Technical methods

A comparative study of laboratory and commercially prepared pregnancy tests

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A laboratory test, which is a modification of the immunological method of Wide and Gemzell (1960), has been developed for the detection of chorionic gonadotrophin in pregnancy urine. This test has been compared with the pregnancy detection kits manufactured by Ortho Pharmaceuticals, Burroughs Wellcome, and Organon, and also with the Hogben biological test.

MATERIALS AND METHODS

Samples of urine were obtained from specimens which hospital clinicians and general practitioners had submitted for examination by the Hogben test. Urine samples were also obtained from patients attending the ante-natal clinic for the first time.

LABORATORY METHOD Antiserum to human chorionic gonadotrophin was prepared by giving rabbits an intravenous injection of 15,000 i.u. human chorionic gonadotrophin (Pregnyl, Organon) in 2 ml. of a 1% bentonite suspension in normal saline (Butt, Crooke, and Cunningham, 1961) at weekly intervals for five weeks. The rabbits were bled one week after the final injection of human chorionic gonadotrophin. Sheep red cells were tanned by the modification of Ling (1960), as described by Butt *et al.* (1961). Pyruvic aldehyde reagent was prepared by adding 1.6 vol. 25% pyruvic aldehyde solution to 3.0 vol. saline, and the pH adjusted to 7.0 with 10% sodium carbonate solution. To this reagent was added 0.7 vol. 0.15 M phosphate buffer (pH 8.0), and 1.0 vol. of a 50% suspension of the washed red cells in saline. The mixture was stored at 4°C. for two days with occasional shaking. The cells were then washed at least six times with saline and stored as a 10% suspension containing 0.1% sodium azide as a preservative. Inefficient washing of the stabilized cells will result in the formation of an ill-defined pattern in the agglutination reaction. To sensitize the cells, 2 ml. of the 10% suspension was centrifuged, the supernatant discarded, and the cells washed twice in saline. Buffered saline, 10 ml. (saline, 10 vol.; 0.15 M Na₂HPO₄, 3.5 vol.; 0.15 M KH₂PO₄, 6.5 vol.), containing 1,500 i.u. human chorionic gonadotrophin was added to the cells and the mixture incubated at 37°C. for one hour. The cells were then washed twice in saline and stored at

4°C. as a 10% suspension in saline, containing 1% normal rabbit serum and 1/10,000 merthiolate.

The antiserum was titred in perspex agglutination trays by adding 0.25 ml. of saline and 0.05 ml. of a 2.5% suspension of the sensitized cells to 0.25 ml. of doubling dilutions of antisera. The tray was left at room temperature for two hours and then read. The highest titre to give complete agglutination was used for routine testing. It was found necessary to test urines of known pregnant and non-pregnant women to ensure that the chosen titre of antisera gave a clear differentiation. The urine specimens were centrifuged and diluted 1:5 with saline. The test was carried out by adding 0.25 ml. of the diluted urine to 0.25 ml. of titred antiserum and 0.05 ml. of a 2.5% suspension of sensitized cells. A control was prepared by substituting 0.25 ml. of saline in place of the antiserum. A pregnancy urine was indicated by a compact ring of cells at the bottom of the well, or by a pattern of non-agglutinated cells identical with that in the control.

ORTHO PREGNANCY TEST Diluted urine was incubated with anti-human chorionic gonadotrophin serum in tubes provided with the kit. Latex particles coated with human chorionic gonadotrophin were added and the mixture incubated for a further period, and then centrifuged. The final turbidity of the solution was then compared with a standard. A turbidity equal to or greater than the standard indicated that agglutination of the latex particles was inhibited by the presence of human chorionic gonadotrophin in the urine. There was no turbidity in the urine of non-pregnant patients due to complete agglutination of the latex particles. To ensure the best results centrifugation had to be carried out exactly as described in the instructions.

BURROUGHS WELLCOME PREPUERIN TEST Samples of urine at three different dilutions were placed in pairs of small test tubes. To one set of tubes was added a test suspension containing formalized red cells sensitized with human chorionic gonadotrophin and agglutinated by anti-human chorionic gonadotrophin serum. A control suspension of formalized cells not sensitized with human chorionic gonadotrophin was added to the second set of tubes. Urines containing human chorionic gonadotrophin reversed the agglutination of the erythrocytes in the test suspension and a ring of cells formed at the bottom of the tube.

ORGANON TEST PREGNOSTICON This test is based on the haemagglutination reaction of Wide and Gemzell (1960). Freeze-dried antiserum is supplied, in ampoules, which are also used as tubes for the reaction. A small sample of filtered urine was added to the antiserum followed by a suspension of sensitized erythrocytes. The contents of the ampoules were shaken in a special rack, and the appearance of the cells in the bottom of the tubes observed after

two hours. A clearly defined ring of cells indicated a pregnancy urine.

RESULTS

Urine specimens from 240 women were tested by the four methods. One hundred and forty-six of these samples were from patients who were later confirmed to be pregnant at the time the specimens were collected.

The results of the tests on urines of known pregnant and non-pregnant patients are given in Table I. All the false negative results were obtained with urine samples which were being tested by the Hogben reaction. Five of these urines were from patients at the end of the second trimester and two from patients less than six weeks pregnant. If these seven urines were not included in the results on the positive pregnancy urines, the Ortho would give 97% correct positive results, and the other tests 99 to 100%.

The laboratory and commercial tests are compared with the Hogben reaction in Table II. The low percentage of correct positive results given by the Ortho and Burroughs Wellcome tests is due mainly to tests being requested on urines from mid-term pregnancies.

The relative sensitivity of the four methods was tested using serial dilutions of human chorionic gonadotrophin in normal male urine. The end-point was the minimum concentration of human chorionic gonadotrophin which gave a reaction indicating a positive pregnancy according to the method used. The modified Wide and Gemzell method detected a minimum of 250 i.u./100 ml. and the Burroughs Wellcome and Ortho tests approximately 750 and 1,000 i.u./100 ml. respectively. The Organon test gave variable results, some of which gave a sensitivity below 20 i.u./100 ml.

DISCUSSION

The Ortho and Burroughs Wellcome pregnancy kits were

found to be suitable methods for the detection of urinary human chorionic gonadotrophin between the seventh and eighteenth week of pregnancy. However, these tests were not sufficiently sensitive to detect the relatively low level of human chorionic gonadotrophin occurring in pregnancies of more than 18 weeks. The Hogben test is sometimes used as an aid to diagnosis of intra-uterine death in mid-term pregnancies, and in this particular investigation the Ortho and Burroughs Wellcome tests were too insensitive to be of any value. The modified Wide and Gemzell technique was considerably more sensitive and could detect urinary human chorionic gonadotrophin in pregnancies between six and 24 weeks. This method duplicated the Hogben test. The Organon test gave similar results with pregnancy urines, but it could not be considered suitable for pregnancy diagnosis because of the large number of false positive results obtained. These poor results may possibly be due to a low and variable concentration of antisera in the ampoules. The other three methods gave negligible false positive results with the urines from non-pregnant patients.

SUMMARY

Urine samples from 147 pregnant and 94 non-pregnant women were tested by four laboratory methods for pregnancy diagnosis. These include a laboratory haem-agglutination reaction based on the method of Wide and Gemzell (1960), and three pregnancy kits manufactured by Ortho Pharmaceuticals, Burroughs Wellcome, and Organon. The laboratory test gave a high degree of accuracy and was sufficiently sensitive to detect pregnancies from six to 24 weeks. The Burroughs Wellcome and Ortho kits were less sensitive but were reliable in detecting pregnancies between seven and 18 weeks. The Organon test was extremely sensitive but gave a large number of false positive results, and was considered to be an unsuitable method for pregnancy diagnosis in its present form.

TABLE I
RESULTS OF PREGNANCY TESTS ON URINES OF CONFIRMED PREGNANT AND
NON-PREGNANT WOMEN

Method	Pregnancy Urines			Non-pregnancy Urines		
	Positive	False Negative	% Correct	Negative	False Positive	% Correct
Wide and Gemzell (modified)	144	2	99	94	0	100
Ortho	135	11	93	94	0	100
Burroughs Wellcome	140	6	96	93	1	99
Organon	144	2	99	79	15	84

TABLE II
COMPARISON OF RESULTS OF LABORATORY PREGNANCY TESTS WITH HOGBEN
BIOLOGICAL TEST ON URINES OF KNOWN PREGNANT AND NON-PREGNANT WOMEN

Method	Pregnancy Urines		Non-pregnancy Urines	
	Positive Results	% Correct	Negative Results	% Correct
Hogben	77	100	93	100
Wide and Gemzell (modified)	76	99	93	100
Ortho	66	86	93	100
Burroughs Wellcome	71	92	92	99
Organon	76	99	81	87

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REFERENCES

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ADDENDUM

Since this paper was submitted for publication it is learned that Organon Laboratories have now reduced the sensitivity of their pregnancy test. A further series of tests have been carried out using this modified Pregnosticon. One false positive result was obtained from 115 non-pregnancy urines, and one false negative result from 78 pregnancy urines. These results indicate that Pregnosticon is now a satisfactory test for pregnancy diagnosis.

CORRECTION

The title of the paper by Jean Bernard, J. Lasneret, J. Chome, J. P. Levy, and M. Boiron (*J. clin. Path.*, **16**, 319) should read: 'A cytological and histological study of acute promyelocytic leukaemia'. Throughout, the nomenclature 'promyelocyte' should be used instead of 'premyelocyte'.

Standardization of haemoglobin solutions by iron determination

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Earlier evaluations of haemoglobin standards in this laboratory utilized a modification of the direct titanium sulphate microtitration of residual Fe in wet- or dry-ashed haemoglobin (McFarlane, 1932; Ramsay, 1944; O'Hagan 1957). In the spectrophotometric determination of a coloured complex formed by interaction of haemoglobin Fe with a sensitive reagent Fe is split from haemoglobin by wet-way oxidative procedures terminating in either partial destruction of the protein, requiring a subsequent filtration step (Wong, 1928; Sunderman, MacFate, MacFadyen, Stevenson, and Copeland, 1953; Dickenson, Crafts, and Zak, 1954), or in complete destruction of the protein followed by evaporation of residual volatile acids (Williams and Zak, 1957). Preliminary trials of the first alternative were not completely satisfactory in our hands, possibly because of partial irreversible absorption of Fe by the precipitated protein. On investigation of the second, certain modifications were introduced, including the elimination of the final evaporative step.

MATERIALS AND METHODS

REAGENTS All are made up in iron-free distilled water.

1 *Buffered 2,2'-dipyridyl solution* One gram of 2,2'-dipyridyl is dissolved with warming in about 700 ml. water, and to this is added 300 g. anhydrous sodium acetate (A.R.). When solution is complete, the whole is transferred to a one-litre volumetric flask and made up to the mark. This solution is stored in a dark glass bottle.

2 *Mineral acids* The concentrated acids used, H_2SO_4 (S.G. 1.83), HNO_3 (S.G. 1.42) and HClO_4 (72%), are each of A.R. quality.

3 *Ascorbic acid* One gram is dissolved in 100 ml. water for each standardization. The solution is stored in a refrigerator when not in use but is discarded after 24 hours.

4 *Standard iron stock solution* This contains 10 mg. Fe per ml.; 86.6 g. $(\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ (A.R.) is dissolved in 400 ml. water, 200 ml. 10% H_2SO_4 is added, and the solution is transferred to a one-litre volumetric flask and diluted to the mark. The iron content is accurately established by a standard oxidimetric procedure (Vogel, 1951).

5 *Standard iron working solution* Accurate 1/100 dilution of stock solution = 100 $\mu\text{g./ml.}$

APPARATUS All glassware must be free of iron. This is accomplished by cleaning with chromic acid, rinsing

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