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.

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.

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.

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