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Holzmuller et al.(10) **Pub. No.: US 2013/0203085 A1**(43) **Pub. Date: Aug. 8, 2013**(54) **ANTIGENIC STRUCTURE AND USES
THEREOF FOR SCREENING
TRYPANOSOMIASIS IN HUMANS AND
ANIMALS**(30) **Foreign Application Priority Data**

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Cuny**, Castries (FR)(51) **Int. Cl.**
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USPC **435/7.92**; 548/496; 530/331(73) Assignee: **Institut de Recherche pour le
Developpment (IRD)**, Marseille (FR)(21) Appl. No.: **13/640,523**(22) PCT Filed: **Apr. 13, 2011**(86) PCT No.: **PCT/IB11/51605**

§ 371 (c)(1),

(2), (4) Date: **Nov. 28, 2012**(57) **ABSTRACT**

The invention relates to an antigenic structure that contains a tryptophan epitope, characterised in that said structure is made up of a tryptophan pattern W or a peptide of 3 or 4 amino acids comprising a pattern W, coupled with glutaraldehyde. The invention can be used for screening trypanosomiasis in humans or animals.

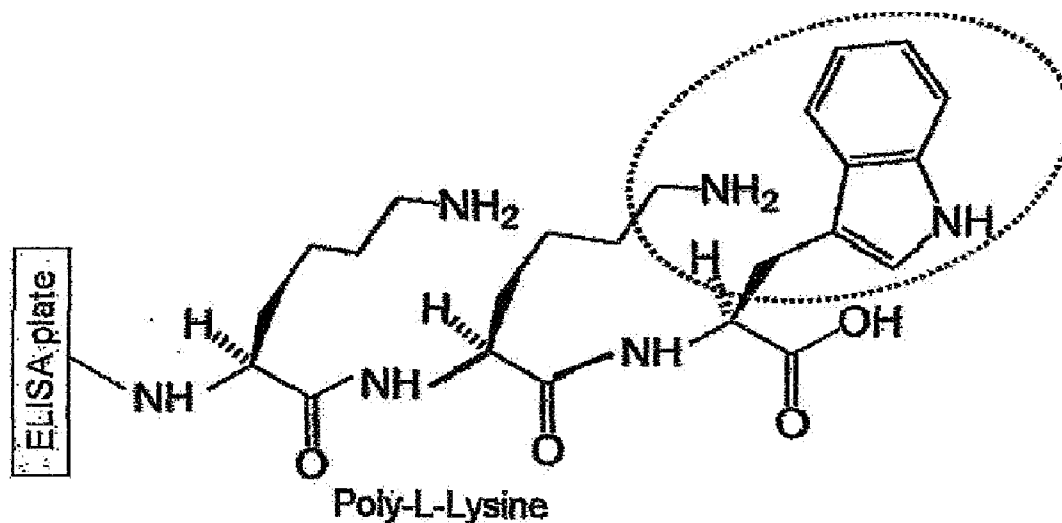


Figure 1A

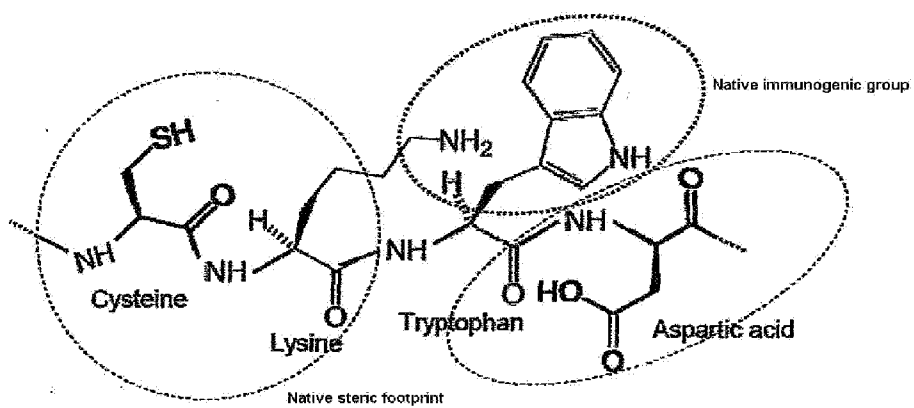


Figure 1B

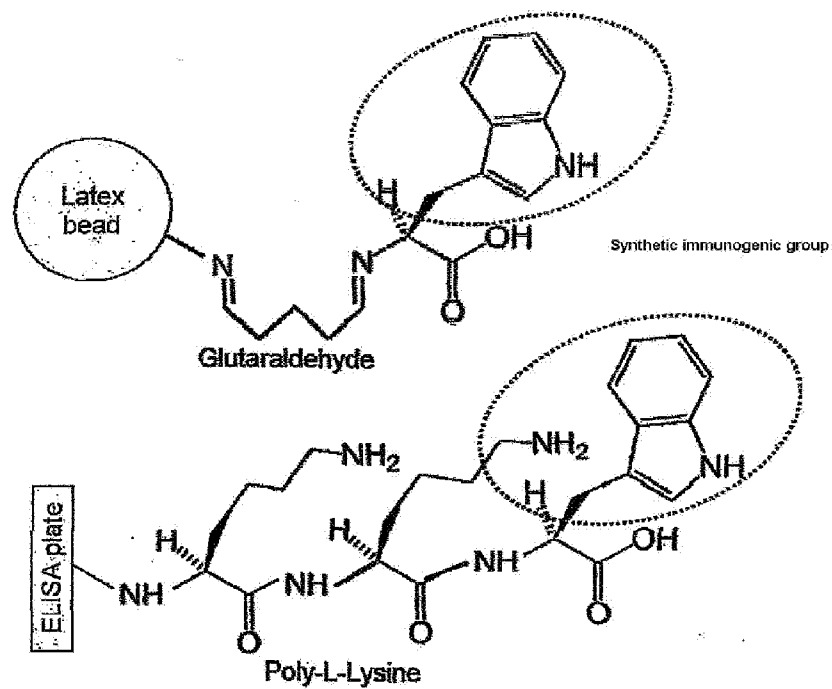


Figure 1C

Figure 2

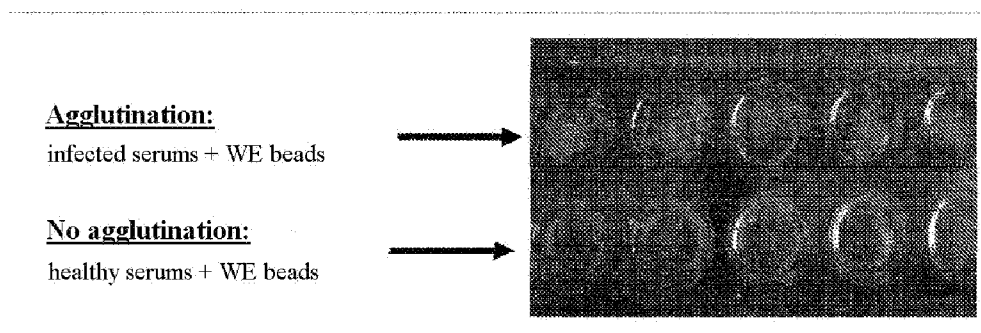
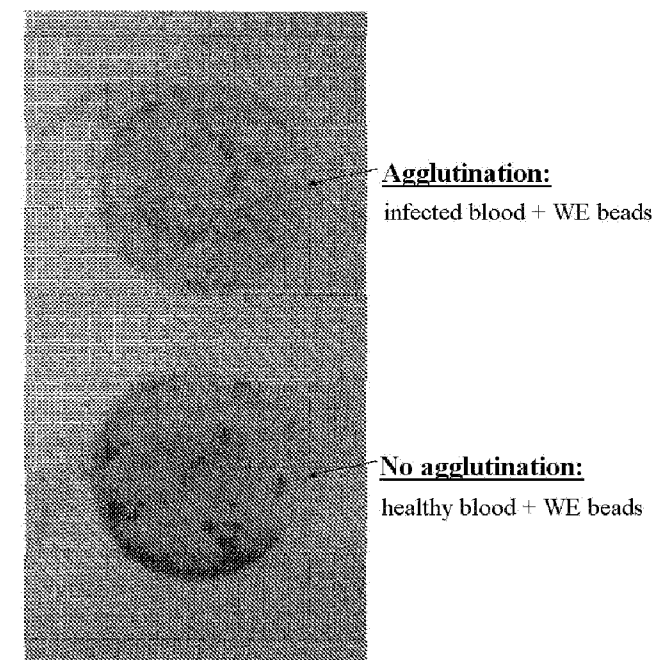


Figure 3



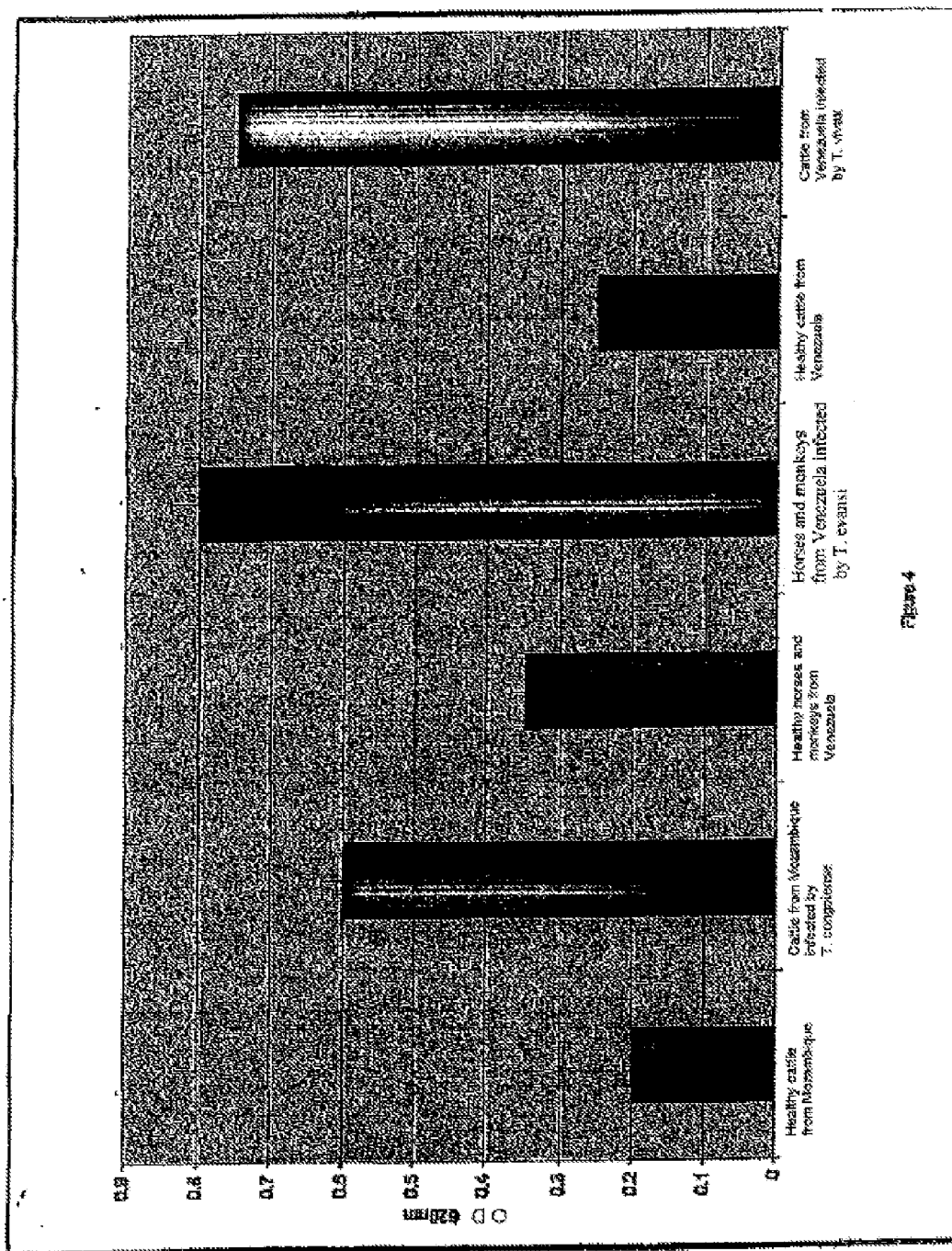
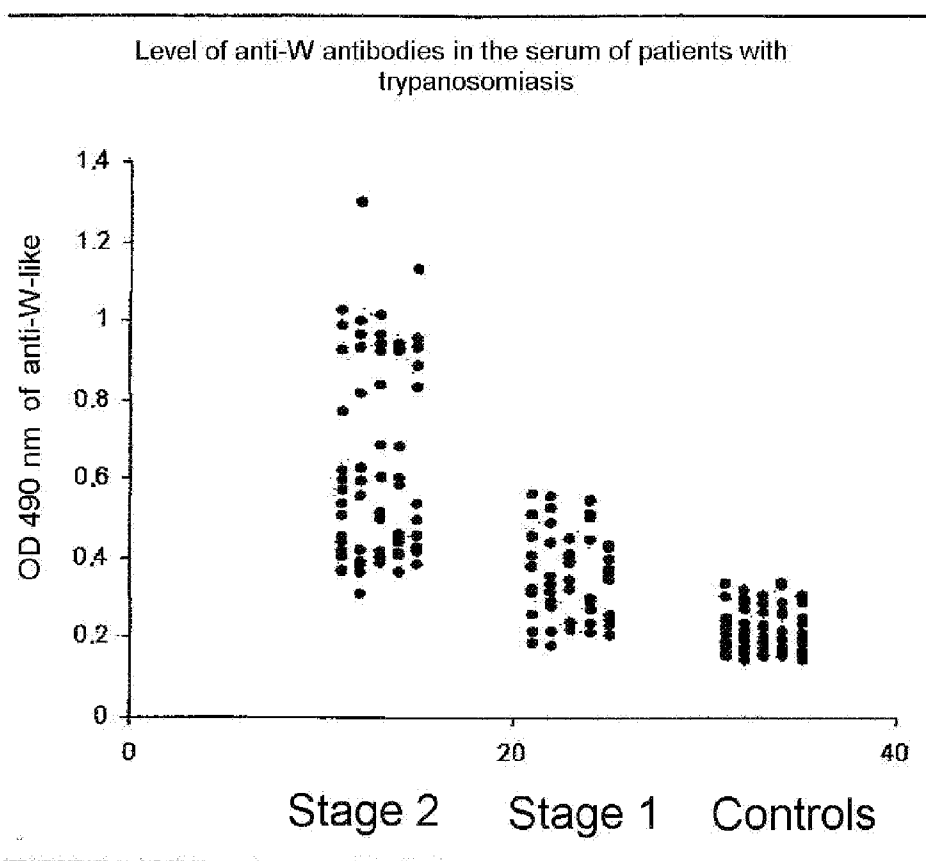


Figure 4

Figure 5

ANTIGENIC STRUCTURE AND USES THEREOF FOR SCREENING TRYPANOSOMIASIS IN HUMANS AND ANIMALS

[0001] The invention relates to an antigenic structure and its use for producing a kit and carrying out a method of screening for trypanosomiasis in humans and animals.

[0002] Human African trypanosomiasis (HAT) and animal trypanosomiasis (AAT) mainly affect poor rural communities and are very often neglected diseases which pose a major public health problem and put a halt to the economic development of many countries. The greatest difficulty in controlling these diseases arises from the great variability of the clinical symptomatology associated with the lack of sensitivity and of specificity or with the burden of carrying out the available screening tests.

[0003] Active screening of the at-risk population is therefore essential if infected individuals are to be identified early and transmission is to be reduced by reducing the parasite reservoir. In addition, screening for HAT must include a diagnosis of stage 1 (haematolymphatic) or 2 (neurological), because patients in stage 2 are treated with melarsoprol, the toxic side-effects of which are considerable (global mortality 5-10%). Even today, stage diagnosis is based on examination of the cerebrospinal fluid after lumbar puncture, and the positivity threshold of the cerebrospinal fluid cell count is still a subject of debate (5, 10 or 20 cells/ μ l).

[0004] At present, the CATT (Card Agglutination Test For Trypanosomiasis)/T. b. gambiense is the most commonly used serological test. The test comprises fixed trypanosomes of the variant LiTat 1.3. A variant of the CATT is composed of semi-purified antigens (LiTat 1.3, 1.5 and 1.6) coupled to latex beads. The main problem associated with the CATT is a lack of specificity, because the antigens used are responsible for numerous cross-reactions and therefore for false positives. In addition, blood, diluted blood or plasma have variable antibody titres against these antigens. Thus, patients who are parasitologically positive may be CATT-negative. Finally, the CATT does not allow stage diagnosis and satisfactory serological monitoring of patients after treatment, in particular because of cross-reactions with other infections.

[0005] The value of having available novel antigens for improving the screening, stage determination and monitoring of HAT and AAT will therefore be appreciated. Although some antigens have been found to be valuable from a pathophysiological point of view, the handling thereof in field conditions (Semballa et al., 2004 (ref. 1)) has proved difficult.

[0006] Tryptophan-like epitopes (WE), which represent constant trypanosome antigens, induce specific antibodies in the host (human or animal). These epitopes include the amino acid L-tryptophan and are situated in constant regions of the C-terminal part of variable surface glycoproteins (VSG). High titres of anti-WE antibodies of isotype IgM have been measured by ELISA in the serum of patients affected by human African trypanosomiasis (HAT), and elevated titres have been found in patients in stage 2 (Okomo-Assoumou et al., 1995 (ref. 2)).

[0007] These pioneering results were obtained by the laboratory of the inventors by virtue of synthetic epitopes proposed for studying their recognition by anti-WE antibodies, especially WE-glutaraldehyde-BSA structures. In these structures, glutaraldehyde acts as the fixer between the synthetic antigen WE and the carrier protein (in this case bovine

albumin BSA). These structures allowed the potential of the epitope WE as a diagnostic target to be demonstrated.

[0008] The work by the inventors in this field has shown an unexpected effect of glutaraldehyde relating to the optimum orientation it imparts to the synthetic antigen. It has become apparent that glutaraldehyde acts as an orientator and spacer arm for the synthetic antigen WE when it is coupled to an inert substrate such as latex beads or ELISA plates. As is shown in FIGS. 1A and 1B, to which the example below relates, the antigenic structure of the invention constitutes a mimotope of the natural antigen when it is coupled solely thereto, which permits better recognition by anti-WE antibodies.

[0009] It is, therefore, an object of the invention to provide a novel antigenic structure. The invention also aims to make use of the properties of this structure in a kit and a method of screening for human and animal trypanosomiasis.

[0010] The antigenic structure of the invention is based on the coupling of an epitope of interest to glutaraldehyde and the fixing thereof to an inert substrate. The invention accordingly relates to an antigenic structure comprising a tryptophan-like epitope, characterised in that it is formed of a tryptophan unit W, or of a peptide of 3 or 4 amino acids including a unit W, coupled to glutaraldehyde.

[0011] Said peptide preferably has the sequence SEQ ID No. 1 C-x-W-y, in which "x" represents K, A, S, V, T, R, I, E, D, N or G and "y" represents D, S, T, E, N, K, G, Q, R or I, it being possible for either "x" or "y" to be absent.

[0012] A representative sequence, SEQ ID No. 2, is of the type CKWD.

[0013] According to an advantageous provision, said coupling product is grafted onto latex beads. This formulation allows the development of a rapid technique of agglutination on the serums of patients or animals affected by HAT or AAT, respectively.

[0014] According to another provision, which is advantageously used with the preceding provision, the coupling product is grafted onto ELISA plates, allowing the development of a quantitative technique for the levels of "anti-trypanosome" antibodies in patients or animals affected by HAT or AAT.

[0015] According to yet another provision, the coupling product as defined above is grafted onto poly-L-lysine deposited in the bottom of ELISA plates.

[0016] This provision allows a wholly synthetic system to be obtained, for development either in the form of beads or in the form of an ELISA test.

[0017] The invention relates also to a kit for screening for HAT or AAT, characterised in that it comprises

[0018] at least one antigenic structure as defined above, optionally with one or more reagents for the antigen/antibody reaction and/or buffer solutions and/or reagents for the detection, quantification or visualisation of antigen/antibody complexes when they are present.

[0019] The above kit advantageously comprises, according to an additional provision of the invention, reagents for performing an immunoenzymatic revelation test, especially an indirect ELISA test or an ELISA inhibition test, in which a monoclonal anti-WE antibody is made to compete with the anti-WE antibodies of the serums of infected hosts.

[0020] In a variant embodiment, the antigenic structure is fixed to a substrate.

[0021] The invention relates further to a method of screening for HAT or AAT, characterised in that it comprises

[0022] contacting of a serum sample from a patient or an animal with an antigenic structure as defined above, advantageously using a kit as described above,

[0023] revelation of an immunological reaction of the antigen-antibody type.

[0024] In the variant in which the antigenic structure is fixed to latex beads, contacting with the serum sample leads to agglutination of the serum if the patient or the animal is affected by HAT or AAT, respectively.

[0025] This serological diagnostic method allows mass screening, stage determination and monitoring of HAT and AAT to be carried out. It has the advantage of being highly sensitive and highly specific, while at the same time avoiding cross-reactions with other infections (false positives) and false negatives (CATT-seronegatives in parasitology).

[0026] It will be possible to use this novel serological diagnostic test, which is inexpensive, stable and easy to carry out, where appropriate combined with an indirect ELISA test or an ELISA inhibition based on anti-WE antibodies, and which has proved its effectiveness, for mass and stage diagnosis (determination of the antibody titres of patients in stages 1 and 2) of HAT or AAT in the field, with a high degree of specificity. The test is particularly suitable for specific screening for human or animal African trypanosomiasis.

[0027] Other features and advantages of the invention are given by way of illustration in the implementation examples of the invention which follow. In these examples, reference is made to FIGS. 1 to 5, in which

[0028] FIGS. 1A to 1C show the native steric footprint of the sequence CKWD (FIG. 1A), a sequence of type inert substrate with amine functional group-glutaraldehyde-W (FIG. 1B), and a sequence fixed to an ELISA plate (FIG. 1C);

[0029] FIGS. 2 and 3 show the results of the diagnostic test on human serum and blood, respectively;

[0030] FIG. 4 shows a graph of results obtained with animal serums infected by different species of Trypanosomes;

[0031] FIG. 5 shows the levels of anti-W antibodies in the serum of patients affected by HAT (stages 1 and 2).

EXAMPLE 1

Synthesis of W-glutaraldehyde or C-x-W-y-glutaraldehyde Coupling Products

Production of Coupled Latex Beads

[0032] 100 μ l of 5% glutaraldehyde are added within a period of about 10 seconds, under a vortex, to 100 μ l of haptene (tryptophan or peptide) in a concentration of 5 mg/ml in a 1.5 M acetate buffer, pH 8.3.

[0033] Without waiting, 50 μ l of aminated latex beads (Sigma) are added, and the mixture is allowed to react for at least 10 minutes, with slow stirring.

[0034] The reaction is stopped by addition of 100 μ l of 1M NaBH₄, and the mixture is stirred slowly for a further 10 minutes.

[0035] The mixture is dialysed against distilled water, to which NaBH₄ (1 spatula/5 l) has been added, for the whole day, the dialysis water being changed 2 to 3 times, and then for about 14 hours (overnight) with water on its own in order to remove excess NaBH₄.

[0036] By way of illustration, the structural chemical formulae of the native epitope sequence and of the chemical sequences used according to the invention have been given in FIGS. 1A and 1B, and they clearly show that the structure of the invention reproduces the molecular configuration of the epitope of interest.

Production of ELISA Plates

[0037] To each well of the ELISA plate (Nunc, PolySorp or MaxiSorp) there are added 200 μ l of a mixture of 100 μ l of haptene (tryptophan or peptide, 5 mg/ml in a 1.5 M acetate buffer, pH 8.3) and 100 μ l of 5% glutaraldehyde added under a vortex within a period of about 10 seconds. The mixture is allowed to react for at least 30 minutes, with slow stirring.

[0038] The reaction is stopped by addition of 100 μ l of 1M NaBH₄, and the mixture is stirred slowly for a further 10 minutes.

[0039] The plates are washed (5 times 1 hour) with distilled water, to which NaBH₄ (1 spatula/5 l) has been added, and then for about 14 hours (overnight) with water on its own in order to remove excess NaBH₄.

[0040] A structure with a poly-L-lysine unit fixed to a LISA plate is given by way of illustration in FIG. 1C.

EXAMPLE 2

Diagnostic Test on Human (or Animal) Serum with Latex Beads

[0041] For rapid screening, 20 μ l of conjugated latex beads are mixed with 20 μ l of diluted or undiluted serum or 20 μ l of whole blood. After 5 to 10 minutes, as shown in FIGS. 2 (with serum) and 3 (with blood), a ring characteristic of the positivity of the reaction is observed. In the absence of a ring, the reaction is considered to be negative.

EXAMPLE 3

Diagnostic Test Combining an ELISA Test for Stage Diagnosis

[0042] The results obtained are shown in FIG. 5.

[0043] The use of the ELISA plates with serums of patients affected by HAT shows an increase in the levels of anti-WE antibodies on passage into stage 2 (neurological involvement).

BIBLIOGRAPHIC REFERENCES

[0044] (1)—Semballa et al., *experimental Parasitology* 115 (2007) 173-180

[0045] (2)—Okomo-Assoumou et al., *Ann. J. Trop. Med. Hyg.*, 1995, pp 461-467 pistage de la trypanosomose humaine ou animale

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1-11. (canceled)

12. An antigenic structure containing a tryptophan-like epitope formed of a tryptophan unit W or of a peptide of 3 or 4 amino acids comprising W, coupled to glutaraldehyde.

13. The antigenic structure of claim **12**, wherein the peptide has the sequence C-x-W-y in which x represents the amino acids K, A, S, V, T, R, I, E, D, N or G and y represents the amino acids D, S, T, E, N, K, G, Q, R or I, wherein one of x or y may be absent.

14. The antigenic structure of claim **12** having the amino acid sequence CKWD.

15. The antigenic structure of claim **12**, wherein the glutaraldehyde-coupling product is grafted onto beads of latex or poly-L-lysine.

16. The antigenic structure of claim **12**, wherein the glutaraldehyde-coupling product is grafted onto ELISA plates.

17. The structure of claim **16**, wherein the ELISA plates comprise poly-L-lysine.

18. A kit for screening for human or animal trypanosomiasis comprising a reagent comprising the antigenic structure of claim **12** and, optionally, a reagent for an antigen-antibody reaction or a buffer a solution or reagent for detection, quantification or visualisation of an antigen-antibody complex.

19. The kit of claim **18** further comprising reagents for performing an immunoenzymatic revelation test in which a monoclonal anti-WE antibody is made to compete with an anti-WE antibody from serum of an infected host.

20. The kit of claim **19**, wherein the immunoenzymatic revelation test is an indirect ELISA test or an ELISA inhibition test.

21. The kit of claim **18**, wherein the antigenic structure is fixed to a substrate.

22. The kit of claim **18**, which enables screening for human or animal African trypanosomiasis.

23. A method of screening for HAT Or AAT, comprising contacting a serum sample from a patient or an animal with the antigenic structure of claim **12**, and revealing an immunological reaction of the antigen-antibody type.

24. The method of claim **23**, wherein at least one of the contacting step or revealing step comprises or further comprises contacting at least one of the serum sample or antigenic structure with at least one of a reagent for an antigen-antibody reaction or a buffer solution or a reagent for detection, quantification or visualisation of antigen-antibody complexes.

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