diagnosed to be suffering from said at least one target disease if the amount of said at least one target antibody is higher than a control value.

In another embodiment, the above disclosed assay method comprises covalently coupling the AGT polypeptide to said solid support, by a coupling method comprising the following steps: a) activating the said solid support, b) adding a substrate of said AGT polypeptide, said substrate being suspended in a buffer containing between 0 and 20 % of DMSO, in appropriate conditions so that the substrate is covalently attached to said support, c) contacting said AGT polypeptide with the substrate-coated support of step b) in a PBS/DTT buffer, wherein unbound molecules are washed out after steps b) and c).

In another embodiment, there is provided a solid support obtained by the method as disclosed above.

In another embodiment, there is provided the use of the solid support as disclosed herein in the assay method defined above.

In another embodiment, there is provided a vector for expressing the N nucleoprotein of the Schmallenberg virus in an host cell, comprising the nucleotide sequence encoding a) a secretion signal peptide, which is functional in said host cells, b) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT), a mutant, a fragment or a catalytic domain thereof, and c) the N nucleoprotein of the Schmallenberg virus of SEQ ID NO: 16.

In another embodiment, there is provided a recombinant cell which is stably transfected by the vector defined above

In another embodiment, there is provided a fusion polypeptide comprising a) a 6-alkylguanine-DNA-alkyltransferase enzyme (AGT) (EC 2.1.1.63), a mutant or a catalytic domain thereof and b) the N nucleoprotein of Schmallenberg virus of SEQ ID NO: 16.

In another aspect, there is provided a method for manufacturing a kit as defined herein, said method comprising the steps of: (a) providing a least a first fusion protein comprising: a polypeptide comprising a first epitope that is recognized by a first target antibody and a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity said AGT polypeptide being the SNAP mutant of SED ID NO:2, (b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide, (c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody, (d) providing at least a second fusion protein comprising: a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and a AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity said AGT polypeptide being the SNAP mutant of SED ID NO:2, (e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide, (f) obtaining a second solid support covalently coupled with a second epitope that is recognized by the second target antibody, but not by said first target antibody, wherein said at least first and at least second solid supports can be specifically identified from each other.

In another aspect, there is provided a multiplex immuno screening assay comprising at least 2, 25, 50, 96 solid supports as disclosed herein and wherein each of said solid supports emits a different and distinguishable wave length after excitation.

In another aspect, there is provided a multiplex immuno screening assay method comprising: a) contacting one or several biological sample(s) with at least 2, 25, 50, 96 solid supports as disclosed herein and wherein each of the solid supports emits a different and distinguishable wave length after excitation, and b) detecting the presence or absence of target antibodies.

In another embodiment, there is provided an apparatus for carrying out an assay defined above or a method defined above, comprising: a technical device for detecting the light sources emitted from the solid supports and the light source emitted from the target antibodies or labeled antibodies binding to the target antibodies, and a calculating or computer device for identifying which solid supports are bound with target antibodies, thereby indicating the presence or absence of antigens, bacteria, virus, or parasites in the analyzed sample.

In another aspect, there is provided a kit for the detection of at least two target antibodies in a biological sample comprising: (a) a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity said AGT polypeptide being the SNAP mutant of SED ID NO:2 and a first epitope that is recognized by a first target antibody; and b) a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity said AGT polypeptide being the SNAP mutant of SED ID NO:2 and a second epitope that is recognized by a second target antibody, but not by said first target antibody.

In another aspect, there is provided a method for detecting at least two target antibodies in a biological sample comprising: (a) contacting a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a O6-alkylguanine-DNA alkyltransferase activity said AGT polypeptide being the SNAP mutant of SED ID NO:2 and a first epitope that is recognized by a first target antibody with the biological sample; (b) contacting a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT

polypeptide having a O6-alkylguanine-DNA alkyltransferase activity said AGT polypeptide being the SNAP mutant of SED ID NO:2 and a second epitope that is recognized by a second target antibody, but not by said first

target antibody with the biological sample; and (c) detecting the presence or absence of the two target antibodies.

Figure legends

Figure 1 represents the oriented coupling of chimeric AGT-antigen proteins to substrate-coated microspheres. First step of coupling consists of coupling the AGT substrate BG-PEG-NH2 to the activated microspheres by amine coupling. The second step consists of contacting the substrate-coated microspheres with fusion proteins containing AGT (for example the SNAP mutant), said enzyme being intended to covalently attach to its BG-PEG-NH2 substrate, that is, to the microspheres.

Figure 2 shows the coupling efficiency of chimeric SNAP-viral antigens proteins (SNAP-DV1.EDIII, SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-ZIKA), as followed by anti-SNAP antibody.

Figure 3 compares the sensitivity of the immunoassay experiment for the detection of purified monoclonal anti-DV2 antibody on chimeric SNAP-DV2.EDIII protein conjugated to microspheres via the substrate of the hAGT protein (coupling of the invention) or coupled through a standard amine coupling procedure, *e.g.* Bio-Plex Amine Coupling Kit, BIORAD.

Figure 4 compares the sensitivity of the immunoassay experiment for the detection of purified monoclonal anti-DV1 antibody on chimeric SNAP-DV1.EDIII protein conjugated to microspheres, either in a singleplex or in a multiplex fomat with other chimeric SNAP-viral Ags proteins (SNAP-DV2.EDIII, SNAP.DV3.EDIII, SNAP.DV4.EDIII, SNAP-WNV, SNAP-YF, SNAP-JE, SNAP-TBE) coupled to microspheres.

concentrations of DMSO above 20 % may affect the detection step of the methods of the invention. Preferably, said buffer is a PBS buffer containing between 0 and 20 % of DMSO, more preferably between 10 % and 20 % of DMSO.

Advantageously, the unspecific sites on the solid supports that have not been covalently attached to the AGT substrate can be further blocked by any conventional means, for example, by using a blocking buffer containing 1 % of bovine serum albumin (BSA) or any saturating protein (*e.g.* casein).

Once the solid supports of the invention have been covalently coupled with the AGT substrate (preferably through a carbodiimide covalent linkage), the solid supports are then contacted by the fusion proteins of the invention, so as to couple the epitopes that are specifically recognized by the target antibodies to said supports.

Again, this coupling step has to be performed under particular conditions. As a matter of fact, the catalytic site of the AGT enzyme and the conformational structure of the antigens / epitopes which are carried by the fusion proteins have to be conserved during the coupling proceedings. The inventors identified that the fusion protein should be suspended in a dithiothreitol (DTT)-containing buffer, preferably a PBS/DTT buffer, for the coupling to be efficient. Advantageously, the said coupling buffer contains tween® 20; indeed, it has been observed by the present inventors that addition of tween® 20 to the coupling medium helps avoiding bead aggregation. Preferably, the coupling buffer contains 0,02 % tween® 20. More preferably, the covalent coupling buffer of the invention is a PBS buffer of pH 7,4, containing 0,02 % tween® 20, and 1 mM DTT.

Other coupling conditions are usual ones. Preferably, the covalent coupling of the AGT substrate and the coupling of the fusion protein to the solid supports are performed at room temperature. If the solid supports are fluorescently labeled, said proceedings are more preferably performed in darkness.

In a second aspect, the present invention is thus drawn to a method for covalently coupling a AGT polypeptide having O^6-alkylguanine-DNA alkyltransferase activity, on a functionalized solid support, comprising the following steps:

a) activating the said functionalized solid support,

b) adding a substrate of said AGT polypeptide, said substrate being suspended in a buffer containing between 0 and 20 % of DMSO, in appropriate conditions so that the substrate is covalently attached to said support,

c) contacting the said AGT polypeptide with the substrate-coated support of step b) in a PBS/DTT buffer,

wherein unbound molecules are washed out after steps b) and c).

Washings can be performed by using any kind of appropriate washing buffers. Such buffers are routinely used by the person of skills in the art and need not be further detailed here. Preferably, a PBS buffer is used.

As used herein, "appropriate conditions" are usual ones. Preferably, the covalent coupling of the AGT substrate is performed at room temperature and, if the solid supports are fluorescently labeled, in darkness.

The functionalization of the solid support can be performed by any conventional means (as those reminded above). The activation of said functionalized solid support is performed accordingly. In a preferred embodiment, the said solid supports are functionalized with surface carboxyl groups and further activated with a classical activation buffer, for example a 50 mg/mL EDAC solution or a 50 mg/mL S-NHS solution.

In a preferred embodiment, DTT is at a concentration of 1 mM in the PBS/DTT buffer.

The present invention is also drawn to a solid support which has been obtained by the said method, and to the use of said solid support in the immunoassay of the invention.

Said solid supports can then be stored in conventional storage buffers, for example containing 0.5 g/L sodium azide, 0.1 % BSA, 0.02 % tween® 20, and/or 1 mM DTT.

All these coupling steps are preferably performed *in vitro*, in buffers which are devoid of living cells, so that there is no need to take into account the reaction with endogenous AGT enzymes, and the reaction of the (exogenous) AGT fusion protein is therefore highly specific.

The solid supports that can be used in the methods of the invention can be of any kind, e.g. test tubes, microtiter wells, sheets, beads, chips, and/or microparticles, provided that

Examples

In the context of the invention, a multiplex bead-based immunoassay was developed for rapid and simultaneous detection of antibodies to arboviruses in biological fluids.

The system is based on the xMAP technology (Luminex corporation) and uses a mixture of antigen-coated microspheres as capture reagents for specific human immunoglobulins. Distinct sets of microspheres (Magplex, Luminex corporation) were coupled with purified AGT fusion proteins, namely the SNAP-tagged viral recombinant proteins: sSNAP-DV1.EDIII, sSNAP-DV2.EDIII, sSNAP-DV3.EDIII, sSNAP-DV4.EDIII, sSNAP-WN.EDIII, sSNAP-JE.EDIII, sSNAP-USU.EDIII, sSNAP-TBE.EDIII, sSNAP-YF.EDIII, sSNAP-MVE.EDIII, sSNAP-Rocio.EDIII, sSNAP-WSL.EDIII, sSNAP-ZIKA.EDIII, SNAP-DV1ectoM, sSNAP-N.RVF, sSNAP-N.TOS, and CHIK.sE2-SNAP. Recombinant antigens were covalently coupled to the carboxyl microsphere surface using a substrate of the AGT protein as linker (BG-PEG-NH2, New England Biolabs), thereby enhancing antibody capture efficiency as compared to standard amine coupling procedures.

Technical validation using anti-SNAP-tag antibodies and specific mouse monoclonal antibodies confirmed coupling efficiency and demonstrated long-term antigen stability (up to six month). This application is not limited to viral antigens as any peptide or polypeptide can be used for bead coating and subsequent antibody capture.

I. <u>Material and methods</u>

1. The following buffers and solutions are used:

a) **PBS buffer**: 100 mL of 10X PBS, pH 7.4 in 1 L H2O sterile

b) **SNAP coupling buffer** (PBS-DTT) : 100 mL of 10 X PBS, pH 7.4, 0.5 mL 10 % tween® 20, 1 mL of 1.0 M DTT, in 1 L H_2O sterile

c) **blocking / assay buffer** (PBS-B): PBS, 1 % BSA, pH 7.4 in 1 L H_2O sterile

d) **storage buffer** (PBS-TBN): 100 mL of 10X PBS, 1 g of BSA, 2 mL of 10 % tween® 20, 500 mg of sodium azide, 1 mL of 1.0M DTT, in 1 L H_2O sterile

CLAIMS

1. An *in vitro* assay method for detecting at least two different target antibodies present in a biological sample from a subject, said method comprising the steps of:

(a) providing a first fusion protein comprising :

- a polypeptide comprising a first epitope that is recognized by a first target antibody and
- a AGT polypeptide having a 06-alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2,

(b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody,

(d) providing a second fusion protein comprising :

- a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and
- a AGT polypeptide having a 06-alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2,

(e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(f) obtaining a second solid support covalently coupled with a second epitope that is recognized by the second target antibody, but not by said first target antibody,

wherein said first and second solid supports can be specifically identified from each other,

(g) contacting said biological sample with the first and second solid supports obtained in steps (c) and (f),

(h) detecting the presence of said at least two target antibodies.

2. The assay method of claim 1, wherein said method is used to detect at least 5, more preferably at least and even more preferably at least 50 target antibodies in a biological sample from a subject.

3. The assay method of claim 1 or 2, wherein said first and second epitopes belong to the same biological species or to unrelated biological species.

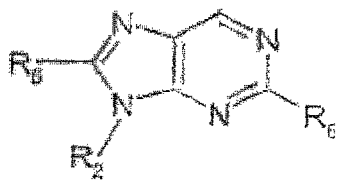
~~4. The assay method of anyone of claim 1 to 3, wherein said AGT polypeptide is the SNAP mutant of SEQ ID NO:2.~~

~~5.~~4. The assay method of any one of claims 1 to 43, wherein said substrate of said AGT polypeptide is a 06 benzyl guanine derivative having the formula I:

R1-X-CH_2-R3-R4-Y

wherein:

- R1 is a heteroaromatic group containing 1 to 5 nitrogen atoms, preferably a purine radical of the formula:



wherein R5 is hydrogen, halogen, e. g. chloro or bromo, trifluoromethyl, or hydroxy; R6 is hydrogen, hydroxy or unsubstituted or substituted amino; and R2 is hydrogen, an alkyl of 1 to 10 carbon atoms, or a saccharide moiety;

- X is an oxygen or sulfur atom; preferably an oxygen atom;
- R3 is an aromatic or a heteroaromatic group, or an optionally substituted unsaturated alkyl, cycloalkyl or heterocyclyl group with the double bond connected to CH_2; preferably a phenyl, e.g. a phenyl substituted by R4 in para or meta position,

- R4 is a linker moiety, preferably -CH_2-NH-C0-NH-[C_2H_4-0]$_n$-, wherein n is comprised between 1 to 8, preferably 2 to 6;
- Y is a reactive group.

6.5. The assay method of any one of claims 1 to 54, wherein said solid supports can be specifically identify by their specific location, size, diameter, weight, granulometry, and/or labeling.

7.6. The assay method of any one of claims 1 to 56, wherein said solid supports are labeled with a fluorochrome, a chromophore, a radioisotope, and/or a mass tag.

8.7. The assay method of any one of claims 1 to 67, wherein said solid supports are made of polystyrene, cellulose, nitrocellulose, glass, ceramic, resin, rubber, plastic, silica, silicone, metal, and/or polymer.

9.8. The assay method of any one of claims 1 to 87, wherein said solid supports are functionalized with a group which is complementary to the reactive group of the AGT substrate before being contacted with the AGT substrate.

10.9. The assay method of any one of claims 1 to 98, wherein said solid supports are functionalized with surface carboxyl groups.

11.10. The assay method of any one of claims 1 to 109, wherein said AGT substrate is covalently coupled to said solid supports by a carbodiimide reaction.

12.11. The assay method of any one of claims 1 to 1110, wherein said solid supports are test tubes, microtiter wells, sheets, beads, chips, and /or microparticles.

13.12. The assay method of any one of claims 1 to 1211, wherein said solid supports are microparticles.

14.13. The assay method of any one of claims 1 to 1312, wherein said solid supports are magnetic.

15.14. The assay method of any one of claims 1 to 1413, wherein said solid supports are microparticles internally labeled with fluorescent dyes.

~~16.~~15. The assay method of any one of claims 1 to ~~15~~14, wherein said solid supports are microparticles internally labeled with fluorescent dyes with magnetite encapsulated in a functional polymer outer coat containing surface carboxyl groups for covalent coupling of ligands.

~~17.~~16. The assay method of any one of claims 1 to ~~16~~15, wherein said first and/or second epitope is present on a viral protein chosen in the group consisting of: the EDIII protein of the dengue virus 1 of SEQ ID NO:3, the EDIII protein of the dengue virus 2 of SEQ ID NO:4, the EDIII protein of the dengue virus 3 of SEQ ID NO:5, the EDIII protein of the dengue virus 4 of SEQ ID NO:6, the EDIII protein of the West Nile virus of SEQ ID NO:7, the EDIII protein of the Yellow Fever virus of SEQ ID NO:8, , the EDIII protein of the Japanese encephalitis virus of SEQ ID NO:9, the EDIII protein of the Zika virus of SEQ ID NO:10, the EDIII protein of the Wesselbron virus of SEQ ID NO:11, the EDIII protein of the Rocio virus of SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus of SEQ ID NO:13, and the EDIII protein of the Saint-Louis encephalitis virus of SEQ ID NO: 14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58, and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

~~18.~~17. The assay method of any one of claims 1 to ~~16~~15, wherein said first and second fusion proteins that are coupled with said first and second solid supports are selected in the group consisting of: SEQ ID NO:21, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID N0:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID N0:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID N0:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID N0:100, SEQ ID NO:102, SEQ ID N0:104, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ

ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO.145, SEQ ID NO:147, SEQ ID NO:149 and SEQ ID NO:151.

~~19.~~18. The assay method of any one of claims 1 to ~~18~~17, wherein said biological sample is chosen from whole blood, serum, plasma, urine, seminal fluid, cerebrospinal fluid and saliva.

~~20.~~19. A kit for detecting at least different two target antibodies present in a biological sample from a subject, comprising the at least two solid supports defined in claim 1:

- a first solid support as obtained in step (c) of claim 1, which is covalently coupled with a first epitope that is recognized by a first target antibody, and
- a second solid support as obtained in step (f) of claim 1, which is covalently coupled with a second epitope that is recognized by a second target antibody, and not by said first target antibody,

wherein the at least two solid supports can be specifically identified from each other and enable to detect two different target antibodies, and further wherein the at least two solid supports are mixed together in at least one single compartment.

20. ~~21.~~ The kit according to claim ~~20~~19, comprising at least 10, preferably at least 50, more preferably at least 100 differently coupled-solid supports.

21. ~~22.~~ The kit according to claim ~~20 or 21~~19 or 20, wherein the said solid supports are microparticles.

22. ~~23.~~ The kit according to any one of claim ~~20 to 22~~19 to 21, wherein the said solid supports are microparticles that are mixed together in at least one well of a microliter plate or at least one tube.

23. ~~24.~~ The kit according to any one of claim ~~20 to 23~~19 to 22, further comprising the means to detect the at least two target antibodies which are bound to the solid supports.

24. ~~25.~~ The kit according to claim ~~24~~23, wherein said means are secondary antibodies recognizing the constant part of the target antibodies, said secondary antibodies being preferably labeled.

25. ~~26.~~ The kit according to any one of claim ~~20 to 25~~19 to 24, wherein said first and/or second epitope is present on a viral protein chosen in the group consisting of: the EDIII protein of the dengue virus 1 of SEQ ID NO:3, the EDIII protein of the dengue virus 2 of SEQ ID NO:4, the EDIII protein of the dengue virus 3 of SEQ ID NO:5, the EDIII protein of the dengue virus 4 of SEQ ID NO:6, the EDIII protein of the West Nile virus of SEQ ID NO:7, the EDIII protein of the Yellow Fever virus of SEQ ID NO:8, , the EDIII protein of the Japanese encephalitis virus of SEQ ID NO:9, the EDIII protein of the Zika virus of SEQ ID N0:10, the EDIII protein of the Wesselbron virus of SEQ ID NO:11, the EDIII protein of the Rocio virus of SEQ ID NO:12, the EDIII protein of the Murray encephalitis virus of SEQ ID NO:13, and the EDIII protein of the Saint-Louis encephalitis virus of SEQ ID NO: 14, the EDIII protein of the Japanese encephalitis virus of genotype 1 encoded by SEQ ID NO:54, the EDIII protein of the Japanese encephalitis virus of genotype 2 encoded by SEQ ID NO:55, the EDIII protein of the Japanese encephalitis virus of genotype 4 encoded by SEQ ID NO:56, the EDIII protein of the Japanese encephalitis virus of genotype 5 encoded by SEQ ID NO:57, the EDIII protein of the Rabensburg virus encoded by SEQ ID NO:58, and the viral protein of HIV1, of HIV2, of the Hepatitis B virus, of the Hepatitis C virus, of the Hepatitis E virus, of the West-Nile virus and of oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

2627. The kit according to any one of claim ~~20 to 25~~19 to 24, comprising at least two solid supports coated with at least two fusion proteins that are selected in the group consisting of: SEQ ID NO:21, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID N0:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO: 129, SEQ ID

NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149 and SEQ ID NO:151.

~~28.~~ 27. Use of the kit as defined in any one of the claims ~~20 to 27~~19 to 26, for detecting at least two target antibodies in a biological sample from a subject.

~~29.~~28. Use of the kit as defined in any one of the claims ~~20 to 27~~19 to 26, for diagnosing at least two target diseases in a subject, wherein said target disease is a viral infection caused by a Papillomavirus or a RNA virus from the family of the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Hepatitis E virus), the *Arenaviridae* (Lassa) or the *Filoviridae* (Ebola, Marburg), a bacterial infection caused by *Leptospirosa Interrogans*, or an infection caused by *Plasmodium falciparum.*

2930. An *in vitro* method for diagnosing at least one target disease in a subject, said target disease being known to induce the synthesis of at least one target antibody in said subject, comprising performing the assay method as defined in any of the claims 1 to ~~19~~18, wherein said subject is diagnosed to be suffering from said at least one target disease if the amount of said at least one target antibody is higher than a control value.

~~31~~30. The *in vitro* diagnosis method according to claim ~~30~~29, wherein said at least one target disease is a viral infection caused by a Papillomavirus or a RNA virus from the family of the *Flaviviridae* (Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C viruses), the *Togaviridae* (Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis viruses), the *Bunyaviridae* (Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg viruses), the *Caliciviridae* (Flepatitis E virus), the *Arenaviridae* (Lassa) or the *Filoviridae* (Ebola, Marburg), a bacterial infection caused by *Leptospirosa Interrogans*, or an infection caused by *Plasmodium falciparum.*

~~32.~~ 31. The *in vitro* diagnosis method according to claim ~~30 or 31~~29 or 30, wherein said method is used to diagnose at least 5, more preferably at least 15 and even more preferably at least 50 viral infections in said subject.

~~33.~~32. The *in vitro* diagnosis method of any one of claims ~~30 to 32~~29 to 31, wherein said control value represents the amount of said target antibody in a sample from a subject which is not suffering from said target disease.

~~34. The assay method according to claims 1 to 19, further comprising covalently coupling said AGT polypeptide to said solid support, by a coupling method comprising the following steps:~~

~~a) activating the said solid support,~~

~~b) adding a substrate of said AGT polypeptide, said substrate being suspended in a buffer containing between 0 and 20 % of DMSO, in appropriate conditions so that the substrate is covalently attached to said support,~~

~~c) contacting said AGT polypeptide with the substrate-coated support of step b) in a PBS/DTT buffer,~~

~~wherein unbound molecules are washed out after steps b) and c).~~

~~35. Method according to claim 34, wherein DTT is at a concentration of ImM in the PBS/DTT buffer.~~

~~36~~33. A method for manufacturing a kit as defined in any one of claims ~~20 to 27~~19 to 26, said method comprising the steps of:

(a) providing a least a first fusion protein comprising :

- a polypeptide comprising a first epitope that is recognized by a first target antibody and
- a AGT polypeptide having a 06-alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2,

(b) contacting said first fusion protein with a first solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(c) obtaining a first solid support covalently coupled with a first epitope that is recognized by the first target antibody,

(d) providing at least a second fusion protein comprising :

- a polypeptide comprising a second epitope, said second epitope being recognized by a second target antibody but not by said first target antibody, and

- a AGT polypeptide having a 06-alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2,

(e) contacting said second fusion protein with a second solid support, said support being covalently coupled with a substrate of said AGT polypeptide,

(f) obtaining a second solid support covalently coupled with a second epitope that is recognized 5 by the second target antibody, but not by said first target antibody,

wherein said at least first and at least second solid supports can be specifically identified from each other.

~~37.~~ 34. A multiplex immuno screening assay comprising at least 2, 25, 50, 96 solid supports as defined in claim 1 and wherein each of said solid supports emits a different and distinguishable wave length after excitation.

~~38.~~ 35. A multiplex immuno screening assay method comprising:

a) contacting one or several biological sample(s) with at least 2, 25, 50, 96 solid supports as defined in claim 1 and wherein each of the solid supports emits a different and distinguishable wave length after excitation, and

b) detecting the presence or absence of target antibodies.

~~39. The method according to claim 38 wherein said target antibodies are selected from antibodies specific to antigen from viruses to be detected in blood bank according to WHO or FDA guidelines, such as for example viruses selected from HBV, HCV, FHV1, HIV2, and WNV.~~

~~40.~~ 36. A method according to claim ~~38~~ 35 wherein said target antibodies are selected from antibodies specific to oncogenic HPV strains such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

~~41.~~37. A method according to ~~any one of~~ claim~~s 38 to 40~~35 or 36, wherein each of said target antibodies are labeled with a detectable label.

~~42.~~38. The multiplex screening assay of claim ~~37 or 38~~34 or 35, wherein said assay is carried out on an apparatus comprising:

- a technical device for detecting the light sources emitted from the solid supports and the light source emitted from the target antibodies or labeled antibodies binding to the target antibodies, and

- a calculating or computer device for identifying which solid supports are bound with target 5 antibodies, thereby indicating the presence or absence of antigens, bacteria, virus, or parasites in the analyzed sample.

~~43.~~39. A kit for the detection of at least two target antibodies in a biological sample comprising:

- (a) a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a 06-alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2 and a first epitope that is recognized by a first target antibody ;

(b) a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a O6 -alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2, and a second epitope that is recognized by a second target antibody, but not by said first target antibody; and wherein said first and second solid supports are mixed together in at least one single compartment.

~~44.~~40. A method for detecting at least two target antibodies in a biological sample comprising:

(a) contacting a first solid support comprising an AGT substrate covalently coupled to a first fusion protein comprising an AGT polypeptide having a 06-alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2 and a first epitope that is recognized by a first target antibody with the biological sample;

(b) contacting a second solid support comprising an AGT substrate covalently coupled to a second fusion protein comprising an AGT polypeptide having a 06-alkylguanine-DNA alkyltransferase activity, said AGT polypeptide being the SNAP mutant of SEQ ID NO:2, and a second epitope that is recognized by a second target antibody, but not by said first target antibody with the biological sample; and

(c) detecting the presence or absence of the two target antibodies.