themselves in complex staining patterns (Avrameas S. *Immunol. Today* 1991). The presence of these natural antibodies can complicate the differentiation of disease-associated antibodies from the complex background of "auto-immune noise", i.e. naturally occurring autoantibodies. As noted in Anderson et al., Methods Mol. Biol. 723:227-238 (2011), cross-talk and interference remain a concern with multiplex assays.

Binding of human antibodies directly to the beads in a multiplex assay has also been reported as a problem. Tainsky et al., Biomarker Insights 2:261-267 (2007); Waterboer et al., J. Immunol Methods 309:200-204 (2006).

Moreover, a low difference between positive and control samples can limit the utility of multiplex assays. Burbelo et al., Exp. Rev Vaccines, 9:567-578 (2010).

Another complication is that ELISA and multiplex assays do not necessary give the same results. For example, Pickering et al., 2002, noted numerous discrepancies between the two assays when measuring protective antibodies against various viral and bacterial antigens. This may reflect alterations in antigenicity relating to how the antigen is attached to the substrate. Ambrosino et al., Malaria Journal 9:317 (2010). Similarly, Cham et al., Malaria Journal 7:108 (2008), reported that some samples showed a marked difference between the ELISA and bead-based assay readings, some of which had higher ELISA readings. This could be due to the fact that different parts of the recombinant proteins are accessible by antibodies when the proteins are bound to a surface or a sphere. *Id.*

The preparation of sufficient quantities of the antigen for commercial applications (i.e., kits) is also required. Burbelo et al., Exp. Rev Vaccines, 9:567-578 (2010).

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) contacting a mixture of at least 2 solid supports with a biological sample; wherein at least one of the solid supports comprises a first AGT-Antigen fusion protein covalently coupled to an AGT substrate, and wherein at least one of the solid supports comprises a second AGT-Antigen fusion protein covalently coupled to an AGT substrate; (b) detecting the presence or absence of binding of antibodies to the first AGT-Antigen; and (c) detecting the presence or absence of binding of antibodies to the second AGT-Antigen wherein the AGT of said first and second fusion proteins is the SNAP mutant of SEQ ID NO: 2.

Another aspect relates to a kit for detecting at least different two target antibodies present in a biological sample from a subject comprising: (a) a first solid support comprising a first AGT-Antigen fusion protein covalently coupled to an AGT substrate, and (b) a second solid support comprising a second AGT-Antigen fusion protein covalently coupled to an AGT substrate, wherein said solid supports are mixed together in at least one single compartment, and wherein the AGT of said first and second fusion proteins is the SNAP mutant of SEQ ID NO: 2.

Yet another aspect relates to the use of the kit as defined herein for detecting at least two target antibodies in a biological sample from a subject.

Still another aspect relates to 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 Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C, Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis, Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg, Hepatitis E virus, Lassa, Ebola, Marburg virus; a bacterial infection caused by *Leptospirosa Interrogans*; or an infection caused by *Plasmodium falciparum*.

Another aspect relates to 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 the subject, the method comprising performing the assay method as defined herein, wherein the 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.

A further aspect relates to a method for manufacturing a kit, said method comprising the steps of: (a) providing a first AGT-Antigen fusion protein that is recognized by a first target antibody, (b) contacting said first AGT-Antigen fusion protein with a first solid support, the support being covalently coupled with a substrate of the AGT-Antigen fusion protein, (c) obtaining a first solid support covalently coupled with the first AGT-Antigen fusion protein, d) providing at a second AGT-Antigen fusion protein that is recognized by a second target antibody, but not by said first target antibody, and (e) contacting said second AGT-Antigen fusion protein with a second solid support, the support being covalently coupled with a substrate of said AGT-Antigen fusion protein, and (f) obtaining a second solid support covalently coupled with the second AGT-Antigen fusion protein, wherein the first and second solid supports can be specifically identified from each other, wherein said solid supports are mixed together in at least one single compartment, and wherein the AGT of said first and second fusion proteins is the SNAP mutant of SEQ ID NO: 2.

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

AMENDED CLAIMS (Art34PCT)

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) contacting a mixture of at least 2 solid supports with a biological sample;
wherein at least one of the solid supports comprises a first AGT-Antigen fusion protein covalently coupled to an AGT substrate, and
wherein at least one of the solid supports comprises a second AGT-Antigen fusion protein covalently coupled to an AGT substrate;
(b) detecting the presence or absence of binding of antibodies to the first AGT-Antigen; and
(c) detecting the presence or absence of binding of antibodies to the second AGT-Antigen.

2. The assay method of claim 1, comprising contacting a mixture of at least 5 solid supports with a biological sample from a subject.

3. The assay method of claim 1, comprising contacting a mixture of at least 10 solid supports with a biological sample from a subject.

4. The assay method of claim 1, comprising contacting a mixture of at least 15 solid supports with a biological sample from a subject.

5. The assay method of any one of claim 1 to 4, wherein both AGT-Antigen fusion proteins comprise the SNAP mutant of SEQ ID NO:2.

6. The assay method of any one of claims 1 to 5, wherein said solid supports can be specifically identify by their specific location, size, diameter, weight, granulometry, or labeling.

7. The assay method of any one of claims 1 to 6, wherein said solid supports are labeled with a fluorochrome, a chromophore, a radioisotope, and/or a mass tag.

8. The assay method of any one of claims 1 to 7, wherein said solid supports are microparticles.

9. The assay method of any one of claims 1 to 8, wherein said solid supports are magnetic.

10. The assay method of any one of claims 1 to 9, wherein said solid supports are microparticles internally labeled with fluorescent dyes.

11. The assay method of any one of claims 1 to 10, wherein each of the solid supports emits a different and distinguishable wave length after excitation.

12. The assay method of any one of claims 1 to 11, wherein said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids 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.

13. The assay method of any one of claims 1 to 12, wherein said first or second AGT-Antigen fusion protein comprises at least 20 consecutive amino acids of a 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 HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

14. The assay method of any one of claims 1 to 13, wherein said first or second AGT-Antigen fusion protein comprises an amino acid sequence selected from 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.

15. The assay method of any one of claims 1 to 14, wherein said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

16. The assay method of any one of claims 1 to 15, wherein said first or second AGT-Antigen fusion protein comprises the amino acid sequence of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

17. The assay method of any one of claims 1 to 16, wherein said biological sample is serum or plasma.

18. A kit for detecting at least different two target antibodies present in a biological sample from a subject comprising:

(a) a first solid support comprising a first AGT-Antigen fusion protein covalently coupled to an AGT substrate, and

(b) a second solid support comprising a second AGT-Antigen fusion protein covalently coupled to an AGT substrate.

wherein the said solid supports are mixed together in at least one single compartment.

19. The kit according to claim 18, comprising at least at least 5 solid supports, each coupled to a different AGT-Antigen fusion protein covalently coupled to an AGT substrate.

20. The kit according to claim 19, comprising at least at least 10 solid supports, each coupled to a different AGT-Antigen fusion protein covalently coupled to an AGT substrate.

21. The kit according to claim 20, comprising at least at least 15 solid supports, each coupled to a different AGT-Antigen fusion protein covalently coupled to an AGT substrate.

22. The kit according to any one of claims 18 to 21, wherein the said solid supports are microparticles.

23. The kit according to any one of claim 18 to 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.

24. The kit according to any one of claim 18 to 23, further comprising labeled secondary antibodies to detect the at least two target antibodies which are bound to the solid supports.

25. The kit according to any one of claim 18 to 24, wherein said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids 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.

26. The kit according to any one of claim 18 to 25, wherein said first or second AGT-Antigen fusion protein comprises at least 20 consecutive amino acids of a 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 HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

27. The kit according to any one of claim 18 to 26, wherein said first or second AGT-Antigen fusion protein comprises an amino acid sequence selected from 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.

28. The kit according to any one of claim 18 to 27, said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

29. The kit according to any one of claim 18 to 28, said first or second AGT-Antigen fusion protein comprises the amino acid sequence of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

30. Use of the kit as defined in any one of the claims 18 to 29, for detecting at least two target antibodies in a biological sample from a subject.

31. Use of the kit as defined in any one of the claims 18 to 30, for diagnosing at least two target diseases in a subject, wherein said target disease is a viral infection caused by a Papillomavirus or a Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C, Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis, Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg, Hepatitis E virus, Lassa, Ebola, Marburg virus; a bacterial infection caused by *Leptospirosa Interrogans*; or an infection caused by *Plasmodium falciparum*.

32. 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 17, 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.

33. A method for manufacturing a kit, said method comprising the steps of:

(a) providing a first AGT-Antigen fusion protein that is recognized by a first target antibody,

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

(c) obtaining a first solid support covalently coupled with the first AGT-Antigen fusion protein,

(d) providing at a second AGT-Antigen fusion protein that is recognized by a second target antibody, but not by said first target antibody, and

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

(f) obtaining a second solid support covalently coupled with the second AGT-Antigen fusion protein,

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

wherein the said solid supports are mixed together in at least one single compartment.

34. The method of claim 33, further comprising following steps a)-c) with at least 5 different AGT-Antigen fusion proteins to generate at least 5 solid supports covalently coupled with 5 different AGT-Antigen fusion proteins.

35. The method of claim 33, further comprising following steps a)-c) with at least 10 different AGT-Antigen fusion proteins to generate at least 10 solid supports covalently coupled with 10 different AGT-Antigen fusion proteins.

36. The method of claim 33, further comprising following steps a)-c) with at least 15 different AGT-Antigen fusion proteins to generate at least 15 solid supports covalently coupled with 15 different AGT-Antigen fusion proteins.

We claim:

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) contacting a mixture of at least 2 solid supports with a biological sample;
wherein at least one of the solid supports comprises a first AGT-Antigen fusion protein covalently coupled to an AGT substrate, and
wherein at least one of the solid supports comprises a second AGT-Antigen fusion protein covalently coupled to an AGT substrate;
(b) detecting the presence or absence of binding of antibodies to the first AGT-Antigen; and
(c) detecting the presence or absence of binding of antibodies to the second AGT-Antigen,
wherein the AGT of said first and second fusion proteins is the SNAP mutant of SEQ ID NO:2.

2. The assay method of claim 1, comprising contacting a mixture of at least 5 solid supports with a biological sample from a subject.

3. The assay method of claim 1, comprising contacting a mixture of at least 10 solid supports with a biological sample from a subject.

4. The assay method of claim 1, comprising contacting a mixture of at least 15 solid supports with a biological sample from a subject.

5. The assay method of any one of claims 1 to 4, wherein said solid supports can be specifically identify by their specific location, size, diameter, weight, granulometry, or labeling.

6. The assay method of any one of claims 1 to 5, wherein said solid supports are labeled with a fluorochrome, a chromophore, a radioisotope, and/or a mass tag.

7. The assay method of any one of claims 1 to 6, wherein said solid supports are microparticles.

8. The assay method of any one of claims 1 to 7, wherein said solid supports are magnetic.

9. The assay method of any one of claims 1 to 8, wherein said solid supports are microparticles internally labeled with fluorescent dyes.

10. The assay method of any one of claims 1 to 9, wherein each of the solid supports emits a different and distinguishable wave length after excitation.

11. The assay method of any one of claims 1 to 10, wherein said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids 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.

12. The assay method of any one of claims 1 to 11, wherein said first or second AGT-Antigen fusion protein comprises at least 20 consecutive amino acids of a 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 HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

13. The assay method of any one of claims 1 to 12, wherein said first or second AGT-Antigen fusion protein comprises an amino acid sequence selected from 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.

14. The assay method of any one of claims 1 to 13, wherein said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

15. The assay method of any one of claims 1 to 14, wherein said first or second AGT-Antigen fusion protein comprises the amino acid sequence of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

16. The assay method of any one of claims 1 to 15, wherein said biological sample is serum or plasma.

17. A kit for detecting at least different two target antibodies present in a biological sample from a subject comprising:

(a) a first solid support comprising a first AGT-Antigen fusion protein covalently coupled to an AGT substrate, and

(b) a second solid support comprising a second AGT-Antigen fusion protein covalently coupled to an AGT substrate.

wherein said solid supports are mixed together in at least one single compartment, and

wherein the AGT of said first and second fusion proteins is the SNAP mutant of SEQ ID NO:2.

18. The kit according to claim 17, comprising at least at least 5 solid supports, each coupled to a different AGT-Antigen fusion protein covalently coupled to an AGT substrate.

19. The kit according to claim 18, comprising at least at least 10 solid supports, each coupled to a different AGT-Antigen fusion protein covalently coupled to an AGT substrate.

20. The kit according to claim 19, comprising at least at least 15 solid supports, each coupled to a different AGT-Antigen fusion protein covalently coupled to an AGT substrate.

21. The kit according to any one of claims 17 to 20, wherein the said solid supports are microparticles.

22. The kit according to any one of claim 17 to 21, 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.

23. The kit according to any one of claim 17 to 22, further comprising labeled secondary antibodies to detect the at least two target antibodies which are bound to the solid supports.

24. The kit according to any one of claim 17 to 23, wherein said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids 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.

25. The kit according to any one of claim 17 to 24, wherein said first or second AGT-Antigen fusion protein comprises at least 20 consecutive amino acids of a 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 HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

26. The kit according to any one of claim 17 to 25, wherein said first or second AGT-Antigen fusion protein comprises an amino acid sequence selected from 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.

27. The kit according to any one of claim 17 to 26, said first or second AGT-Antigen fusion protein comprises at least 30 consecutive amino acids of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

28. The kit according to any one of claim 17 to 27, said first or second AGT-Antigen fusion protein comprises the amino acid sequence of SEQ ID NOs: 155, 157, 159, 161, 163, 165, 167, 171, 173, 175, 177, and 179.

29. Use of the kit as defined in any one of the claims 17 to 28, for detecting at least two target antibodies in a biological sample from a subject.

30. Use of the kit as defined in any one of the claims 17 to 29, for diagnosing at least two target diseases in a subject, wherein said target disease is a viral infection caused by a Papillomavirus or a Dengue, Yellow fever, West Nile, Japanese encephalitis, Tick-Borne Encephalitis, Hepatitis C, Chikungunya, Ross River, Mayaro, Western Equine encephalitis, Eastern Equine Encephalitis, Venezuela Equine Encephalitis, Crimean-Congo hemorrhagic fever, Rift Valley Fever, Schmallenberg, Hepatitis E virus, Lassa, Ebola, Marburg virus; a bacterial infection caused by *Leptospirosa Interrogans*; or an infection caused by *Plasmodium falciparum.*

31. 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 16, 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.

32. A method for manufacturing a kit, said method comprising the steps of:

(a) providing a first AGT-Antigen fusion protein that is recognized by a first target antibody,

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

(c) obtaining a first solid support covalently coupled with the first AGT-Antigen fusion protein,

(d) providing at a second AGT-Antigen fusion protein that is recognized by a second target antibody, but not by said first target antibody, and

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

(f) obtaining a second solid support covalently coupled with the second AGT-Antigen fusion protein,

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

wherein said solid supports are mixed together in at least one single compartment, and

wherein the AGT of said first and second fusion proteins is the SNAP mutant of SEQ ID NO:2.

33. The method of claim 32, further comprising following steps a)-c) with at least 5 different AGT-Antigen fusion proteins to generate at least 5 solid supports covalently coupled with 5 different AGT-Antigen fusion proteins.

34. The method of claim 32, further comprising following steps a)-c) with at least 10 different AGT-Antigen fusion proteins to generate at least 10 solid supports covalently coupled with 10 different AGT-Antigen fusion proteins.

35. The method of claim 32, further comprising following steps a)-c) with at least 15 different AGT-Antigen fusion proteins to generate at least 15 solid supports covalently coupled with 15 different AGT-Antigen fusion proteins.