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 ~~aspect~~ embodiment, ~~there is provided a~~the above disclosed assay method comprises ~~for~~ covalently coupling ~~a~~ the AGT polypeptide ~~having a O6-alkylguanine-DNA alkyltransferase activity, on a functionalized~~to said solid support, by a coupling method 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).

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

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

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

In another ~~aspect~~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, (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, (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 ~~aspect~~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 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 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 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 and a second epitope that is recognized by a second target antibody, but not by said first

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

20. 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.

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

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

~~23. The kit according to any one of claim 20 to 22, wherein the said solid supports are mixed together in at least one single compartment.~~

~~24~~23. The kit according to any one of claim 20 to ~~23~~22, wherein the said solid supports are microparticles that are mixed together in at least one well of a microtiter plate or at least one tube.

~~25~~24. The kit according to any one of claim 20 to ~~24~~23, further comprising the means to detect the at least two target antibodies which are bound to the solid supports.

2625. The kit according to claim 2524, wherein said means are secondary antibodies recognizing the constant part of the target antibodies, said secondary antibodies being preferably labeled.

2726. The kit according to any one of claim 20 to 2625, 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.

2827. The kit according to any one of claim 20 to 2625, 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 NO: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.

2928. Use of the kit as defined in any one of the claims 20 to 2827, for detecting at least two target antibodies in a biological sample from a subject.

3029. Use of the kit as defined in any one of the claims 20 to 2827, 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*.

3130. 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, 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.

3231. The *in vitro* diagnosis method according to claim 3130, 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* (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*.

~~33~~32. The *in vitro* diagnosis method according to claim 30 or 31 ~~or 32~~, 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.

~~34~~33. The *in vitro* diagnosis method of any one of claims 30~~1~~ to 32~~3~~, 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.

~~35~~34. The assay method according to claims 1 to 19, further comprising ~~Method for~~ covalently coupling ~~a~~said AGT polypeptide ~~having a O6-alkylguanine-DNA alkyltransferase activity, on~~ to said ~~a functionalized~~ solid support, by a coupling method 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).

~~36~~35. Method according to claim ~~35~~34, wherein DTT is at a concentration of 1mM in the PBS/DTT buffer.

~~37. The method of any one of claims 35 to 36, wherein said solid support is microsphere internally labeled with a fluorescent dye with magnetite encapsulated in a functional polymer outer coat containing surface carboxyl groups.~~

~~38. A solid support obtained by the method of any one of claims 35 to 37.~~

~~39. The solid support of claim 38, wherein it is a microsphere.~~

~~40. Use of the solid support of claim 38 or 39 in the assay method of claims 1 to 19.~~

~~41. 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.~~

~~42. The vector of claim 41, comprising the nucleotide sequence SEQ ID NO:35 or the nucleotide sequence of SEQ ID NO:36.~~

~~43. A recombinant cell which is stably transfected by the vector of claim 41 or 42.~~

~~44. The recombinant cell of claim 43, wherein it is an insect cell, preferably a S2 cell.~~

~~45. The recombinant cell of claim 43 or 44, wherein it is the S2 cell which has been deposited at the Centre National de Culture et de Microorganismes (CNCM), Institut Pasteur (25 rue du Docteur Roux, 75724 Paris cedex 15, France) on April 24, 2012, under the number CNCM I-4616.~~

~~46. 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.~~

~~47. The fusion polypeptide of claim 46, having the SEQ ID NO: 41, the SEQ ID NO:42 or the SEQ ID NO:46.~~

~~48~~36. A method for manufacturing a kit as defined in any one of claims 20 to ~~28~~27, 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,

(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,

(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.

~~49~~37. A multiplex immuno screening assay comprising at least 2, 25, 50, 96 solid supports as defined in claim 1 ~~or 48~~ and wherein each of said solid supports emits a different and distinguishable wave length after excitation.

~~50~~38. 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 ~~or 48~~ 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.

~~51~~39. The method according to claim ~~50~~ 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, HIV1, HIV2, and WNV.

~~52~~40. A method according to claim ~~50~~ 38 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.

~~53~~41. A method according to any one of claims ~~50~~ 38 to ~~52~~40, wherein each of said target antibodies are labeled with a detectable label.

~~54~~42. The multiplex screening assay of claim 37 or 38, wherein said assay is carried out on ~~A~~an apparatus ~~for carrying out the assay of claim 49 or the method of claim 50,~~ 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.

~~55~~43. 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 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 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.

~~56~~44. 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 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 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.