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(54) TEST STRIP AND KIT FOR TESTING MYCOPHENOLIC ACID AND PREPARATION METHOD OF TEST STRIP

TESTSTREIFEN UND KIT ZUM TESTEN VON MYCOPHENOLSÄURE UND HERSTELLUNGSVERFAHREN FÜR TESTSTREIFEN

BANDE DE TEST ET KIT POUR TESTER L'ACIDE MYCOPHÉNOLIQUE ET PROCÉDÉ DE PRÉPARATION DE BANDE DE TEST

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Description

TECHNICAL FIELD

[0001] The present invention belongs to the technical field of mycophenolic acid testing, and specifically relates to a test strip and kit for testing mycophenolic acid (MPA) and a preparation method of the test strip.

TECHNICAL BACKGROUND

[0002] MPA, also called mycophenolic acid, can noncompetitively bond with inosine monophosphate dehydrogenase, and the latter one is a key enzyme to the denovo synthesis of guanine nucleotides of T/B lymphocyte in its proliferation process. As immunosuppressors, the mycophenolate mofetil derivatives have been widely used in the prevention and treatment of acute rejection of the transplanted organs at home and abroad. Mycophenolate mofetil is transformed to mycophenolic acid in vivo to exert its immunosuppression activity. In different transplanted groups, great disparities of mycophenolic acid pharmacokinetics can be found among individuals, and moreover, its pharmacokinetics will be influenced by in vivo/vitro factors.

[0003] With the proposal of precision medicine and individualized medicine, various kinds of drug concentration testing get more and more attention. Excessive metabolic concentration of mycophenolic acid in blood will inhibit immunological competence excessively, resulting in hypoimmunity and other problems; too low metabolic concentration of mycophenolic acid in blood will not bring due immunosuppression, resulting in the failure of a therapeutic action to acute rejection of the transplanted organs. Therefore, the significance of mycophenolic acid testing becomes particularly outstanding.

[0004] At present, the common testing methods for mycophenolic acid mainly include mass-spectrography, high performance liquid chromatography, chemiluminescent immunoassay, homogeneous enzyme immunoassay, etc. Mass-spectrography refers to a method that moving ions are separated by electric field and magnetic field for testing according to the mass-to-charge ratio, and the testing is conducted by a mass spectrometer. High performance liquid chromatography refers to a method that with liquid as mobile phase, a high-pressure transfusion system is taken to pump a single solvent with different polarities or a mixed solvent different proportions, a buffer solution and other mobile phase into a chromatographic column loaded with stationary phase, each component is put into a detector after being separated in the column, thus achieving sample analysis, and the analytical instrument is high performance liquid chromatograph. Chromatography and mass-spectrography have precise testing results, but the sample needs to be pretreated before loading, taking too much detection time, and only one sample can be loaded every time, incapable of satisfying high throughput requirement, and the testing instrument used is relatively expensive, restricting its clinical popularization to some extent. Turbidimetry is mainly applied by Roche for testing, the method needs to be equipped with a high-cost biochemical analyzer and the reagent needs to be refrigerated, which is incapable of satisfying the increasing clinical testing requirement and achieving single testing in any time. Mycophenolic acid was tested by homogeneous enzyme immunoassay (EVERMED, application No.: 201510039618.3). By an immune method, multiple samples can be measured on an automatic biochemical analyzer at the same time to achieve high-throughput and rapid measurement. The method requires an automatic biochemical analyzer, 2-8°C storage temperature and cold-chain transportation, therefore, the method fails to achieve a single package and timely monitoring in medication departments.

[0005] US 2014/0017812 A1 discloses in general a lateral flow immunoassay device for detecting antineoplastic drugs, while it does not disclose how to test the content of mycophenolic acid.

SUMMARY

[0006] In view of this, an objective of the present invention is to provide a test strip and kit for testing mycophenolic acid and a preparation method of the test strip; the test strip can effectively make up the gaps of the several methods above and can achieve normal temperature preservation, rapid, high throughput and single testing in any time, thus greatly improving the test cost, operation convenience and clinical use simplicity of the test strip.

[0007] To achieve the above objective of the invention, the present invention provides the following solution.

[0008] A test strip for testing the content of mycophenolic acid includes a bottom plate and a sample pad, a glassfiber membrane, a nitrocellulose membrane and an absorbent paper which are successively lapped on a surface of the bottom plate, where

the sample pad is treated by a sample pad treatment fluid, and the sample pad treatment fluid includes a buffer solution, an active protein and a surfactant; the active protein is selected from one or more of bovine serum albumin, casein and ovalbumin;

the glassfiber membrane is treated by a glassfiber membrane treatment fluid, and the glassfiber membrane is coated by a mycophenolic acid specific-antibody conjugate; and the mass concentration of the mycophenolic acid specific-

antibody conjugate is 0.1-0.3%, and the coating concentration of the mycophenolic acid specific-antibody conjugate is 0.5-1 mg/mL;
the nitrocellulose membrane is provided with a detection line and quality control line, a mycophenolic acid protein conjugate is sprayed on the detection line, the concentration of the mycophenolic acid protein conjugate is 1-3 mg/ml, and the spraying amount is 1-3 μL/cm.

[0009] Preferably, the buffer solution in the sample pad treatment fluid is selected from one or more of PBS buffer solution, TRIS buffer solution and glycine buffer solution; the surfactant in the sample pad treatment fluid is selected from one or more of Tween 20, Tween 80 and Triton-X-100.

[0010] Preferably, the conjugate in the mycophenolic acid specific-antibody conjugate is a colloidal gold particle or a fluorescent microsphere.

[0011] Preferably, the glassfiber membrane treatment fluid includes a basal solution and the following components with the following concentrations: 0.5-5 g/L polyethylene glycol 6000, 3-15 g/L mannitol, 0.1-1.5 g/L alum and 0.8%-1.0% sodium chloride; the basal solution is selected from one or more of PBS buffer solution, TRIS buffer solution and glycine buffer solution.

[0012] A preparation method of the above test strip includes the following steps of:

1) treating the sample pad by the sample pad treatment fluid to obtain a treated sample pad;
2) mixing mycophenolic acid monoclonal antibodies and the conjugate for coupling and sealing to obtain a mycophenolic acid specific-antibody conjugate; spraying the mycophenolic acid specific-antibody conjugate on the treated glassfiber membrane to obtain the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate;
3) spraying the mycophenolic acid protein conjugate on the detection line of the nitrocellulose membrane and spraying goat-anti-mouse IgG on the quality control line to obtain a nitrocellulose membrane sprayed with the detection line and quality control line;
4) successively lapping and pasting the treated sample pad, the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate, the nitrocellulose membrane provided with the detection line and quality control line as well as the absorbent paper on the surface of the bottom plate to obtain the test strip;

where, there is no limitation on an execution sequence of steps 1), 2) and 3).

[0013] Preferably, the concentration of mycophenolic acid monoclonal antibodies of the coupling system in step 2) is 100-500 μg/mL; and the coupling time is 2.5-3.5h.

[0014] Preferably, the mycophenolic acid protein conjugate is sprayed after mixed with a spraying buffer solution; components of the spraying buffer solution are the same as those of the glassfiber membrane treatment fluid.

[0015] A kit including the above test strip is provided, where the kit further includes a mycophenolic acid QC substance.

[0016] Preferably, the mycophenolic acid QC substance is obtained by mixing and lyophilizing pure mycophenolic acid and a lyophilized buffer solution.

[0017] Preferably, the lyophilized buffer solution includes a basal solution and the following components with the following concentrations: 2-6 g/L polyethylene glycol, 3-8 g/L trehalose, 5-20 g/L mannitol, 1wt% polyvinylpyrrolidone; the basal solution is selected from one or more of TRIS buffer solution, phosphoric acid buffer solution, boric acid buffer solution and phosphate buffer solution; pH of the basal solution is 7.6-8.6; the solute concentration of the basal solution is 50-200 mmol/L.

[0018] Beneficial effect of the present invention: the test strip provided by the present invention can rapidly test the content of mycophenolic acid in blood samples, and the testing instrument has low cost, simple and rapid operation; the test strip has good stability and can be stored at room temperature for transportation with 18 months of shelf life at 2-30°C; meanwhile test samples are extensive, including serum samples, plasma samples and whole blood samples, while the whole blood sample cannot be tested by the method of prior art; the linear range is wide, being up to 0-20 μg/mL; the test strip has a stronger anti-interference capability, after 50 mg/ml hemoglobin and 50mg/dl triglyceride are added for interference, compared with the results obtained before adding interfering substances, the relative deviation is less than 5% within the acceptable range, therefore, the anti-interference capability is strong.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019]

FIG. 1 is a curve chart showing clinic correlation of reagents.

DETAILED DESCRIPTION

[0020] The present invention provides a test strip according to independent claim 1.

[0021] There is no special limitation to texture and source of the bottom plate in the present invention, and conventional texture and source of the test strip in this field are available.

[0022] In the present invention, the sample pad is treated by a sample pad treatment fluid, and the sample pad treatment fluid includes a buffer solution, an active protein and a surfactant; in the present invention, the buffer solution in the sample pad treatment fluid is preferably selected from one or more of PBS buffer solution, TRIS buffer solution and glycine buffer solution; the active protein in the sample pad treatment fluid is selected from one or more of bovine serum albumin, casein and ovalbumin; the surfactant in the sample pad treatment fluid is preferably selected from one or more of Tween 20, Tween 80 and Triton-X-100. In specific implementation process of the present invention, the sample pad treatment fluid is preferably a PBS buffer solution added 0.1% Tween 20. In the present invention, the sample pad treatment is preferably to soak the sample pad into the sample pad treatment fluid for 0.5h and then put it into an oven for drying for 2h at 65°C.

[0023] In the present invention, the glassfiber membrane is coated by a mycophenolic acid specific-antibody conjugate, and the mass concentration of the mycophenolic acid specific-antibody conjugate is 0.1-0.3%, and the coating concentration of the mycophenolic acid specific-antibody conjugate is 0.5-1 mg/mL. In the present invention, the conjugate in the mycophenolic acid specific-antibody conjugate is preferably a colloidal gold particle or fluorescent microsphere; in the present invention, grain size of the colloidal gold particle is preferably 10-50nm, and grain size of the fluorescent microsphere is preferably 100-200nm; there is no special limitation to the source of the fluorescent microsphere in the present invention, and the conventional commercial fluorescent microsphere in this field is available.

[0024] In the present invention, the glassfiber membrane is treated by a glassfiber membrane treatment fluid. Preferably, the glassfiber membrane treatment fluid includes a basal solution and the following components at concentrations below: 0.5-5 g/L polyethylene glycol 6000, 3-15 g/L mannitol, 0.1-1.5 g/L alum and 0.8%-1.0% sodium chloride; the basal solution is selected from one or more of PBS buffer solution, TRIS buffer solution and glycine buffer solution; the basal solution is preferably selected from one or more of PBS buffer solution, TRIS buffer solution and glycine buffer solution. In the present invention, when the marked conjugate is a fluorescent microsphere, preferably, TRIS buffer solution is served as the basal solution of the glassfiber membrane treatment fluid, the concentration of the TRIS buffer solution is preferably 40-60 mmol/L, more preferably 50 mmol/L; the glassfiber membrane treatment fluid more preferably includes the following components: 3 g/L polyethylene glycol, 1.2 g/L alum, 4 g/L mannitol and 0.9% sodium chloride. In the present invention, when the marked conjugate is a colloidal gold particle, boric acid buffer solution is served as the basal solution of the glassfiber membrane treatment fluid, the concentration of the boric acid buffer solution is preferably 15-25 mmol/L, more preferably 20 mmol/L; the glassfiber membrane treatment fluid more preferably includes the following components at concentrations below: 0.5 g/L polyethylene glycol, 0.2 g/L alum, 4 g/L mannitol and 0.9% sodium chloride.

[0025] In a specific implementation process of the present invention, the glassfiber membrane is dried after being soaked in the glassfiber membrane treatment fluid; the soaking time is preferably 12-17 min, more preferably 15 min; the drying temperature is preferably 63-68°C and the drying time is preferably 2h. In the present invention, the nitrocellulose membrane is provided with a detection line and quality control line, a mycophenolic acid protein conjugate is sprayed on the detection line, the concentration of the mycophenolic acid protein conjugate is 1-3 mg/ml, and the spraying amount is 1-3 μL/cm. In the present invention, the mycophenolic acid protein conjugate is preferably mycophenolic acid-coupled bovine serum albumin or mycophenolic acid-coupled ovalbumin; preferably, goat-anti-mouse IgG is sprayed on the quality control line, and the concentration of the goat-anti-mouse IgG is preferably 1-3 mg/ml, and the spraying amount is 1-3 μL/cm.

[0026] The present invention further provides a preparation method of the test strip, including the following steps of: 1) treating the sample pad by the sample pad treatment fluid to obtain a treated sample pad; 2) mixing mycophenolic acid monoclonal antibodies and the conjugate for coupling and sealing to obtain a mycophenolic acid specific-antibody conjugate; spraying the mycophenolic acid specific-antibody conjugate on the treated glassfiber membrane to obtain the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate; 3) spraying the mycophenolic acid protein conjugate on the detection line of the nitrocellulose membrane and spraying goat-anti-mouse IgG on the quality control line to obtain a nitrocellulose membrane sprayed with the detection line and quality control line; 4) successively lapping and pasting the treated sample pad, the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate, the nitrocellulose membrane provided with the detection line and quality control line as well as the absorbent paper on the surface of the bottom plate to obtain the test strip; there is no limitation on an execution sequence of steps 1), 2) and 3).

[0027] In the present invention, the sample pad is treated by the sample pad treatment fluid to obtain the treated sample pad. In the present invention, the sample pad treatment is preferably to soak the sample pad into the sample pad treatment fluid for 0.5h and then put it into an oven for drying for 2h at 65°C.

[0028] Preferably, mycophenolic acid monoclonal antibodies are mixed with the conjugate for coupling and sealing to obtain the mycophenolic acid specific-antibody conjugate. The mycophenolic acid monoclonal antibodies are preferably purchased from Beijing Deaoping Biotech Co., Ltd. with the item number of ATDMMPA-01. In the present invention, when the conjugate is a fluorescent microsphere, the mycophenolic acid monoclonal antibodies, EDC and fluorescent microsphere solution are mixed, and in the coupling system of the present invention, the concentration of the mycophenolic acid monoclonal antibodies is preferably 100-500 μg/mL, more preferably 200-400 μg/mL; the concentration of the fluorescent microsphere is preferably 0.5%-1.5%, more preferably 1%; the concentration of the EDC is preferably 8-12 mg/mL, more preferably 10 mg/mL; in the present invention, 20 mmol/L phosphate buffer is served as a solvent of the coupling system. The coupling time is preferably 2.5-3.5h, more preferably 3.0h. In the present invention, preferably, the coupling system is centrifuged after coupling and before sealing to remove supernatant; there is no special limitation to the centrifugal speed and time in the present invention as long as solid can be separated from liquid available. In the present invention, sealing is preferably conducted after the centrifugation. In the present invention, the sealing is preferably conducted by bovine serum albumin solution, and the sealing time is preferably 50-70 min, more preferably 60 min. In the present invention, the mass concentration of the bovine serum albumin solution is preferably 0.8%-1.2%, more preferably 1.0%.

[0029] In the present invention, when the conjugate is a colloidal gold particle, grain size of the colloidal gold particle is preferably 10-50nm; in the present invention, the colloidal gold particle is preferably a commercial product or homemade product, when the colloidal gold particle is a homemade product, it is preferably prepared by a conventional reduction method of trisodium citrate. In the present invention, when the colloidal gold particle is served as the conjugate for coupling, preferably, the colloidal gold solution is mixed with the mycophenolic acid monoclonal antibodies well, no EDC is added and a physical absorption principle is taken; the coupling time is preferably 2.5-3.5h, more preferably 3.0h; In the present invention, preferably, the coupling system is centrifuged after coupling and before sealing to remove supernatant; there is no special limitation to the centrifugal speed and time in the present invention as long as solid can be separated from liquid available. After the mycophenolic acid specific-antibody conjugate is prepared in the present invention, the mycophenolic acid specific-antibody conjugate is sprayed on the treated glassfiber membrane to obtain the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate. In the present invention, preferably, the mycophenolic acid protein conjugate is sprayed after mixed with a spraying buffer solution; components of the spraying buffer solution are preferably the same as those of the glassfiber membrane treatment fluid. In the present invention, the concentration of the mycophenolic acid specific-antibody conjugate in the spraying liquor is 0.1 %-0.3%, more preferably 0.2%. In the present invention, the spraying is preferably conducted by a gold dispenser system, at the end of the spraying, drying is conducted preferably, the drying temperature is preferably 45-65°C, the drying time is preferably 2-6h, and the drying is preferably conducted in an air dry oven.

[0030] In the present invention, preferably, mycophenolic acid protein conjugate is sprayed on the detection line of the nitrocellulose membrane and goat-anti-mouse IgG is sprayed on the quality control line to obtain the nitrocellulose membrane sprayed with detection line and quality control line. In the present invention, the mycophenolic acid bovine serum albumin is preferably mycophenolic acid-coupled bovine serum albumin or mycophenolic acid-coupled ovalbumin. In the present invention, the spraying concentration of the mycophenolic acid protein conjugate is 1-3 mg/mL, the spraying amount of the mycophenolic acid protein conjugate is 0.5-1 mg/mL, the spraying concentration of the goat-anti-mouse IgG is preferably 1-3 mg/mL, and the spraying amount of the goat-anti-mouse IgG is 0.5-1 mg/mL. In the present invention, at the end of the spraying, drying is conducted preferably, the drying temperature is preferably 45-65°C, the drying time is preferably 2-6h, and the drying is preferably conducted in an air dry oven.

[0031] In the present invention, after preparing the treated sample pad, the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate, the glassfiber membrane provided with the detection line and quality control line, the treated sample pad, the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate, the glassfiber membrane provided with the detection line and quality control line as well as the absorbent paper are successively lapped and pasted on the surface of the bottom plate to obtain the test strip. In the present invention, when the test strip is used to test whole blood, a blood filtering membrane is added between the sample pad and the dried nitrocellulose membrane, the blood filtering membrane is a commercial product, namely, the sample pad, the blood filtering membrane, the dried glassfiber membrane, the dried nitrocellulose membrane and the absorbent paper are successively pasted on the surface of the bottom plate to obtain the test strip.

[0032] The present invention preferably further includes a trimming step of the test strip, there is no special limitation to the trimming step in the present invention, and a conventional trimming method in this field is available. In the present invention, after preparation, the trimmed test strip is preferably put into a reagent card, drying agent is added and sealed by an aluminium foil bag to obtain a final test strip.

[0033] The present invention further provides a kit including the test strip, further including a mycophenolic acid quality control (QC) substance. In the present invention, the mycophenolic acid QC substance is preferably obtained by mixing and lyophilizing pure mycophenolic acid and a lyophilized buffer solution. In the present invention, the lyophilized buffer solution preferably includes a basal solution and the following components at concentrations below: 2-6 g/L polyethylene

glycol, 3-8 g/L trehalose, 5-20 g/L mannitol, 1wt% polyvinylpyrrolidone; the basal solution is selected from one or more of TRIS buffer solution, phosphoric acid buffer solution, boric acid buffer solution and phosphate buffer solution; pH of the basal solution is preferably 7.6-8.6; the solute concentration of the basal solution is preferably 50-200 mmol/L. In the present invention, the mycophenolic acid QC substance preferably includes several mycophenolic acid QC substances at different concentrations. The test strip is applied to test mycophenolic acid QC substances at different concentrations to obtain a curvilinear relationship between C/T values and concentrations of mycophenolic acid, thus quantitatively testing the content of mycophenolic acid in samples.

Embodiment 1

Preparation of a test strip:

[0034]

1) The sample pad was soaked into a sample pad treatment fluid for 0.5h and put into an oven for drying for 2h at 65°C;
2) Fluorescent microsphere (grain size=100nm) was taken; 1% fluorescent microsphere, 10 mg/mL EDC and 200 μg/mL mycophenolic acid monoclonal antibodies were added to 20 mmol/L phosphate buffer solution as a solvent for mixing well and coupling for 3h, centrifuged to remove the supernatant, then 1% BSA was added for sealing for 1h.
3) The prepared marked conjugate was centrifuged and resuspdended by a resuspending buffer solution (50mm TRIS buffer solution, 3 g/L polyethylene glycol, 1.2 g/L alum, 4 g/L mannitol and 0.9% sodium chloride) to concentration= 0.2%, and the glassfiber membrane was soaked by a pretreating buffer solution (its components and content are consistent with the resuspending buffer solution) and dried for 1h; a gold dispenser system was used to spray the resuspended solution on the glassfiber membrane, and then it was dried by an air dry oven for 2h at 65°C;
4) Line T represented mycophenolic acid-coupled BSA and the concentration was 1 mg/ml; line C represented goat-anti-mouse IgG and the concentration was 1 mg/ml; the conjugate and quality control line are respectively dispersed on line T and line C of the nitrocellulose membrane, and then it was dried by an air dry oven for 2h at 65°C;
5) The sample pad, blood filtering membrane, dried glassfiber membrane, dried nitrocellulose membrane and absorbent paper were successively pasted on the bottom plate; a big reagent board was trimmed and loaded into a reagent card, was added a drying agent and sealed by an aluminium foil bag to obtain the test strip;

[0035] The blood filtering membrane is used for measuring whole blood, not for the measurement of serum in normal condition, and it is a commercial product.

Preparation of a calibration curve:

[0036] Mycophenolic acid calibration products at concentrations of 0, 1.25, 2.5, 5, 10, 20 μg/mL were dropwise added to a reagent card and mixed well for standing chromatography for 15 min, where 3 reagent cards were set for each concentration, the fluorescence signal value was read by an immune fluoroanalyzer, and T/C value was calculated to establish a calibration curve, and the results were shown in FIG. 1, where X-axis is the concentration of calibration products, Y-axis is T/C value. It can be seen from FIG. 1 that sam ples at different concentrations are tested within the whole linear range, test ing results CVs are less than 10%, indicating good repeatability and satisfying test requirements.

Test of sample repeatability:

[0037] Test samples were dropwise added to loading holes, 10 repetitions were set for each sample, the test samples were serum samples, due to the metabolism of mycophenolic acid, it failed to obtain natural high-value samples, and the high-value samples were only obtained by adding pure mycophenolic acid to clinical serum samples. Specific data was shown in table 1.

Table 1 Test results of sample repeatability

Sample No.	Sample concentration (μg/mL)										Mean value	Standard deviation	cv
	Measurement 1	Measurement 2	Measurement 3	Measurement 4	Measurement 5	Measurement 6	Measurement 7	Measurement 8	Measurement 9	Measurement 10			
1	1.35	1.42	1.27	1.28	1.21	1.41	1.35	1.36	1.56	1.25	1.346	0.10 1893	7.57 %
2	1.47	1.32	1.23	1.45	1.21	1.58	1.23	1.33	1.42	1.33	1.357	0.12 1019	8.92 %
3	2.23	2.57	2.12	2.63	2.53	2.47	2.65	2.78	2.55	2.43	2.496	0.19 6932	7.89 %
4	3.23	3.45	3.67	3.21	3.55	3.63	3.72	3.81	3.65	3.24	3.516	0.22 1068	6.29 %
5	4.11	4.26	4.38	4.57	4.81	4.21	4.35	4.27	4.71	4.32	4.399	0.22 5854	5.13 %
6	5.05	5.21	5.03	5.47	5.28	5.33	5.64	5.28	5.31	5.42	5.302	0.18 3836	3.47 %
7	7.23	7.53	7.28	7.4	7.21	7.22	7.83	7.08	7.73	7.09	7.36	0.25 9272	3.52 %
8	9.67	9.48	10.02	9.58	9.77	9.12	10.35	10.67	10.58	9.99	9.923	0.49 7997	5.02 %
9	15.33	13.77	14.32	15.03	14.68	13.97	14.25	15.97	14.31	15.08	14.671	0.67 9206	4.63 %
10	18.21	19.22	19.98	17.23	19.32	18.25	19.32	19.25	18.36	18.71	18.785	0.79 0713	4.21 %

Embodiment 2

Preparation of a test strip:

[0038]

1) 100mL of 0.01% HAuCl4 aqueous solution was taken, boiled and rapidly added to 0.75mL of 1% trisodium citrate aqueous solution, and then continuously boiled for about 5 min till orange red appeared, 50nm colloidal gold particles were prepared at this time.
2) Colloidal gold solution and 200 μg/mL of mycophenolic acid monoclonal antibodies were mixed well and coupled for 3h, then centrifuged to remove the supernatant, 1% BSA was added for sealing for 1h.
3) The prepared marked conjugate was centrifuged and resuspdended by a resuspending buffer solution (20mm boric acid buffer solution, 0.5 g/L polyethylene glycol, 0.2 g/L alum, 4 g/L mannitol and 0.9% sodium chloride), a gold pad was soaked by a pretreating buffer solution (its components and content are consistent with the resuspending buffer solution) and dried for 2h; a gold dispenser system was used to spray the resuspended solution on the gold pad, and then it was dried by an air dry oven for 6h at 45°C;
4) Line T on the nitrocellulose membrane represented mycophenolic acid-coupled BSA and the concentration was 1 mg/ml; line C represented goat-anti-mouse IgG and the concentration was 1 mg/ml; the mycophenolic acid-coupled BSA and goat-anti-mouse IgG are respectively dispersed on line T and line C of the nitrocellulose membrane, and then it was dried by an air dry oven for 6h at 45°C;
5) The sample pad (treated the same as embodiment 1), dried glassfiber membrane, blood filtering membrane, dried nitrocellulose membrane and absorbent paper were successively pasted on the bottom plate; a big reagent board was trimmed and loaded into a reagent card, was added a drying agent and sealed by an aluminium foil bag to obtain the test strip;

[0039] Preparation of a calibration curve was the same as embodiment 1.

Test of anti-interference capacity

[0040] Conventional blood was added to test samples for testing (50 mg/mL hemoglobin, 10 mmol/L triglyceride) the values obtained before and after adding interfering substances, and moreover, conventional clinical interfering substances were observed whether influencing the measured results.

Table 2 Test results of anti-interference capacity

Sample No.	Before adding interfering substances				After adding interfering subsstances				Relative deviation
	Measurement 1	Measurement 2	Measurement 3	Mean value	Measurement 1	Measurement 2	Measurement 3	Mean value	
1	1.45	1.32	1.36	1.376667	1.38	1.46	1.41	1.416667	2.91%
2	1.67	1.73	1.82	1.74	1.65	1.72	1.88	1.75	0.57%
3	1.37	1.54	1.42	1.443333	1.36	1.51	1.42	1.43	-0.92%
4	1.89	1.82	1.67	1.793333	1.76	1.62	1.81	1.73	-3.53%

[0041] It can be seen from table 2 that the relative deviation before and after adding interfering substances is less than 5% within the acceptable range.

Embodiment 3

[0042] The test strip in embodiment 1 was applied to test whole blood samples and serum samples. Contrast of the test results between whole blood samples and serum samples:
The selected whole blood samples and serum samples were obtained from the same source, by contrasting the test results of the both two, the test reliability of the reagent to whole blood samples can be judged.

Table 3 Contrast of the test results between whole blood samples and serum samples:

Sample No.	Measured value of serum				Measured value of whole blood				
	Measurement 1	Measurement 2	Measurement 3	Mean value	Measurement 1	Measurement 2	Measurement 3	Mean value	Relative deviation
1	1.45	1.32	1.36	1.376666667	1.36	1.35	1.51	1.406667	2.18%
2	1.67	1.73	1.82	1.74	1.65	1.83	1.72	1.733333	-0.38%
3	1.37	1.54	1.42	1.443333333	1.41	1.43	1.5	1.446667	0.23%
4	1.89	1.82	1.67	1.793333333	1.84	1.76	1.72	1.773333	-1.12%
5	0.58	0.61	0.68	0.623333333	0.58	0.67	0.65	0.633333	1.60%
6	0.74	0.65	0.72	0.703333333	0.68	0.78	0.72	0.726667	3.32%
7	1.05	1.03	1.02	1.033333333	1.05	0.99	1.03	1.023333	-0.97%
8	1.13	1.24	1.15	1.173333333	1.07	1.24	1.32	1.21	3.13%
9	2.32	2.24	2.36	2.306666667	2.31	2.27	2.41	2.33	1.01%
10	1.32	1.41	1.37	1.366666667	1.35	1.32	1.34	1.336667	-2.20%

[0043] It can be seen from the above table that the deviation between whole blood samples and serum samples is within 5%, which is acceptable.

Contrast of stability:

[0044] The shelf life of the test strip prepared in the present invention is 18 months at 2-30°C, and the shelf life of the contrast reagent, namely, the mycophenolic acid kit (enzyme multiplied immunoassay technique) produced by Siemens Medical Diagnosis Products (Shanghai) Co., Ltd. is 18 months at 2-8°C. In the aspect of storage temperature, the test strip of the present invention is superior to the contrast reagent.

[0045] Contrast of linear range: the linear range of this experiment reagent is 0-20 μg/ml, the linear range of the contrast reagent, namely, the mycophenolic acid kit (enzyme multiplied immunoassay technique) produced by Siemens Medical Diagnosis Products (Shanghai) Co., Ltd. is 0-15 μg/ml, and THE linear range of the contrast reagent, namely, the mycophenolic acid homogeneous enzyme immunoassay reagent produced by EVERMED is 0-16 μg/ml. In the aspect of linear range, the test strip of the present invention is wider than the contrast reagent.

[0046] In the aspect of clinic correlation: test samples of the test strip provided by the present invention include serum, plasma and whole blood, while the test samples of the contrast reagent exclude whole blood. Due to the existence of hemocyte in whole blood, whole blood cannot be loaded on the machine. The contrast reagent is the mycophenolic acid kit (enzyme multiplied immunoassay technique) produced by Siemens Medical Diagnosis Products Co., Ltd. Contrast results of the clinic correlation are shown in table 4 and FIG. 1, and it can be seen that the clinic correlation is more than 0.9, conforming to clinical needs.

Table 4 Contrast results of the clinic correlation (unit: μg/mL)

Sample No.	Test strip in embodiment 1	Contrast reagent
1	0.31	0.41

(continued)

Sample No.	Test strip in embodiment 1	Contrast reagent
2	11.35	11.12
3	5.31	5.28
4	3.42	3.27
5	2.54	2.36
6	4.35	4.21
7	0.72	0.75
8	1.24	1.31
9	1.33	1.28
10	2.53	2.36
11	2.42	2.31
12	5.36	5.28
13	10.25	10.11
14	7.35	7.21
15	15.24	15.11
16	16.25	13
17	7.25	6.83
18	1.56	1.32
19	1.47	1.58
20	12.11	11.67
21	1.45	1.33
22	3.24	3.54
23	6.31	6.52
24	10.26	9.47
25	2.57	2.61
26	3.1	2.91
27	3.11	3.01
28	14.32	13.99
29	8.67	8.21
30	9.22	9.01

[0047] It can be seen from the above embodiments that the test strip provided by the present invention can rapidly test the content of mycophenolic acid in blood samples, and the testing instrument has low cost, simple and rapid operation; the test strip has good stability and can be stored at room temperature for transportation with 18 months of shelf life at 2-30°C; meanwhile test samples are extensive, including serum samples, plasma samples and whole blood samples.

Claims

1. A test strip for testing the content of mycophenolic acid, comprising a bottom plate and a sample pad, a glassfiber membrane, a nitrocellulose membrane and an absorbent paper which are successively lapped on a surface of the bottom plate, wherein

the sample pad is treated by a sample pad treatment fluid, and the sample pad treatment fluid comprises a buffer solution, an active protein and a surfactant; the active protein is selected from one or more of bovine serum albumin, casein and ovalbumin;
the glassfiber membrane is treated by a glassfiber membrane treatment fluid, and the glassfiber membrane is coated by a mycophenolic acid specific-antibody conjugate; and the mass concentration of the mycophenolic acid specific-antibody conjugate is 0.1-0.3%, and the coating concentration of the mycophenolic acid specific-antibody conjugate is 0.5-1 mg/mL;
the nitrocellulose membrane is provided with a detection line and quality control line, a mycophenolic acid protein conjugate is sprayed on the detection line, the concentration of the mycophenolic acid protein conjugate is 1-3 mg/ml, and the spraying amount is 1-3 μL/cm.

2. The test strip according to claim 1, wherein the buffer solution in the sample pad treatment fluid is selected from one or more of PBS buffer solution, TRIS buffer solution and glycine buffer solution; the surfactant in the sample pad treatment fluid is selected from one or more of Tween 20, Tween 80 and Triton X-100.

3. The test strip according to claim 1, wherein the conjugate in the mycophenolic acid specific-antibody conjugate is a colloidal gold particle or a fluorescent microsphere.

4. The test strip according to claim 1, wherein the glassfiber membrane treatment fluid comprises a basal solution and the following components with the following concentrations: 0.5-5 g/L polyethylene glycol 6000, 3-15 g/L mannitol, 0.1-1.5 g/L alum and 0.8%-1.0% sodium chloride; the basal solution is selected from one or more of PBS buffer solution, TRIS buffer solution and glycine buffer solution.

5. A preparation method of the test strip according to any one of claim 1-4, comprising the following steps of:

1) treating the sample pad by the sample pad treatment fluid to obtain a treated sample pad;
2) mixing mycophenolic acid monoclonal antibodies and the conjugate for coupling and sealing to obtain a mycophenolic acid specific-antibody conjugate; spraying the mycophenolic acid specific-antibody conjugate on the treated glassfiber membrane to obtain the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate;
3) spraying the mycophenolic acid protein conjugate on the detection line of the nitrocellulose membrane and spraying goat-anti-mouse IgG on the quality control line to obtain a nitrocellulose membrane sprayed with the detection line and quality control line;
4) successively lapping and pasting the treated sample pad, the glassfiber membrane coated by the mycophenolic acid specific-antibody conjugate, the nitrocellulose membrane provided with the detection line and quality control line as well as the absorbent paper on the surface of the bottom plate to obtain the test strip;

wherein, there is no limitation on an execution sequence of steps 1), 2) and 3).

6. The preparation method according to claim 5, wherein the concentration of mycophenolic acid monoclonal antibodies of the coupling system in step 2) is 100-500 μg/mL; and the coupling time is 2.5-3.5h.

7. The preparation method according to claim 5, wherein the mycophenolic acid protein conjugate is sprayed after mixed with a spraying buffer solution; components of the spraying buffer solution are the same as those of the glassfiber membrane treatment fluid.

8. A kit comprising the test strip according to any one of claim 1-5, wherein the kit further comprises a mycophenolic acid QC substance.

9. The kit according to claim 8, wherein the mycophenolic acid QC substance is obtained by mixing and lyophilizing pure mycophenolic acid and a lyophilized buffer solution.

10. The kit according to claim 9, wherein the lyophilized buffer solution comprises a basal solution and the following components with the following concentrations: 2-6 g/L polyethylene glycol, 3-8 g/L trehalose, 5-20 g/L mannitol, 1wt% polyvinylpyrrolidone; the basal solution is selected from one or more of TRIS buffer solution, phosphoric acid buffer solution, boric acid buffer solution and phosphate buffer solution; pH of the basal solution is 7.6-8.6; the solute concentration of the basal solution is 50-200 mmol/L.

Patentansprüche

1. Teststreifen zum Prüfen des Gehalts an Mycophenolsäure, umfassend eine Bodenplatte und ein Proben-Pad, eine Glasfasermembran, eine Nitrozellulosemembran und ein Saugpapier, die nacheinander auf einer Fläche der Bodenplatte übereinandergelegt sind, wobei das Proben-Pad mit einem Proben-Pad-Behandlungsfluid behandelt ist und das Proben-Pad-Behandlungsfluid eine Pufferlösung, ein aktives Protein und ein Netzmittel umfasst; das aktive Protein gewählt ist aus einem oder mehreren von Rinderserumalbumin, Kasein und Eieralbumin; die Glasfasermembran mit einem Glasfasermembran-Behandlungsfluid behandelt ist und die Glasfasermembran mit einem Mycophenolsäure-spezifischen Antikörper-Konjugat beschichtet ist; und die Massenkonzentration des Mycophenolsäure-spezifischen Antikörper-Konjugats 0,1-0,3 % beträgt und die Beschichtungskonzentration des Mycophenolsäure-spezifischen Antikörper-Konjugats 0,5-1 mg/ml beträgt;
   die Nitrozellulosemembran mit einer Nachweislinie und einer Qualitätskontrolllinie versehen ist, ein Mycophenolsäure-Protein-Konjugat auf die Nachweislinie gesprüht ist, die Konzentration des Mycophenolsäure-Protein-Konjugats 1-3 mg/ml beträgt und die Sprühmenge 1-3 μl/cm beträgt.

2. Teststreifen nach Anspruch 1, wobei die Pufferlösung in dem Proben-Pad-Behandlungsfluid gewählt ist aus einer oder mehreren aus PBS-Pufferlösung, TRIS-Pufferlösung und Glycin-Pufferlösung; das Netzmittel im Proben-Pad-Behandlungsfluid gewählt ist aus einem oder mehreren aus Tween 20, Tween 80 und Triton X-100.

3. Teststreifen nach Anspruch 1, wobei das Konjugat in dem Mycophenolsäure-spezifischen Antikörper-Konjugat ein kolloidales Goldpartikel oder eine fluoreszierende Mikrosphäre ist.

4. Teststreifen nach Anspruch 1, wobei das Glasfasermembran-Behandlungsfluid eine basale Lösung und die folgenden Bestandteile mit den folgenden Konzentrationen umfasst: 0,5-5 g/l Polyethylenglycol 6000, 3-15 g/l Mannitol, 0,1-1,5 g/l Alaun und 0,8 %-1,0 % Natriumchlorid; wobei die basale Lösung gewählt ist aus einer oder mehreren aus PBS-Pufferlösung, TRIS-Pufferlösung und Glycin-Pufferlösung.

5. Zubereitungsverfahren des Teststreifens gemäß einem beliebigen der Ansprüche 1 bis 4, umfassend die folgenden Schritte des:

   1) Behandelns des Proben-Pads mit dem Proben-Pad-Behandlungsfluid, um ein behandeltes Proben-Pad zu erhalten;
   2) Mischens von monoklonalen Mycophenolsäure-Antikörpern und des Konjugats zum Koppeln und Versiegeln, um ein Mycophenolsäurespezifisches Antikörper-Konjugat zu erhalten; Sprühens des Mycophenolsäure-spezifischen Antikörper-Konjugats auf die behandelte Glasfasermembran, um die mit dem Mycophenolsäure-spezifischen Antikörper-Konjugat beschichtete Glasfasermembran zu erhalten;
   3) Sprühens des Mycophenolsäure-Protein-Konjugats auf die Nachweislinie der Nitrozellulosemembran und Sprühens von Ziege-Anti-Maus-IgG auf die Qualitätskontrolllinie, um eine mit der Nachweislinie und der Qualitätskontrolllinie besprühte Nitrozellulosemembran zu erhalten;
   4) nacheinander Übereinanderlegens und Verklebens des behandelten Proben-Pads, der mit dem Mycophenolsäure-spezifischen Antikörper-Konjugat beschichteten Glasfasermembran, der mit der Nachweislinie und der Qualitätskontrolllinie versehenen Nitrozellulosemembran sowie des Saugpapiers auf der Fläche der Bodenplatte, um den Teststreifen zu erhalten;

   wobei keine Einschränkung hinsichtlich einer Ausführungsfolge der Schritte 1), 2) und 3) besteht.

6. Zubereitungsverfahren nach Anspruch 5, wobei die Konzentration der monoklonalen Mycophenolsäure-Antikörper des Koppelsystems in Schritt 2) 100-500 μg/ml beträgt; und die Koppelzeit 2,5-3,5 h beträgt.

7. Zubereitungsverfahren nach Anspruch 5, wobei das Mycophenolsäure-Protein-Konjugat nach einem Mischen mit einer Sprüh-Pufferlösung gesprüht wird; Bestandteile der Sprüh-Pufferlösung dieselben sind wie diejenigen des Glasfasermembran-Behandlungsfluids.

8. Ausstattungssatz, umfassend den Teststreifen gemäß einem beliebigen der Ansprüche 1 bis 5, wobei der Satz weiter eine Mycophenolsäure-Qualitätskontrollsubstanz umfasst.

9. Satz nach Anspruch 8, wobei die Mycophenolsäure-Qualitätskontrollsubstanz erhalten ist durch ein Mischen und Lyophilisieren reiner Mycophenolsäure und einer lyophilisierten Pufferlösung.

10. Satz nach Anspruch 9, wobei die lyophilisierte Pufferlösung eine basale Lösung und die folgenden Bestandteile mit den folgenden Konzentrationen umfasst: 2-6 g/l Polyethylenglycol, 3-8 g/l Trehalose, 5-20 g/l Mannitol, 1 Gew-% Polyvinylpyrrolidon; die basale Lösung gewählt ist aus einer oder mehreren aus TRIS-Pufferlösung, Phosphorsäure-Pufferlösung, Borsäure-Pufferlösung und Phosphat-Pufferlösung; der pH-Wert der basalen Lösung 7,6-8,6 beträgt; die Lösungskonzentration der basalen Lösung 50-200 mmol/l beträgt.

Revendications

1. Bande de test destinée à tester la teneur en acide mycophénolique, comprenant une plaque inférieure et un support d'échantillon, une membrane en fibres de verre, une membrane de nitrocellulose et un papier absorbant qui sont superposés successivement sur une surface de la plaque inférieure, dans laquelle
le support d'échantillon est traité par un fluide de traitement de support d'échantillon, et le fluide de traitement de support d'échantillon comprend une solution tampon, une protéine active et un tensioactif ; la protéine active est choisie parmi une ou plusieurs parmi l'albumine de sérum bovin, la caséine et l'ovalbumine ;
la membrane en fibres de verre est traitée par un fluide de traitement de membrane en fibres de verre, et la membrane en fibres de verre est tapissée avec un conjugué d'anticorps spécifique de l'acide mycophénolique ; et la concentration en masse du conjugué d'anticorps spécifique de l'acide mycophénolique est de 0,1-0,3 %, et la concentration d'application du conjugué d'anticorps spécifique de l'acide mycophénolique est de 0,5-1 mg/ml ;
la membrane de nitrocellulose est dotée d'une ligne de détection et d'une ligne de contrôle de qualité, un conjugué protéine-acide mycophénolique est pulvérisé sur la ligne de détection, la concentration du conjugué protéine-acide mycophénolique est de 1-3 mg/ml, et la quantité de pulvérisation est de 1-3 μl/cm.

2. Bande de test selon la revendication 1, dans laquelle la solution tampon dans le fluide de traitement de support d'échantillon est choisie parmi une ou plusieurs parmi une solution tampon PBS, une solution tampon TRIS et une solution tampon glycine ; le tensioactif dans le fluide de traitement de support d'échantillon est choisi parmi un ou plusieurs parmi le Tween 20, le Tween 80 et le Triton X-100.

3. Bande de test selon la revendication 1, dans laquelle le conjugué dans le conjugué d'anticorps spécifique de l'acide mycophénolique est une particule d'or colloïdal ou une microsphère fluorescente.

4. Bande de test selon la revendication 1, dans laquelle le fluide de traitement de la membrane en fibres de verre comprend une solution de base et les composants suivants aux concentrations suivantes : 0,5-5 g/l de polyéthylèneglycol 6000, 3-15 g/l de mannitol, 0,1-1,5 g/l d'alun et 0,8 %-1,0 % de chlorure de sodium ; la solution de base est choisie parmi une ou plusieurs parmi une solution tampon PBS, une solution tampon TRIS et une solution tampon glycine.

5. Procédé de préparation de la bande de test selon l'une quelconque des revendications 1-4, comprenant the étapes suivantes consistant à :

1) traiter le support d'échantillon par le fluide de traitement de support d'échantillon, pour obtenir un support d'échantillon traité ;
2) mélanger des anticorps monoclonaux anti-acide mycophénolique et le conjugué pour le couplage et la soudure afin d'obtenir un conjugué d'anticorps spécifique de l'acide mycophénolique ; pulvériser le conjugué d'anticorps spécifique de l'acide mycophénolique sur la membrane en fibres de verre traitée, afin d'obtenir la membrane en fibres de verre tapissée avec le conjugué d'anticorps spécifique de l'acide mycophénolique ;
3) pulvériser le conjugué protéine-acide mycophénolique sur la ligne de détection de la membrane de nitrocellulose et pulvériser de l'anticorps de chèvre anti-IgG de souris sur la ligne de contrôle de qualité afin d'obtenir une membrane de nitrocellulose sur laquelle ont été pulvérisées la ligne de détection et la ligne de contrôle de qualité ;
4) superposer successivement et faire adhérer le support d'échantillon traité, la membrane en fibres de verre tapissée avec le conjugué d'anticorps spécifique de l'acide mycophénolique, la membrane de nitrocellulose dotée de la ligne de détection et de la ligne de contrôle de qualité ainsi que le papier absorbant sur la surface de la plaque inférieure, pour obtenir la bande de test ;

dans lequel il n'y a pas de limitation à une séquence d'exécution des étapes 1), 2) et 3).

6. Procédé de préparation selon la revendication 5, dans lequel la concentration d'anticorps monoclonaux anti-acide

mycophénolique du système de couplage dans l'étape 2) est de 100-500 pg/ml ; et le temps de couplage est de 2,5-3,5 h.

7. Procédé de préparation selon la revendication 5, dans lequel le conjugué protéine-acide mycophénolique est pulvérisé après avoir été mélangé avec une solution tampon de pulvérisation ; les composants de la solution tampon de pulvérisation sont les mêmes que ceux du fluide de traitement de la membrane en fibres de verre.

8. Trousse comprenant la bande de test selon l'une quelconque des revendications 1-5, dans laquelle la trousse comprend en outre une substance pour le contrôle de qualité de l'acide mycophénolique.

9. Trousse selon la revendication 8, dans laquelle la substance pour le contrôle de qualité de l'acide mycophénolique est obtenue par mélange et lyophilisation d'acide mycophénolique pur et d'une solution tampon lyophilisée.

10. Trousse selon la revendication 9, dans laquelle la solution tampon lyophilisée comprend une solution de base et les composants suivants aux concentrations suivantes : 2-6 g/l de polyéthylèneglycol, 3-8 g/l de tréhalose, 5-20 g/l de mannitol, 1 % en poids de polyvinylpyrrolidone ; la solution de base est choisie parmi une ou plusieurs parmi une solution tampon TRIS, une solution tampon acide phosphorique, une solution tampon acide borique et une solution tampon phosphate ; le pH de la solution de base est 7,6-8,6 ; la concentration du soluté de la solution de base est de 50-200 mmoles/l.

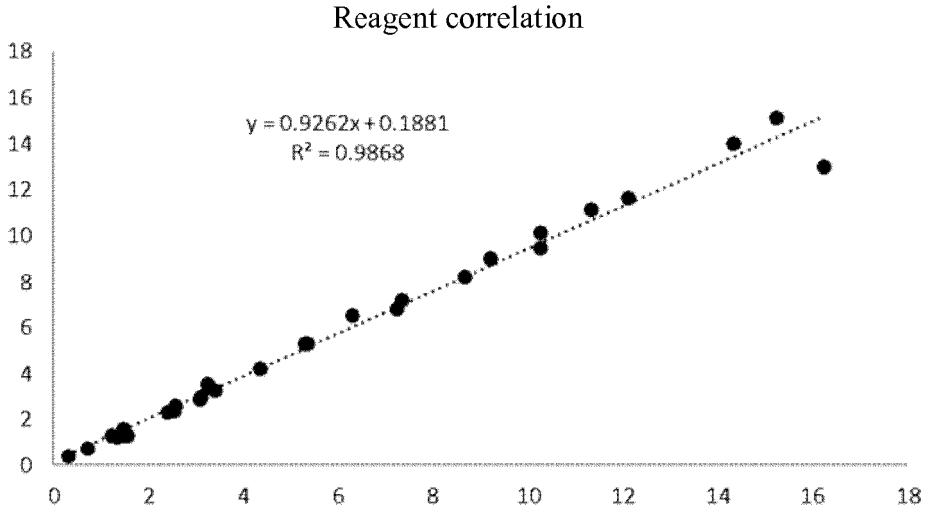
Reagent correlation
y = 0.9262x + 0.1881
R² = 0.9868
0
2
4
6
8
10
12
14
16
18

FIG. 1

REFERENCES CITED IN THE DESCRIPTION

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