

SPECIFICATION

A method for pretreating a subject fluid in a pregnancy test

- 5 The present invention relates to an improvement in the pretreatment of subject fluids for immunologic pregnancy tests. 5

Immunologic tests have been used for the diagnosis of pregnancy, which estimate the human chorionic gonadotropin (hereinafter briefly, HCG) in the urine or body fluid (serum, plasma, etc.) of a pregnant woman.

- The principle of the anti-HCG antibody-sensitized latex agglutination test (latex direct agglutination reaction, hereinafter briefly, LDAR) is that, as latex particles carrying anti-HCG antibody adsorbed thereon are admixed with a subject urine, serum or plasma sample, the latex particles are agglutinated if HCG is present in the sample, so that the detection of such agglutination establishes a diagnosis of pregnancy. On the other hand, the principle of the HCG-sensitized latex agglutination inhibition test (latex agglutination inhibition reaction; hereinafter briefly, LAIR), is that, as a latex carrying HCG adsorbed thereon is admixed with a given amount of anti-HCG antiserum, the latex particles are agglutinated, but if the latex is added after the admixture of an HCG-containing subject fluid with a given amount of anti-HCG antiserum, the latex will not be agglutinated because the anti-HCG antibody has already been coupled to the HCG in the subject fluid. Thus, in LAIR the absence of latex agglutination establishes a diagnosis of pregnancy. Another method is the haemagglutination inhibition test (hereinafter briefly, HAIR) in which a diagnosis of pregnancy is performed using HCG-sensitized human or animal red blood cells instead of latex particles. 10 15 20

- These immunologic pregnancy tests tend to give false results in early stages of pregnancy because the secretion of HCG in this period is so small that only a weak agglutination (or inhibition) reaction takes place. Moreover, sensitized latex and sensitized red blood cells may undergo non-specific reactions independently of the immunologic reaction of HCG with anti-HCG antibody. Therefore, when urine or serum containing a variety of components is assayed by the above methods, the agglutination (or inhibition) reaction is interfered with by components other than HCG in the sample, thereby leading to a false diagnosis. 25

The subject fluids may also be turbid and, if such fluids are directly assayed, false results may ensue. To prevent such false results, the fluids are usually filtered prior to the agglutination (inhibition) reaction.

- The filter materials conventionally employed are cellulosic filter paper, absorbent cotton, cellulose acetate, polyacrylonitrile, carboxymethylcellulose and other fibers but none of them is fully satisfactory. For instance, cellulosic filter paper and absorbent cotton are capable of removing the turbidity but have the disadvantage that HCG is also absorbed. Cellulose acetate and polyacrylonitrile fiber are not efficient enough thoroughly to remove interfering components. While fibrous carboxymethylcellulose is highly capable of removing the turbidity and interfering components, it entails the adsorption of HCG. 30

- With the above technical difficulties by way of background, we have unexpectedly found that the interfering components and the elements of turbidity present in the subject fluids can be specifically removed without entailing a substantial loss of HCG by contacting the subject fluids with a carboxylic acid-type cation-exchange resin fiber or a siliconized glass fiber. 35

We have sought to provide an improved method for pretreating the subject fluid.

- According to the present invention, we provide a method for pretreating a subject fluid for a pregnancy test by means of an immunological assay of human chorionic gonadotrophin, which comprises contacting the subject fluid with a carboxylic acid-type cation exchange resin fiber or a siliconized glass fiber. The carboxylic acid-type cation exchange resin fiber used in this invention preferably has a cation-exchange capacity of at least 1.5 meq/g and, especially, from 5 to 8 meq/g. For example, a preferred fiber may be prepared by reacting a polymer moulding containing at least 40 weight percent of acrylonitrile and hydrazine, or a homologue of hydrazine, to provide the polymer moulding with an anion-exchange capacity of from 0.01 to 3.0 meq/g and, then, hydrolysing the residual nitrile groups with alkali or mineral acid to introduce cation-exchanging groups into the moulding [cf. Japanese Patent Application As-Laid-Open No. 14389/1974 (Tokukai Sho 49-14389)]. 40 45

- The siliconized glass fiber may be prepared by treating an ordinary glass fiber with a compound of silicon or a silicon-containing composition such as, e.g., a silane coupling agent (organofunctional silane), a silicone oil, a silicone oil emulsion, silicone varnish or a silicone varnish emulsion. This siliconization treatment is generally carried out by impregnating a glass fiber with an aqueous or organic solvent solution of the compound of silicon or the silicon-containing composition, and heating the fiber (usually at from 100°C to 300°C). 50 55

The carboxylic acid-type cation-exchange resin fiber and siliconized glass fiber may be used alone or in various combinations. The carboxylic acid-type cation exchange resin fiber is advantageously used in the form of filament or staple fiber (preferably 0.1 to 2mm in length) with a denier number of from 2 to 15. The siliconized glass fiber is preferably used in the form of filament with a diameter of from 5 to 15 μ m.

- In contacting the subject fluid, such as the urine, serum or plasma of a woman, with the above-mentioned fiber, the fluid may be filtered through a layer of the fiber or, alternatively, the fluid may be admixed with the fiber and then separated (e.g., by filtration or centrifugal separation), although it is generally preferable to filter the subject fluid through the fibrous layer. A typical filtration procedure is as follows. A tube (preferably made from polyethylene) is packed with from 20 to 100 mg, most preferably 50 mg, of said fiber, and is connected to the lower end of a suction tube (preferably made from polyethylene) fitted with a rubber 60 65

suction cap. Then, the tube is dipped into the subject fluid and the rubber cap is squeezed to fill the suction tube with a suitable quantity (0.3 ml) of the test fluid to obtain a filtrate. The tube is disconnected and the filtrate is directly used for the pregnancy diagnostic test. For instance, two drops of the above filtrate are dripped onto a clean glass plate for LDAR or LAIR, of a HAIR test is carried out with 0.1 ml in a test tube.

- 5 The present invention provides for the removal of interfering components and turbidity elements without causing any substantial loss of HCG in the subject fluid, thus leading to remarkable improvements in sensitivity and reliability of the pregnancy test, especially LDAR. 5

The beneficial results of this invention will be further explained by the following tests and working examples.

- 10 The carboxylic acid-type cation-exchange resin fiber and siliconized glass fiber used in these tests and examples are as follows: 10

The carboxylic acid-type cation-exchange resin fiber was prepared by the procedure described in Example 1 of Japanese Patent Application As-Laid-Open No. 14389/1974. It has a triazine-or tetrazine-cross linked structure and a cation-exchange capacity of 6.4 meq/g, and has a pale pinkish appearance. In the 15 infrared region of the spectrum, this fiber showed a strong carbonyl band at 1600-1700 cm^{-1} but showed no nitrile absorption (2250 cm^{-1}). The resin fiber was used in the form of a filament of 6 denier except in Example 4 in which it was used in the form of staple fiber of 1 mm in length and of 3 denier. 15

The siliconized glass fiber was prepared as follows. A glass fiber with a diameter of about 10 μm was dewaxed with acetone and diethyl ether, immersed in a 1% solution of dimethylpolysiloxane in 20 trichloroethylene for one minute, heat-treated at 200°C for one hour and finally washed with 20% ethanol. 20

In the following tests and examples, LDAR and HAIR tests were respectively performed in conformity with the procedures described in the following literature:

LDAR test: J. Pharm. Soc. Japan, 98 376 (1978)

HAIR test: Acta Endocr. (Kbh), Suppl. 70(1962)

- 25 25

Test Example 1

Lower portions of polyethylene tubes with an inside diameter of 5 mm and a height of 45 mm were respectively packed with 50 mg of the above fibers according to this invention, and the filtration procedure described hereinbefore was followed to yield 0.3 ml of filtered urine or serum. As the cellulosic filter paper, 30 Toyo Filter No. 50 (dia. 5.5 cm, wt. 237 mg) was used. The paper was pursed and placed over a funnel and 1.5 ml of urine or serum were passed. 30

As regards subject fluids, 10 urine specimens, with especially high degrees of interference, were chosen from fluids of non-pregnant women. Five serum specimens from non-pregnant women were also employed. Each specimen was filtered as described hereinbefore and submitted to LDAR and HAIR tests. As 35 controls, unfiltered samples were also tested. The results are presented in Tables 1 and 2 appearing below. 35

The unfiltered urine specimens of non-pregnant women used in these tests showed overtly positive results in 5 out of 10 cases in LDAR, with the remaining 5 cases giving doubtfully positive results, i.e. intermediate between negative and positive reactions. In HAIR, one case was positive and 3 cases were doubtfully positive. The influence of components leading to false results is particularly evident in LDAR but 40 their incidence is reduced by filtration. The incidences of false results with various filter materials, when doubtfully positive results were added to positive results, were as follows. Cellulosic filter paper 5/10; absorbent cotton 5/10; wool 3/10; untreated glass fiber 3/10; viscose rayon 5/10; nylon 5/10; polyester 5/10; polypropylene 5/10; polyvinyl chloride 5/10; polyacrylonitrile 3/10; polyurethane 4/10; cellulose acetate 2/10; and carboxymethylcellulose 1/10. 40

45 When the carboxylic acid-type cation-exchange resin fiber or siliconized glass fiber was employed, all cases were found to be negative. In HAIR runs using filtered urine, all cases were found to be negative. The results of LDAR runs on non-pregnant sera, filtered through different filters, are shown in Table 3. Without filtration, the incidence of false results was 2/5 but with filtered sera all specimens were found to be negative, irrespective of the filter materials employed. 45

50 As mentioned above, when tests are carried out on unfiltered urine or serum, specimens which ought to be negative tend to give positive reactions, thus leading to misdiagnoses. Moreover, even with cellulosic filter paper, absorbent cotton, untreated glass, viscose rayon, nylon, polyester, polypropylene, polyvinyl chloride, polyacrylonitrile, polyurethane, cellulose acetate or carboxymethylcellulose fibers, for example, specimens which ought to be negative sometimes produce positive results, thus leading again to 55 misdiagnoses. 55

LDAR tests were performed on 10 urine samples from women in early stages of pregnancy which were lean in HCG. The results are shown in Table 4 below. When the urine samples were not filtered, all cases were found to be positive. However, when specimens were filtered through cellulosic filter paper or absorbent cotton, the reaction was considerably weakened so that pregnant women who ought to give 60 positive results were falsely found to be non-pregnant. With viscose rayon and carboxymethylcellulose, the incidence of false results was 2/10. The reaction was invariably weak when wool, untreated glass fiber, nylon, polyester, polypropylene, polyvinyl chloride and polyurethane were employed, the incidence of false results being 1/10 in each case. In contrast, all cases were found to be positive with the carboxylic acid-type cation-exchange resin and siliconized glass fibers according to this invention as well as with polyacrylonitrile 65 and cellulose acetate. 65

It is necessary to filter subject fluids in order to remove turbidity and interfering components. However, depending on the kind of filter material used, the HCG in the test fluid is adsorbed on the filter to cause a misdiagnosis.

When the carboxylic acid-type cation-exchange resin or siliconized glass fiber is employed, substantially no HCG adsorption takes place. Moreover, the filters of this invention effectively remove the components which would interfere with the reactions. Thus, they reduce considerably the incidence of misdiagnosing non-pregnancy as pregnancy.

Test Example 2

HCG was added to 3 urine samples from healthy non-pregnant women to give an HCG concentration of one I.U./ml, and each sample was filtered as described hereinbefore. The residual HCG (%) in each filtered urine sample was measured by radioimmunoassay.

As shown in Table 5 below, cellulosic filter paper, absorbent cotton, wool, untreated glass fiber, viscose rayon, nylon, polyester, polypropylene, polyvinyl chloride, polyurethane and carboxymethylcellulose showed up to 65% adsorption of HCG. Substantially no adsorption of HCG occurred with carboxylic acid-type cation-exchange resin fiber, siliconized glass fiber, cellulose acetate or polyacrylonitrile fiber.

Example 1

Polyethylene tubes, 5.0 mm in inside diameter, were respectively packed with 50 mg of carboxylic acid-type cation-exchange resin fiber or siliconized glass fiber of the present invention, absorbent cotton, cellulose acetate fiber, polyacrylonitrile fiber and carboxymethylcellulose fiber, and 400 urine samples from non-pregnant women were filtered with the above tubes by the procedure described hereinbefore, to obtain 0.3 ml of filtered urine from each urine sample. Two drops of each filtered urine sample were subjected to the LDAR tests.

As shown in Table 6 below, for the urine samples filtered through absorbent cotton, 10 out of 400 samples showed positive reactions and 35 doubtfully positive reactions; with cellulose acetate, 7 positive and 8 doubtfully-positive; with polyacrylonitrile, 7 positive and 13 doubtfully positive. With carboxymethylcellulose, too, 1 showed a positive reaction and 2 doubtfully positive reactions. On the contrary, with the carboxylic acid-type cation-exchange resin fiber or siliconized glass fiber of the invention, all the samples (400 samples) gave negative results. In this manner, those components which possibly interfere with the diagnostic reaction can be removed to a very great extent by the method of the present invention.

Example 2

Urine samples from 200 women definitely diagnosed as pregnant by clinical diagnoses were subjected to the LDAR test after filtration under the same conditions as in Example 1 using the carboxylic acid-type cation-exchange resin fiber or siliconized glass fiber of the present invention or absorbent cotton, cellulose acetate fiber, polyacrylonitrile fiber or carboxymethylcellulose fiber.

As shown in Table 7 below, in the case of absorbent cotton, cellulose acetate, polyacrylonitrile and carboxymethylcellulose, 10, 3, 3 and 5 cases, respectively, were erroneously diagnosed as non-pregnant. When the carboxylic acid-type cation-exchange resin fiber was used as the filter material according to the invention, all the samples reacted positively in agreement with the results of the definite clinical diagnosis. Also, in the case where the siliconized glass fiber was used, all the cases showed positive results except for one case in which the reaction was doubtfully positive.

Example 3

Serum samples from 50 non-pregnant women and 50 pregnant women were filtered through the carboxylic acid-type cation-exchange resin fiber or siliconized glass fiber used as a filter material under the same conditions as in Example 1, and two drops of each filtered serum sample were subjected to the LDAR test. All the serum samples from non-pregnant women reacted negatively, while all the serum samples from pregnant women showed positive reactions.

Example 4

Urine samples from 50 non-pregnant women and 50 pregnant women were filtered through the carboxylic acid-type cation-exchange resin staple fiber of 1 mm in length and of 3 denier used as a filter material under the same conditions as in Example 1, and two drops of each filtered urine sample were subjected to the LDAR test. All the urine samples from non-pregnant women reacted negatively, while all the urine sample from pregnant women showed positive reactions.

TABLE 1

Comparison of different pretreatments of urine samples from non-pregnant women (LDAR)

5	Urine No.	1	2	3	4	5	6	7	8	9	10	5
	Pretreatment											
10	Without filtration	+	+	±	±	+	±	±	±	+	+	10
15	Filtration through Cellulosic filter paper	-	+	-	±	-	-	±	-	±	+	15
	Absorbent cotton	-	+	-	±	-	-	±	-	±	+	
	Wool	-	+	-	-	-	-	-	-	±	+	
20	Untreated glass fiber	-	+	-	-	-	-	-	-	±	+	20
	Viscose rayon	-	+	-	±	-	-	±	-	±	+	
25	Nylon	-	+	-	±	-	-	±	-	±	+	25
	Polyester	-	+	-	±	-	-	±	-	+	+	
	Polypropylene	-	+	-	±	-	-	±	-	+	+	
30	Polyvinyl chloride	-	±	-	±	-	-	±	-	+	+	30
	Polyacrylonitrile	-	±	-	-	-	-	-	-	±	+	
35	Polyurethane	-	+	-	-	-	-	±	-	±	+	35
	Cellulose acetate	-	-	-	-	-	-	-	-	±	+	
	Carboxymethylcellulose	-	-	-	-	-	-	-	-	-	+	
40	Carboxylic acid-type cation-exchange resin fiber	-	-	-	-	-	-	-	-	-	-	40
45	Siliconized glass fiber	-	-	-	-	-	-	-	-	-	-	45
50	Remarks: + Positive results - Negative results.	± Doubtfully-positive results										50
NB These symbols have the same meaning below.												

TABLE 3

Comparison of different pretreatments of serum samples from non-pregnant women (LDAR)

5	Serum No.						5
	Pretreatment	1	2	3	4	5	
10	Without filtration	-	±	±	-	+	10
	Filtration through						
	Cellulosic filter paper	-	-	-	-	-	
15	Absorbent cotton	-	-	-	-	-	15
	Wool	-	-	-	-	-	
20	Untreated glass fiber	-	-	-	-	-	20
	Viscose rayon	-	-	-	-	-	
	Nylon	-	-	-	-	-	
25	Polyester	-	-	-	-	-	25
	Polypropylene	-	-	-	-	-	
30	Polyvinyl chloride	-	-	-	-	-	30
	Polyacrylonitrile	-	-	-	-	-	
	Polyurethane	-	-	-	-	-	
35	Cellulose acetate	-	-	-	-	-	35
	Carboxymethylcellulose	-	-	-	-	-	
40	Carboxylic acid-type cation-exchange resin fiber	-	-	-	-	-	40
	Siliconized glass fiber	-	-	-	-	-	
45							45

Comparison of different pretreatments of urine samples from women in early stages of pregnancy

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TABLE 5

Residual HCG (%) in filtered urine samples

5	Urine No.	1	2	3	5
	Pretreatment				
10	Filtration through Cellulosic filter paper	47	45	50	10
	Absorbent cotton	41	35	40	
15	Wool	62	65	59	15
	Untreated glass fiber	55	60	60	
20	Viscose rayon	40	40	35	20
	Nylon	92	85	88	
	Polyester	79	80	80	
25	Polypropylene	85	80	75	25
	Polyvinyl chloride	90	88	88	
30	Polyacrylonitrile	100	95	97	30
	Polyurethane	95	86	90	
	Cellulose acetate	100	99	98	
35	Carboxymethylcellulose	56	60	65	35
	Carboxylic acid-type cation-exchange resin fiber	100	101	100	
40	Siliconized glass fiber	100	96	99	40

TABLE 6

Comparison of different pretreatments of urine samples from 400 non-pregnant women

5	Pretreatment	Diagnosis (Number of cases)			5
		Positive	Doubtfully-positive	Negative	
10					10
	Filtration through				
	Absorbent cotton	10	35	355	
15	Cellulose acetate	7	8	385	15
	Polyacrylonitrile	7	13	380	
20	Carboxymethyl-cellulose	1	2	397	20
	Carboxylic acid-type cation-exchange resin fiber	0	0	400	
25	Siliconized glass fiber	0	0	400	25
30					30

TABLE 7

Comparison of different pretreatments of urine samples for 200 pregnant women

40	Pretreatment	Diagnosis (Number of cases)			40
		Positive	Doubtfully positive	Negative	
45					45
	Filtration through				
	Absorbent cotton	181	9	10	
	Cellulose acetate	195	2	3	
50	Polyacrylonitrile	193	4	3	50
	Carboxymethyl-cellulose	189	6	5	
55	Carboxylic acid-type cation-exchange resin fiber	200	0	0	55
60	Siliconized glass fiber	199	1	0	60

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CLAIMS

1. A method for pretreating a subject fluid for a pregnancy test by means of an immunological assay of human chorionic gonadotropin, which comprises contacting the subject fluid with a carboxylic acid-type cation exchange resin fiber or a siliconized glass fiber. 5
2. A method according to claim 1, wherein the subject fluid is filtered through a layer of the carboxylic acid-type cation exchange resin fiber or the siliconized glass fiber.
3. A method according to claim 2, wherein the layer is of the carboxylic acid-type cation-exchange resin fiber having a cation exchange capacity of at least 1.5 meq/g.
- 10 4. A method according to claim 3, wherein the resin fiber is in the form of a filament or staple fiber with a denier number of from 2 to 15. 10
5. A method according to claim 2, wherein the layer is of the siliconized glass fiber in the form of filament with a diameter of 5 to 15 μm .
6. A method according to claim 2, wherein the amount of the fibrous layer is from 20 to 100 mg per 0.3 15 ml of the subject fluid. 15
7. A method according to claim 1, wherein the subject fluid is woman's urine or serum.
8. A method according to claim 1, substantially as herein described with reference to any of the specific examples.

ERRATUM

SPECIFICATION NO 2040045B

Front page Heading (73) Proprietor
after Kogyo delete KK,
insert Kabushiki Kaisha also known as
Takeda Chemical Industries Limited

THE PATENT OFFICE
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