

# Comparative study of immunological tests for pregnancy diagnosis

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**SYNOPSIS** The reliability of five commercially produced immunological pregnancy diagnosis methods has been investigated. The tests used were Pregnosticon (a tube test) and four slide tests, Hyland, Pregslide, Gravindex, and Planotest. In the series described, Planotest gave 0.5% false positives, Gravindex had 2.1%, Pregnosticon 2.6%, and Pregslide 4.7%, Hyland A (sensitivity 4,500 iu/l.) gave 3.6% and Hyland B (sensitivity 2 to 3,000 iu/l.) had 8.7% false positives. Pregnosticon had 0.5% false negatives, Planotest 2.0%, Gravindex 3.5%, Hyland B 6.5%, Pregslide 9%, and Hyland A 19.5% false negatives.

Planotest and Pregnosticon were found to be less influenced by protein and blood in the urine than the other pregnancy tests investigated.

Although many papers have been written on individual immunological pregnancy tests, such as the Gravindex slide test,<sup>1</sup> and such tube tests as Pregnosticon,<sup>2</sup> UCG,<sup>3</sup> Prepuerin,<sup>4</sup> and the Ortho test,<sup>1</sup> there is a lack of papers comparing these tests with each other. In many papers also, clinical confirmation of the diagnosis was not obtained and the patients were not divided according to the state of the pregnancy. Recently, some new slide tests, which have advantages over the tube tests in simplicity and time taken to obtain a result, have been produced and these have not been adequately investigated. For these reasons it was thought that it would be of interest to compare all the available slide tests with one tube test, Pregnosticon, which had been in use in this laboratory for some years.

## METHODS

Pregnancy tests were carried out on 686 early morning specimens of urine. Final diagnoses on all patients were obtained either from the case notes or by correspondence with the general practitioner. Four hundred and eighty-six urines were tested with four different pregnancy

diagnosis tests: Pregnosticon<sup>2</sup> (a haemoagglutination inhibition tube test); and three-complement fixation slide tests, Hyland HCG-test,<sup>5</sup> Pregslide,<sup>3</sup> and Gravindex.<sup>1</sup> In addition, 238 of these urines were tested with Planotest,<sup>2</sup> another complement-fixation slide test. Two hundred further urines were tested with Planotest alone.

The sensitivities of the tests were:

Gravindex, 3,000 to 3,500 iu/l. (stated on the product).

Pregnosticon, 1,000 iu/l. (stated on the product).

Planotest, 2,500 iu/l. (manufacturer's personal communication).

Pregslide, 3,000 iu/l. (manufacturer's personal communication).

Hyland, 2,000 to 6,000 iu/l. (manufacturer's personal communication).

It was noted during the preparation of this paper that the pattern of results with the Hyland HCG test had changed during the course of the work, the earlier results showing fewer false positives and more false negatives than the later results. Baxter laboratories were contacted and informed us that they had changed the sensitivity of their test from 6,000 iu/l. through 4,500 iu/l. to 2 to 3,000 iu/l., which they are currently supplying. We were unable to obtain the exact dates when these changes were made and are thus unable to include all the results with the Hyland slide test in this paper. Few tests were carried out with the Hyland test at a sensitivity of 6,000 iu/l. and these have been excluded. The results obtained using the Hyland test at a sensitivity of 4,500 iu/l. are referred to as type A and with a sensitivity of 2 to 3,000 iu/l. as

<sup>1</sup>Ortho Diagnostics, Raritan, New Jersey, USA (English address, Saunderton, Bucks.).

<sup>2</sup>N.V. Organon, Oss, Holland (English address, Organon Laboratories, Crown House, Morden Surrey).

<sup>3</sup>Wampole Laboratories, Stamford, Connecticut, USA (English address, Denver Laboratories, 12 Carlisle Road, London, N.W.9).

<sup>4</sup>Burroughs Wellcome & Co., Euston, London, N.W.1.

<sup>5</sup>Hyland Laboratories, Los Angeles, California, USA (English address, Baxter Laboratories Ltd, Thetford, Norfolk).

type B. The importance of pregnancy diagnosis kits carrying an approximate estimate of their sensitivity cannot be overemphasized and users should be notified if a change is made. Without this, mistakes and confusion can arise when interpreting results.

All urines were filtered before testing and the urines were tested for protein by the salicyl sulphonic acid test and Albustix,<sup>6</sup> blood by Haemostix<sup>6</sup> and for salicylates with ferric chloride. No urine was tested earlier than 35 days after the stated date of the last menstrual period. All urines not tested immediately were refrigerated and all glassware used in the tests was thoroughly washed in tap water followed by distilled water. Soap and detergents were not used.

**SLIDE TESTS** The tests were carried out according to the manufacturers' instructions. It was found easier to read the result in daylight but when artificial light was used it was found best to use a Tungsten bench lamp, the slide being held near, but not directly under, the light. The results were read as positive (no agglutination), negative (agglutination present), and inconclusive (doubtful agglutination).

**TUBE TEST** The test was carried out according to the manufacturers' instructions. The result was read as positive (definitive, well formed brown ring), negative (no ring formation), and inconclusive (a very open, thin or irregularly formed ring). The end point of the tube test was easy to read and tests could be carried out either in daylight or artificial light.

#### RESULTS

**UNCOMPLICATED PREGNANCIES** Table I shows the results in 203 uncomplicated pregnancies. Pregnosticon was the most sensitive test used, giving only one false negative (0.5%). The patient giving the false negative result was tested at 37 days after the date of the last menstrual period, and gave a positive result with Pregnosticon and Gravindex, an inconclusive result with Pregslide and a negative

result with Hyland A when tested 21 days later. Planotest and Gravindex had a similar sensitivity, Planotest giving 2.0% false negatives and Gravindex 3.5%. Pregslide had 9% false negatives. Hyland A was the most insensitive test, giving 19.5% false negatives, Hyland B gave 6.5% false negatives.

**COMPLICATED PREGNANCIES AND PATHOLOGICAL CONDITIONS** All pregnancy tests were positive in the patient with a hydatidiform mole and negative in the follow up patients who were tested between three and 18 months after the original diagnosis and treatment of the mole. The latter patients had no evidence for recurrence of the mole.

Four patients were diagnosed clinically as ectopic pregnancies. With one of these patients no chorionic tissue was found histologically and the diagnosis is therefore in doubt. This patient had a negative pregnancy test by all five methods. One patient had a positive test with Pregnosticon and a negative result with the other tests. This was presumably due to the greater sensitivity of Pregnosticon. One patient was positive with all tests and the other with all tests except Hyland A. Sixteen patients had a threatened abortion. The patient with a negative result by all tests was tested at 40 days after the date of the last menstrual period; this patient is still pregnant. Of the 11 patients with a positive result with each of the tests carried out, seven aborted within 16 to 33 days of the test, while four are still pregnant. The remaining four patients were positive with Pregnosticon and had varying results with the other tests. A single pregnancy test, therefore, appears to be of little help in the management of a threatened abortion.

The four patients with incomplete abortions all had positive results, while patients with complete abortions generally gave negative results. Pregnosticon and Pregslide, however, gave inconclusive results

<sup>6</sup>Ames Co., Inc., Elkhart, Indiana, USA (English address, Division of Miles Laboratories Ltd, Stoke Poges, Slough, Bucks.).

TABLE I

RESULTS OF PREGNANCY TESTS IN PREGNANT PATIENTS (EXCLUDING URINES CONTAINING BLOOD AND/OR PROTEIN)

Days after Last Menstrual Period	Pregnosticon					Hyland A					Hyland B				
	+	-	±	False Negative (%)	In-conclusive (%)	+	-	±	False Negative (%)	In-conclusive (%)	+	-	±	False Negative (%)	In-conclusive (%)
35-49	42	1	3	2.2	6.5	10	6	1	35	6	15	2	1	11	6
50-59	52	0	1	0	1.9	13	2	1	12	6	20	2	1	8.7	4.3
60-69	45	0	0	0	0	13	4	0	24	0	11	1	0	8.3	0
70-79	18	0	0	0	0	5	1	0	17	0	4	0	0	0	0
80-89	13	0	0	0	0	4	0	1	0	20	5	0	0	0	0
90-99	7	0	0	0	0	2	0	0	0	0	5	0	0	0	0
100-199	13	0	0	0	0	3	0	0	0	0	8	0	0	0	0
>200	3	0	0	0	0	1	0	0	0	0	2	0	0	0	0
Longstanding amenorrhoea	2	0	0	0	0	1	0	0	0	0	1	0	0	0	0
No amenorrhoea	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Postpartum amenorrhoea	2	0	0	0	0	1	0	0	0	0	1	0	0	0	0

with one patient each, two and three days respectively after the abortion.

**NON-PREGNANT PATIENTS EXCLUDING URINES WITH PROTEIN AND BLOOD** This group of patients was divided into menopausal and premenopausal because of the observation (Wide, Roos, and Gemzell, 1961; Lunenfeld, Isersky, and Shelesnyak, 1962; Hamashige and Arquilla, 1963; Taymor, Goss, and Buytendorp, 1963) that antisera to human chorionic gonadotrophin will crossreact with pituitary gonadotrophin. Patients with postpartum amenorrhoea were considered separately because of the observation (Bertini, 1965) that false positives are found in these patients. There were eight patients with postpartum amenorrhoea; all results were negative except one inconclusive with Pregnosticon.

Pregnosticon gave two inconclusive results in the menopausal group which became negative on diluting the urine. These were presumably due to the greater sensitivity of Pregnosticon causing pituitary gonadotrophins to be detected. In the premenopausal group, 0.5% false positives and 0.5% inconclusive were found with Planotest; 2.1% false positives, 4.1% inconclusive with Gravindex; 2.6% false positives, 6.2% inconclusive with Pregnosticon; 4.7% false positives, 8% inconclusive with Pregslide; 3.6% false positives none inconclusive with Hyland A, and 8.7% false positives, 1.4% inconclusive with Hyland B.

The 32 patients who gave false positive or inconclusive results with one or more of the pregnancy tests were thoroughly investigated to find if any cause for these false positives could be found. Three patients were taking steroids, two patients were on contraceptive pills, two on barbiturates, two on penicillin, and one each on chlordiazepoxide

and Prochloroperazine and Nitrazepam. Eight patients had infertility, six menstrual irregularities, and two chronic active hepatitis. There were, however, 25 patients on barbiturates, seven patients on a contraceptive pill, four on chlordiazepoxide and three on penicillin, who did not give false positive results, so these compounds do not appear to be the cause of the false positives seen. No false positives were found in patients on salicylates.

**NON-PREGNANT PATIENTS WITH URINE CONTAINING BACTERIA, PROTEIN, AND/OR BLOOD** In this group of patients, Planotest was the only pregnancy test which did not give a false positive result. False positives with the other pregnancy tests appeared to be related more to protein in the urine than to bacteria but some urines with an *E. coli* infection gave a positive or inconclusive pregnancy test even in the absence of protein with Pregslide or Gravindex.

**PREGNANCY TESTS ON BACTERIAL CULTURES** Preparations of cultured staphylococci, streptococci, and of an *E. coli*-*Proteus* mixture were prepared with a bacterial count several times higher than that which would be present in a highly infected urine. These cultures were then tested undiluted and at a dilution of 1 in 10 and 1 in 20. The only pregnancy test to give a positive was Hyland B which reacted down to a 1 in 10 dilution with the staphylococci and gave an inconclusive result with undiluted streptococci.

**EFFECT ON PREGNANCY TESTS OF ADDITION OF PROTEIN TO URINE** Human albumin,<sup>7</sup>  $\gamma$  globulin,<sup>7</sup> and glycoprotein<sup>8</sup> were added to urines at varying

<sup>7</sup>A.B. Kabi, Stockholm, Sweden (English address, Bilton House Uxbridge Road, W.5).

<sup>8</sup>Koch-Light Laboratories Ltd, Colnbrook, Bucks.

TABLE I—continued

## RESULTS OF PREGNANCY TESTS IN PREGNANT PATIENTS (EXCLUDING URINES CONTAINING BLOOD AND/OR PROTEIN)

Days after Last Menstrual Period	Pregslide					Gravindex					Planotest				
	+	-	±	False Negative (%)	Inconclusive (%)	+	-	±	False Negative (%)	Inconclusive (%)	+	-	±	False Negative (%)	Inconclusive (%)
35-49	36	6	4	13	9	41	5	0	11	0	48	3	4	6	8
50-59	44	5	4	9	7.5	52	1	0	1.9	0	55	1	0	2	0
60-69	36	6	3	13	6.7	44	1	0	2.2	0	37	0	0	0	0
70-79	18	0	0	0	0	18	0	0	0	0	10	0	0	0	0
80-89	11	0	2	0	15	13	0	0	0	0	18	0	0	0	0
90-99	7	0	0	0	0	7	0	0	0	0	6	0	0	0	0
100-199	13	0	0	0	0	13	0	0	0	0	9	0	0	0	0
>200	2	1	0	—	0	3	0	0	0	0	2	0	0	0	0
Longstanding amenorrhoea	2	0	0	0	0	2	0	0	0	0	1	0	0	0	0
No amenorrhoea	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Postpartum amenorrhoea	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
<b>Overall results</b>	<b>Pregnosticon</b>					<b>Hyland A</b>					<b>Hyland B</b>				
No. tested	203					68					78				
False negative (%)	0.5					19.5					6.5				
Inconclusive (%)	2.0					4.5					2.6				
	<b>Pregslide</b>					<b>Gravindex</b>					<b>Planotest</b>				
No. tested	203					203					195				
False negative (%)	3.5					9					2				
Inconclusive (%)	0					6					2				

concentrations to investigate the relative non-specificities of the pregnancy tests. Using human albumin at a concentration of 2 g/100 ml urine, positive results were obtained with Hyland B, Pregslide, and Gravindex but not with Pregnosticon or Planotest. At a concentration of 1 g/100 ml negative results were found with all tests. Using human  $\gamma$  globulin, Planotest and Pregnosticon gave inconclusive results at concentrations of 2 and 1 g  $\gamma$  globulin/100 ml. Pregslide and Gravindex gave positive results down to 250 mg/100 ml and Gravindex gave a further two inconclusive results at 125 and 62.5 mg/100 ml. Hyland B gave positive results at concentrations down to 125 mg/100 ml. Human glycoprotein (Cohn fraction VI) gave positive results only with Gravindex and Hyland B. Gravindex and Hyland B gave positive results down to 500 mg/100ml. Gravindex also gave an inconclusive result at 250 mg/100 ml.

**TESTS ON FILTERED AND UNFILTERED URINE** Although all tests in this series were on filtered urine, a series of tests was carried out with the slide methods to find if filtering was absolutely necessary. Six urines with varying degrees of cloudiness were tested. The only difference found was with one moderately cloudy urine, which gave a positive result with Pregslide on the filtered urine and an inconclusive result on the unfiltered urine. It would appear from this small series that filtration may not be necessary before pregnancy testing with Planotest, Hyland, Pregslide, or Gravindex.

#### DISCUSSION

To be completely satisfactory a pregnancy test should give neither false positive nor false negative results.

In this survey Planotest was the most satisfactory method for eliminating false positives, giving only 0.5% false positives in protein and blood-free urine from non-pregnant patients compared with 2.1% for Gravindex, 2.6% with Pregnosticon, 4.7% with Pregslide, 3.6% with Hyland A, and 8.7% with Hyland B. False positive immunological tests could be due to a number of different reasons: technical factors (Noto and Miale, 1964), impurity of reagents (Lunenfeld *et al*, 1962; Loewitt, 1966), cross reaction of the antihuman chorionic gonadotrophin (anti-HCG) preparation with antigens other than HCG (Wide *et al*, 1961; Wide and Gemzell, 1962; Goss and Lewis, 1964), and other as yet undetermined causes. Tests using albumin,  $\gamma$  globulin, glycoprotein, and various bacteria have shown that Pregnosticon and Planotest contain the purest reagents, only giving inconclusive results with

1 g  $\gamma$  globulin/100 ml. Since a fairly high proportion of pregnant females may have non-symptomatic bacteriuria and/or proteinuria, a pregnancy test which does not give false positives under these circumstances is wanted (Godts and Mighorst, 1964; Kew, Seftel, and Bloomberg, 1967), otherwise it is necessary to maintain a biological test. The lower limit of sensitivity of immunological pregnancy tests needs to be set at such a level that cross reactions with pituitary gonadotrophins do not interfere. Wide and Gemzell (1962) found that the level of luteinizing hormone in fertile and menopausal urine would be detected as 100 to 400 iu of HCG and it has been suggested that the lower limit of sensitivity should therefore be set at 1,000 iu, which is the limit for Pregnosticon. It would appear, however, from the two inconclusive results seen in the menopausal urine and the inconclusive results seen in some patients with infertility that even at this level pituitary gonadotrophins may sometimes interfere.

A test which does not give a large number of false negatives is also required, especially in such cases as ectopic pregnancies and threatened abortions when the level of HCG may be much lower than that in normal pregnancy. In this series Pregnosticon was the most suitable method, giving only 0.5% false negatives in normal pregnancies. Planotest gave 2.0%, Gravindex 3.5%, Pregslide 9%, Hyland A 19.5%, and Hyland B 6.5%. False negative results using immunological pregnancy tests have been reported in ectopic pregnancies (Southam, Sultzberg and Cohen, 1963; Gusdon, 1964; Islami, Fisher, and Kupfer, 1964) and in threatened abortions (Hutcherson, Schwartz, and Bates, 1964; Sato and Greenblatt, 1965; Fink and Frie, 1966). Pregnosticon gave the smallest number of false negatives in these conditions in the present work.

The type of pregnancy test used will depend on the type of pregnancy being tested. As a general pregnancy test, Planotest would appear to be the most efficient. In ectopic pregnancies and threatened abortions it may be preferable to use a more sensitive test, such as Pregnosticon or a similar tube test, but the number of false positives will then be greater.

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