

Department of Medical and Public Affairs, The George Washington University Medical Center, 2001 S Street, N.W., Washington, D.C. 20009

— **Pregnancy Tests** —
The Current Status

SUMMARY

Accurate diagnosis of pregnancy soon after conception offers the woman with an unwanted pregnancy the option of terminating that pregnancy by simple, safe, effective and inexpensive procedures. It also permits early initiation of prenatal maternity care such as treatment of ectopic pregnancy, threatened abortion, and other pregnancy-related disorders and provides knowledge to women who are uncertain of their pregnancy status.

Traditionally, diagnosis of pregnancy is based on missed menstrual periods, subjective feelings of nausea, and observation of the visible signs and symptoms of pregnancy. Scientific testing supplements these and is especially important very early in pregnancy when clinical signs are often ambiguous. Scientific testing techniques, however, are not yet widely available in developing countries, especially in areas which lack basic family planning and health services.

If the use of pregnancy tests is to be expanded for general use in developing countries, a simple, inexpensive test, capable of early diagnosis, and suitable for field use by relatively unskilled personnel or the woman herself is required. A reliable pregnancy test which meets these criteria would be a valuable addition to general family planning and health services. It would:

- provide knowledge and reassurance to the patient by confirming pregnancy or nonpregnancy;
- permit early, safe termination, if indicated, and would avoid needless termination procedures;
- screen patients prior to oral contraceptive prescription, IUD insertion, rubella vaccination and the use of drugs which may be associated with side effects in pregnant patients or with increased risk of congenital malformation in developing fetuses;
- detect ectopic pregnancy, threatened abortions, trophoblastic tumors, and other pregnancy-related disorders at an early stage and would therefore allow prompt treatment.

None of the pregnancy tests currently available is ideal. The tests which are most accurate in early diagnosis—the bio-

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assays and immunologic tube tests—are expensive, time-consuming to perform and process, and generally unsuitable for field use in rural areas. They require laboratories or offices equipped with refrigeration and other expensive equipment. The immunologic slide tests are less bulky and easier to transport, produce results rapidly, and are relatively inexpensive and easier to perform, but are also less capable of early diagnosis than the tube tests. They are generally unreliable until two or more weeks following a missed period. By that time pregnancy, if it exists, can no longer be terminated by menstrual regulation (soft cannula vacuum aspiration of the uterine contents, without dilatation of the cervix, performed within six weeks after the last menses). Beyond that period termination must be accomplished by more complicated, hazardous and expensive procedures (see **Population Reports F-2, F-4**).

Recent research in developed countries has focused primarily on radioimmunoassays (RIAs) capable of very early diagnosis of pregnancy prior to a missed period. One procedure, the radioreceptorassay (RRA) developed by Brij Saxena and Robert Landesman at the Cornell University Medical Center, will be commercially available in the USA

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in early 1976. Another is already available in Great Britain for approximately \$10 (US) per test. The RIAs are believed to be accurate in diagnosing pregnancy at or around the time of a missed period. Currently, RIAs can be processed only in research centers and laboratories which have the relatively expensive equipment and specially trained personnel required. Research to develop a simpler RRA which does not require special facilities and personnel is underway at Cornell.

Sponsored by the US Agency for International Development (USAID), Dr. Lorrin Lau at The Johns Hopkins University (USA) has developed a new immunoassay using a capillary tube. In preliminary trials it has reportedly demonstrated greater accuracy in early diagnosis of pregnancy than any pregnancy test now available commercially. The capillary test appears to offer advantages in terms of shipping, storage, and cost to consumers which should make it particularly valuable for use in family planning programs in both developed and developing countries. Extensive laboratory and field trials of this new test are underway.

HISTORY

Pregnancy testing has evolved during the past half century from a time-consuming, complicated laboratory procedure using test animals into a rapid and convenient technique suitable for general office use. The historical trend has been towards the development of simpler pregnancy tests which are less expensive and easier to perform but not, until recently, towards development of tests with earlier, accurate diagnostic capability.

Early Diagnosis of Pregnancy

For centuries there has been interest in the diagnosis of pregnancy. Ancient Egyptian records describe a pregnancy test in which a woman drank a mixture of pounded watermelon and breast milk from a woman who had borne a son. If, as a result, she vomited, pregnancy was assumed. According to Hebrew scriptures, if a woman's feet sank deeply into soft ground, she was thought to be pregnant. Until the 20th century, diagnosis of pregnancy—primarily to satisfy curiosity—was based on missed menses and subjective observations of criteria such as turgidity of the veins of the breast and increased pleasure during sexual intercourse (29). Only within the past 50 years have scientists begun to develop objective tests that detect physiological changes associated with pregnancy.

Scientific Basis

The familiar signs and symptoms of pregnancy—missed menses, morning sickness, weight gain, and increased body temperature—reflect numerous physiological adaptations and adjustments, most of which protect and nurture the developing fetus. Although the precise nature of many physiological changes remains to be established, their existence and general patterns have been identified and documented (39).

For example, one of the best known patterns—and the basis for most pregnancy tests—involves the fluctuations during pregnancy of human chorionic gonadotropin (HCG). Produced by the placenta during pregnancy, the hormone can be found in blood and urine samples from pregnant women (15,19,55,77). Detection of HCG, by observing the effects of injections of a pregnant woman's blood or urine into test animals, marked the beginning of more accurate diagnosis of pregnancy using bioassays.

Apparently, HCG functions to preserve the developing corpus luteum and thus maintain pregnancy by preventing menstrual shedding. In normal pregnancy, the production of HCG begins within 48 hours after implantation, ascends to a peak between 50 and 90 days after the first day of the last menstrual period (LMP), falls to a much lower level which is maintained throughout pregnancy, and then ceases 3-10 days after delivery (39,55,60) (see Fig. 1).

In addition to pregnancy, however, there are other conditions that trigger the production of HCG. For example, placental (trophoblastic) tumors such as hydatidiform mole excrete particularly large amounts of HCG (see Fig. 2). On the other hand, ectopic (extra-uterine) pregnancies and threatened abortions are associated with abnormally low levels of HCG (see Fig. 3 and 4).

Bioassays

The first bioassay (biological test) was introduced in 1928 by German gynecologists, Selmar Aschheim and Bernhardt Zondek, who observed that urine from pregnant women injected into immature female mice caused premature maturation of the ovarian follicles and subsequent swelling, congestion, and ovarian hemorrhages. After repeated injections, the test animals were killed 4-5 days later and examined for evidence of HCG activity (15,19,78).

In 1932 Friedman and Lapham reported that rabbits as well as mice were sensitive to urinary HCG from pregnant women. Injections of urine containing HCG into white female rabbits produced ovarian hemorrhages. Because the Friedman test for pregnancy required less time—the rabbits were killed and examined 48 hours following injection—it replaced the Aschheim-Zondek test (15,19). The search continued for another test animal, however, because of the expense and difficulty involved in maintaining a large colony of rabbits.

During the 1940s and 1950s two other bioassays with demonstrated reliability and relative ease of performance gained widespread acceptance—the rat hyperemic test and the male toad test.

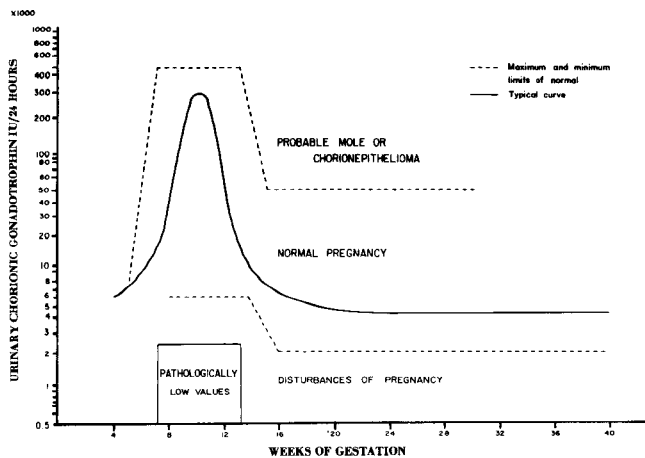


Fig. 1. During normal pregnancy excretion of HCG begins soon after implantation, ascends to a peak between the 50th and 90th days, then falls to a much lower level. HCG excretion ceases soon after delivery. Trophoblastic tumors which excrete HCG cause abnormally high levels while ectopic pregnancy and threatened spontaneous abortion are associated with low HCG levels. SOURCE: Courtesy of Organon, Inc., West Orange, N.J.

The rat test, first reported by Frank and Berman in 1941, uses white immature female rats which are injected with urine or blood, then killed and examined 16-24 hours later. Hyperemic ovaries (congested with blood) in the rats, caused by the presence of HCG in the woman's urine or blood sample, constitute a positive pregnancy test (15,19).

The male toad test uses the species *Bufo arenarum*, as reported by Galli-Mainini in 1947, or the *Rana pipiens* reported by Wiltberger and Miller in 1948. Injections of blood containing HCG cause the appearance of sperm in the toad's urine, indicating pregnancy. The male toad test reduced reporting time from several days to 1-5 hours and the same test animals could be used again and again since dissection was not required for analysis. (15,60,78,81,91).

Hormonal-Withdrawal Tests

The use of hormonal-withdrawal regimens as tests for pregnancy is based on the work of Zondek, who reported in 1942 that injections of estrogen-progestogen preparations could induce uterine bleeding in women with functional amenorrhea (absence of menstruation), many of whom had not menstruated for several months or even years (92). This discovery led to the development of tests which use synthetic hormones, either injected or taken orally, in an attempt to confirm or exclude pregnancy in women with delayed menstrual periods.

Women with delayed menstrual periods may or may not be pregnant. Repeated doses of estrogen-progestogen preparations, during a 3-5 day period, were thought to cause the body to simulate the normal hormonal fluctuations that precede the menstrual cycle when estrogen and progesterone levels increase. Withdrawing the hormones causes a decrease, as in the normal cycle, which may result in menstrual bleeding in the nonpregnant patient. If the patient is pregnant, however, the corpus luteum will continue to furnish progesterone and estrogen, thus maintaining the hormones at a level that will obviate bleeding despite withdrawal of the synthetic preparation (26,55,78).

Contrary to previous assumptions, new data suggests that the administration of hormones will not hasten, and may even delay the onset of menstrual bleeding (88). This lack of efficacy, together with some evidence of increased incidence of congenital malformations associated with the administration of hormones during early pregnancy, suggests that hormonal-withdrawal tests should be abandoned (17, 83,88).

Immunoassays

Immunological tests (tests which utilize antibodies to react with another substance) for the detection of HCG of pregnancy have gained wide acceptance as convenient substitutes for the bioassays in the diagnosis of pregnancy.

Immunological pregnancy tests first became available in 1960, when it was independently reported by Wide and Gemzell, McKean, and Brody and Carlstrom that in vitro tests could detect HCG in blood and urine samples from pregnant women (7,20).

The immunoassays (immunological tests) are based on the capability of HCG to stimulate antibody production. HCG extracted from the blood or urine of pregnant women, and injected into test animals, causes the production of antibodies. These antibodies to HCG can then be extracted from the animal's blood and are capable of neutralizing HCG in blood and urine samples from pregnant women. Thus, the immunoassays, like the bioassays, indicate pregnancy by detecting the presence of HCG in blood or urine samples. Instead of injecting a woman's blood or urine into test animals, however, the sample is analyzed in vitro. The presence or absence of HCG in the woman's urine is demonstrated by the reaction of the sample with the antibodies to HCG. Commercial production of anti-HCG (produced by injecting rabbits with purified HCG from urine of pregnant women) eliminated the need for maintaining an animal colony in each laboratory and testing center.

In 1960 Wide and Gemzell introduced the first tube hemagglutination inhibition pregnancy test. A few drops of urine are mixed with HCG antibody in a test tube. If urinary HCG is present, suggesting pregnancy, it will neutralize the anti-HCG. Red blood cells from sheep, sensitized with HCG, are then added. If HCG has neutralized the antibody,

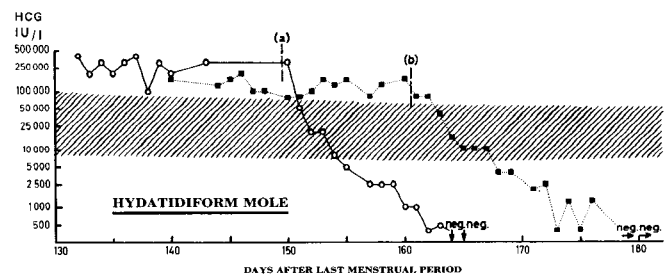


Fig. 2. Trophoblastic diseases are characterized by the excretion of abnormally large amounts of HCG as illustrated in the diagram, demonstrating HCG activity in 57 morning urines from two women with hydatidiform moles. In one of the women the mole was removed by curettage (a) and in the other the mole was delivered spontaneously (b). The shaded area represents the range (P=0.5) of the immunological HCG activity in morning urines from women with a normal pregnancy. SOURCE: Courtesy of Organon, Inc., West Orange, N.J.

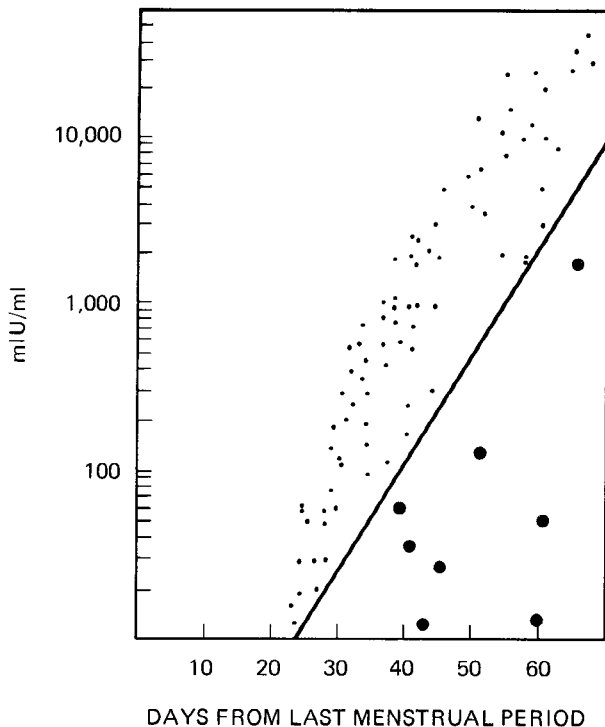


Fig. 3. Ectopic (extra-uterine) pregnancies produce abnormally low levels of HCG. Large circles (in red) represent HCG values of ectopic pregnancies. Small dots represent HCG levels of pregnancies progressing normally, while solid line represents lower limit of HCG in a pregnancy progressing normally.

SOURCE: Adapted from Kosasa et al. (46)

there will be no reaction. In other words, the cells will not agglutinate because the antibody has already been "inhibited" by the HCG in the woman's urine. If the HCG antibody has not been neutralized because of the absence or lack of sufficient quantities of HCG in the urine, it will react with the HCG-coated blood cells, causing them to agglutinate (7,62) (see Fig. 5).

Agglutinated cells—implying no HCG and therefore that the woman is not pregnant—remain in suspension (see Fig. 6). Nonagglutinated cells, because they are not clumped together, will roll down the sides of the test tube until they come to rest on the slope of the hemispherical bottom, producing a readily identifiable "ring" pattern (see Fig. 6). This ring pattern constitutes a positive pregnancy test.

In 1962 Robbins and associates described the first slide test for pregnancy, also dependent upon inhibition of agglutination. Urine is mixed with anti-HCG serum on a slide. Instead of red blood cells, however, latex particles coated with HCG are then added. As with the tube tests, agglutination indicates that HCG is not present in the urine and, conversely, inhibition of agglutination indicates that HCG is present, thus suggesting pregnancy (7,62) (see Fig. 7).

Radioimmunoassays

Radioimmunoassays (RIAs), capable of detecting the extremely low levels of HCG that exist in very early pregnancy, were developed in the late 1960s.

RIAs are based on the same principle as the immunoassays—that HCG in the woman's blood or urine will react

immunologically with HCG antibodies. Instead of using HCG-coated latex particles or red blood cells, however, the RIA method utilizes purified preparations of HCG which have been labeled (bound) with radioisotopes of iodine.

The woman's blood is combined with a pre-determined amount of antibody to HCG; the labeled HCG is then added. If HCG is not present in the blood, the unreacted antibody is available to combine with the radioactive HCG. The amount of reacted or unreacted labeled HCG is measured by counting the gamma rays emitted by the radioactive HCG, indicating the amount of antibody neutralized by HCG in the blood sample (14,52,84).

EFFECTIVENESS

The ideal pregnancy test would make early, accurate diagnosis possible, thus enabling the potential mother to seek medical supervision and prenatal care. It would also allow intervention at a time of minimal risk if termination were indicated, and permit prompt identification and early treatment of ectopic pregnancy, trophoblastic tumors, threatened spontaneous abortion, and even testicular malignancy in men.

Functions

A positive diagnosis of pregnancy serves to:

- confirm pregnancy for women who wish to be pregnant and who wish to establish a plan of prenatal care;
- signal the need for intervention if pregnancy termination is indicated;
- alert the physician to take special precautionary measures to maintain pregnancy in women with a history of miscarriage;
- direct the doctor's attention to the possible existence of HCG-secreting trophoblastic tumors, and to aid in treatment and follow-up.

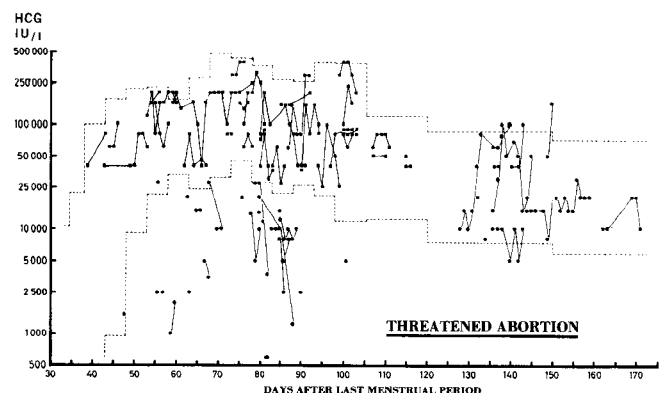


Fig. 4. Threatened spontaneous abortions (red circles) are usually associated with abnormally low levels of HCG excretion, as illustrated in the diagram by the HCG activity in 229 morning urines from 72 women hospitalized for threatened abortion. Thirty-three women aborted spontaneously (results represented by red circles). In the other 39 women pregnancy continued normally (results represented by solid squares). The dotted lines represent the fiducial limits of error ($P=0.05$) of the immunological HCG activity in morning urines from women with a normal pregnancy.

SOURCE: Courtesy of Organon, Inc., West Orange, N.J.

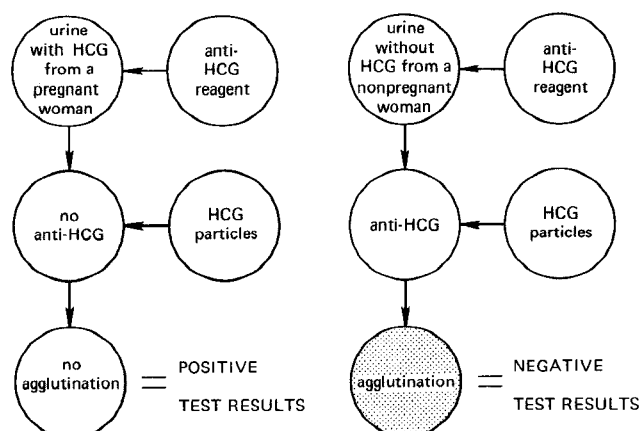


Fig. 5. Most of the immunoassays depend on inhibition of agglutination methods. If the level of urinary HCG is high enough to be detected by the test, the anti-HCG reagent is neutralized and no agglutination will occur when HCG coated particles or red blood cells are added. If HCG is not present or is below the level which the test can detect, the anti-HCG is not neutralized and will agglutinate the HCG coated particles.

Negative test results serve to:

- reassure a woman who has missed her period and does not want a pregnancy that she is not pregnant;
- screen patients prior to beginning oral contraception, IUD insertion, German measles vaccination, X-ray therapy or tests, or drug therapy which might harm a developing fetus;
- if amenorrhea continues, direct the physician's attention to the possible existence of ectopic pregnancy or threatened abortion.

No test is 100 percent accurate. All will occasionally produce false results. False-positive test results, which incorrectly diagnose pregnancy in women who are not pregnant, are rare. False-negative results, which incorrectly indicate nonpregnancy in women who are pregnant, are relatively common with many tests and are increasingly so in very early pregnancy. Both false-negatives and false-positives have serious implications. False-positives may result in unnecessary treatment for nonexistent conditions. False-negatives, which may become true-positives at a later date, may result in failure to seek early treatment or abortion, if indicated or desired.

A woman who is told incorrectly that she is pregnant may undergo an unnecessary abortion procedure or she may stop using contraceptives and, as a result, become pregnant. Incorrectly assured that she is not pregnant, a pregnant woman may needlessly expose the fetus to possible danger from contraindicated drugs, German measles vaccination, or X-ray. False-negatives may, in fact, be dangerous or even life-threatening if they result in failure to treat ectopic pregnancy prior to rupture.

For family planning programs, it is particularly useful to eliminate false-negatives which result in failure to take advantage of early, uncomplicated termination procedures. Menstrual regulation (MR) is a less expensive and less complicated method of termination compared to uterine aspiration or dilatation and curettage performed later in pregnancy. For maximum effectiveness and safety MR should

be performed within two weeks following the missed period (see **Population Reports F-2, F-4**) (12,66,79,85,88). Because with the current commercially available tests the possibility of a false-negative is great during the first two weeks following a missed period, MR is generally performed regardless of test results. Studies indicate that as many as 25-50 percent of women with delayed menstrual periods of one week undergo MR needlessly because of the high incidence of false-negatives with the standard tests (63,79,85,88). A pregnancy test which is accurate within this crucial period would rule out these unnecessary procedures. In some countries, however, proof of pregnancy could make a physician legally liable for prosecution under anti-abortion statutes, while if the same physician evacuates the uterus in the absence of evidence of pregnancy, the act is likely to be judged legal (58).

Abnormal Pregnancy

Reliable pregnancy tests can aid in the diagnosis of ectopic pregnancy and threatened spontaneous abortion, both of which are associated with abnormally low levels of HCG production (see Figs. 3 and 4). Early diagnosis of ectopic pregnancy allows surgical intervention before rupture and the subsequent severe inter-abdominal hemorrhage, thus reducing the danger faced by the woman.

Early diagnosis of pregnancy in women with a history of miscarriage allows for institution of measures designed to maintain pregnancy (4,28,89).

Trophoblastic Tumors

Pregnancy tests can also be valuable for identification and treatment of HCG-producing tumors. Trophoblastic diseases such as hydatidiform mole and choriocarcinoma often produce abnormally high levels of HCG (see Fig. 2). Pregnancy tests and quantitative assays of HCG can be useful in diagnosing trophoblastic disease, in monitoring the response of tumors to chemotherapy over a period of time, and as follow-up after surgical evacuation to insure that removal is complete and that there is no recurrence (45,55,84).

Testicular Malignancy

Pregnancy tests based on levels of HCG can be used to detect testicular malignancy in men. Hobson reported in

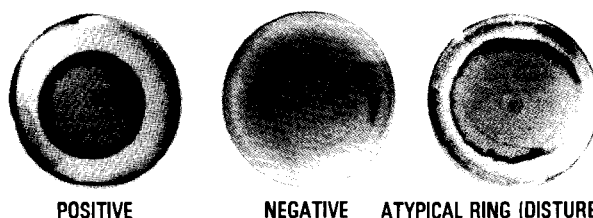


Fig. 6. Hemagglutination inhibition tube tests are more reliable than the slide tests in early diagnosis of pregnancy because they are capable of detecting lower levels of HCG. If the HCG antibody is neutralized by urinary HCG, no agglutination occurs when HCG coated red blood cells are added, signifying a positive test for pregnancy. Non-agglutinated cells are free to roll down the sides of the tube and settle in a "ring" pattern on the bottom. Agglutinated cells remain in suspension and indicate a negative pregnancy test. If the tube is jarred during the two-hour incubation period, the vibration may cause inconclusive test results characterized by atypical "rings".

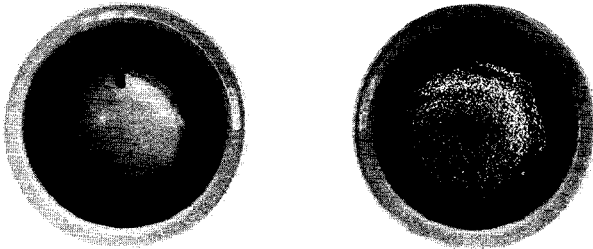


Fig. 7. The latex agglutination inhibition slide tests are popular because they are easy to perform and produce results within minutes. If the antibody is neutralized by HCG in urine from a pregnant woman, no agglutination occurs when latex particles coated with HCG are added. Agglutination indicates a negative result.

1965 that tests using female toads as test animals could be used to identify testicular tumors which excrete HCG, such as seminoma and choriocarcinoma, and to monitor the effectiveness of chemotherapy and X-ray treatment (33). Further research is needed concerning the use of pregnancy tests to screen men for cancer (55).

BIOASSAYS

The biologic pregnancy tests are accurate, but they are time-consuming, complicated, and expensive. Thus, in most places they have been replaced by the immunoassays as primary pregnancy tests.

Diagnostic Capability

Accurate diagnosis of pregnancy by standard bioassays—the Friedman rabbit, the rat, and the male toad tests—is well established (15). The rabbit and rat tests are sensitive to (capable of detecting) as little as one International Unit (IU) of HCG per milliliter of urine. They are most reliable beginning 14 days following a missed period although they may detect pregnancy before that (15,81) (see Table 1). The Delfs assay, a bioassay using rats to measure levels of HCG in blood rather than HCG in urine, has been reported to be sensitive to .4 IU of HCG/ml (55).

The male frog and toad tests are less sensitive, but capable of detecting 1-5 IU of HCG/ml. The assays have been reported to be 92-95 percent accurate beginning two to four weeks after a missed period (9,60,78,81,91).

Rat and rabbit assays are especially valuable in identification and management of hydatidiform mole, chorioadenoma, choriocarcinoma, and other pathologic conditions associated with abnormally high levels of HCG because they can provide quantitative estimates of HCG activity. The bioassays, however, are usually sensitive to only 1-2 IU of HCG/ml and thus may fail to indicate ectopic pregnancy or threatened abortion associated with abnormally low HCG levels.

Convenience and Cost

For general use the bioassays have several disadvantages:

- They are time-consuming: the toad test requires 1-5 hours; the rat test, 16-24 hours; and the Friedman rabbit test 48 hours before results can be analyzed;
- Bioassays require maintenance of an animal colony and personnel skilled in animal husbandry, since the animal's sensitivity to HCG varies according to season of

the year, mating periods, and temperature fluctuation (15,60,77,91);

- The more sensitive bioassays—the rat and rabbit tests—require experienced technicians and special facilities for preparing injections, killing the animals, and analyzing the ovaries;
- The tests are relatively expensive because animals must be replaced constantly, and because special facilities, costly equipment and specially trained personnel are required.

HORMONAL-WITHDRAWAL TESTS

The diagnostic capability and safety of the hormonal-withdrawal tests have been frequently questioned, and evidence suggests that they are unreliable and possibly unsafe. For these reasons the tests are no longer widely used in developed countries where other pregnancy tests—such as the immunoassays—are available. Hormonal-withdrawal tests continue to be used extensively in developing countries, at least in urban areas, where alternative tests are expensive and often difficult to obtain. In many areas women assume, and sometimes the physicians imply, that hormonal-withdrawal tests are abortifacient. This belief, and the fact that the tests are easy and inexpensive to perform, may account for their popularity (26,30,72).

Diagnostic Capability

The nature of the hormonal-withdrawal pregnancy tests has inhibited scientific investigation of their effectiveness. Because a negative result is indicated by menstrual bleeding within a period of several days to two weeks following the use and withdrawal of the hormonal preparations, it is difficult to establish that bleeding was actually induced by the test and not simply delayed menses which would have occurred anyway.

In fact, in the first and so far only controlled study, Vengadasalam et al. in Singapore found that among women whose menstrual periods were delayed less than 14 days bleeding occurred sooner for those who did not receive hormonal injections than for those who did. In the study if amenorrhea persisted one week after the injections, menstrual regulation was performed and the uterine contents were examined for evidence of pregnancy. Based on these examinations, a false-positive rate of approximately 19 percent was associated with the use of the test; that is, the injections failed to induce bleeding in 19 of 95 women subsequently found to be nonpregnant (88).

The possibility that administering exogenous hormones to a pregnant woman induces menstrual shedding has caused some speculation that hormonal "tests" may act as abortifacients (13,26,30,72). In a 1972 study of spontaneous abortion cases in the London area, Brotherton and Craft found that 7.6 percent of aborters had had an oral hormonal pregnancy test (13). Gal has reported a similar association of spontaneous abortion with the use of the tests (26). The investigators did not offer these observations as proof of a causal relationship, however, and there is no evidence that hormonal-withdrawal tests act as abortifacients.

A factor that encourages use of the hormonal-withdrawal tests is that they are easy to perform. Administering injections or tablets requires no training for the personnel involved and no special facilities for preparation, performance, or interpretation of results. No refrigeration is necessary; the packages are not bulky and can be transported easily, thus facilitating use in rural health centers and other remote areas in the field (73).

Hormonal-withdrawal pregnancy tests have not been commercially available in the USA since 1973 when they were withdrawn at the request of the US Food and Drug Administration (USFDA) for lack of substantial evidence of effectiveness (83). The Singapore study supports the FDA decision and also recommends discontinuing use of the tests for diagnosis of pregnancy (88).

Table 1—Selected Studies of Biologic Tests for Pregnancy, 1963-1971*

Author and Date	Ref. No.	Test Animal	Methodology	Performance Time (hours)	Sensitivity (IU of HCG/ml)	Diagnostic Capability	Special Requirements
Cabrera 1969	15	white immature female rats	Rats injected with urine or blood (filtered) are killed after 16-24 hours. Hyperemic ovaries indicate presence of HCG.	16-24	1	High accuracy beginning 2 weeks following a missed period.	All require: <ul style="list-style-type: none"> • laboratory facilities for performance, including facilities for maintenance of animal colonies; • expertise in animal husbandry, due to animal sensitivity variations according to season, species; • special facilities, skills and experience for preparation of sample (filtering, centrifuging) processing (injections, killing and dissecting animals, where indicated), and analysis (microscopes, scales).
Driscoll 1971	20	immature male rats	Rats injected with urine are killed after 24 hours. Ventral prostate glands are weighed for increases due to HCG.	24	1.5-2.0	High accuracy beginning 2 weeks following a missed period.	
Cabrera, 1969 Tietz, 1965	15 81	white female rabbits (the Friedman test)	Rabbits injected with urine or blood (filtered) are killed 48 hours later. Corpora hemorrhagica in ovaries indicates presence of HCG.	48	NR	High accuracy beginning 2 weeks following a missed period.	
Mayo, 1965 Tietz, 1965	60 81	male frog (Rana pipiens)	Blood injected into dorsal lymph sac. Presence of sperm in fluid aspirated from cloaca indicates HCG in sample.	1-5	NR	High accuracy beginning 3-4 weeks following a missed period.	
Barnett, 1963 Spadoni, 1964 Yahia, 1964	9 78 91	male toad (Bufo marinus)	Urine (filtered and/or centrifuged) injected into dorsal lymph sacs of two toads. Presence of sperm in fluid aspirated from cloaca indicates HCG in sample.	3-5	NR	High accuracy beginning 2 weeks following a missed period.	

NR = Not Reported.

*Bioassays diagnose pregnancy by observing and analyzing the effects of urine or blood injected into test animals. Samples from pregnant women, which contain HCG, cause a variety of changes in the animals which have been documented as indicative of pregnancy, although the exact nature of the causal relationship is unknown.

Safety

Although principally concerned with the diagnostic capability of the hormonal tests, the USFDA also concluded that a question of safety existed concerning the possible association of hormone administration in early pregnancy with congenital malformation (83). The tests do alter the hormonal levels within the uterus and therefore may affect the pregnant as well as the nonpregnant woman.

A number of retrospective studies suggest that there is an association between teratogenic effects and exposure to hormonal pregnancy tests during a vulnerable period of fetal development. Studies indicate an increased incidence of multiple congenital anomalies described by the acronym VACTEL (Vertebral, Anal, Cardiac, Tracheal, Esophageal, Limb) among children exposed to hormonal tests during the first trimester of pregnancy (2,26,27,30,67,68,71). Data is inconclusive, however. More research is needed in this area.

The administration of hormonal-withdrawal pregnancy tests has also been associated with side effects. Both pregnant and nonpregnant women have reported headache, nausea, and irregular vaginal bleeding (26,88).

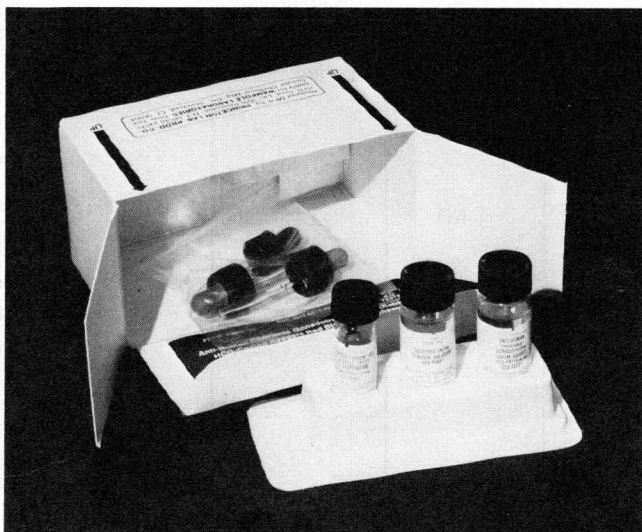
Even if effective, the results may not be known for as long as two weeks, thus prolonging the woman's anxiety, delaying medical attention for possible disorders, and limiting the option for early termination.

IMMUNOASSAYS

Because they are accurate, convenient and inexpensive, the immunoassays have replaced the bioassays for routine screening and become the most widely used pregnancy tests in the world. There are two basic categories of immunoassays—the tube tests and the slide tests.

Tube Tests

All commercially available tube tests are based on principles of agglutination inhibition, but differ in the HCG carrier particles used (latex or red blood cells). In the hemagglutination inhibition tests urine is mixed in a tube with HCG



antibody; then HCG sensitized sheep red blood cells are added, and the tube is left in a rack for two hours. If HCG is present in the sample in sufficient quantity to neutralize the antibody no agglutination will occur and pregnancy can be presumed. In the absence of HCG, the antibody will react with the HCG coated cells and agglutination will occur (see Fig. 6). Hemagglutination inhibition tube tests available in the USA and many countries overseas include the Pregnosticon Tube (Organon, Inc., West Orange, N.J.), Pregnosticon Accuspheres (Organon, Inc., West Orange, N.J.), UCG Tube (Wampole Laboratories, Stamford, Conn.), and UCG Lyphotest Tube (Wampole Laboratories, Stamford, Conn.) (see Fig. 8).

Placentex (Roche Diagnostics, Nutley, N.J.) is a unique latex agglutination inhibition tube test which uses latex particles to which HCG has been chemically bound as carrier particles, rather than red blood cells. The negative endpoint is bold, distinct flocculation (agglutinated clumps) while the positive endpoint is translucent (as opposed to the "rings" seen in the hemagglutination inhibition tests). The test has a 90 minute incubation period and requires a heating bath or block (34).

Slide Tests

The latex agglutination inhibition slide tests—such as Gravindex Slide (Ortho Diagnostics, Raritan, N.J.), Pregna-Chek (Hyland, Los Angeles, Calif.), Pregnosis Slide (Roche Diagnostics, Nutley, N.J.), Pregnosticon Slide (Organon, Inc., West Orange, N.J.), and UCG Slide (Wampole Laboratories, Stamford, Conn.)—substitute latex particles sensitized with HCG for the red blood cells used in most of the tube tests. A few drops of the woman's urine is mixed with antibody on a slide and the latex particles are added. Results are read in 1-3 minutes. Agglutination indicates absence of HCG required to neutralize the antibody—suggestive of nonpregnancy. Lack of agglutination indicates that the level of urinary HCG is high enough to assume pregnancy (see Figs. 7 and 9).

In the direct agglutination slide test, DAP (Wampole Laboratories, Stamford, Conn.), the anti-HCG is directly ab-

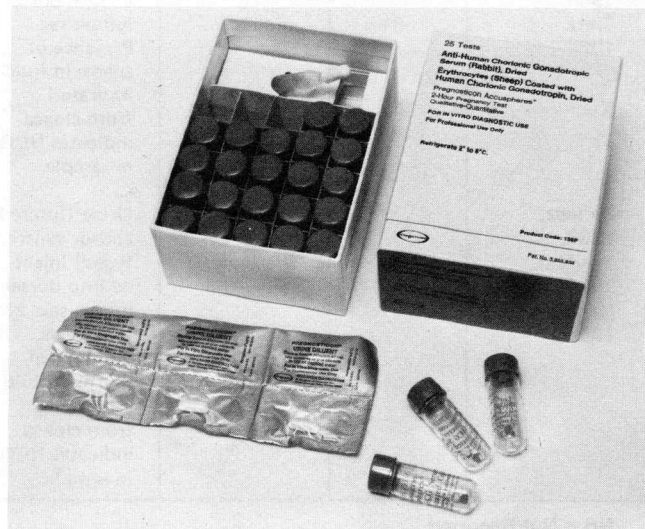


Fig. 8. Immunological tube tests, which are available in many countries throughout the world, include the UCG Tube test (left), and the Pregnosticon Accuspheres test (right). Both depend on hemagglutination inhibition. The Pregnosticon Accuspheres test kit includes individual test tubes containing freeze-dried reagents. (Test kits furnished by manufacturers.)

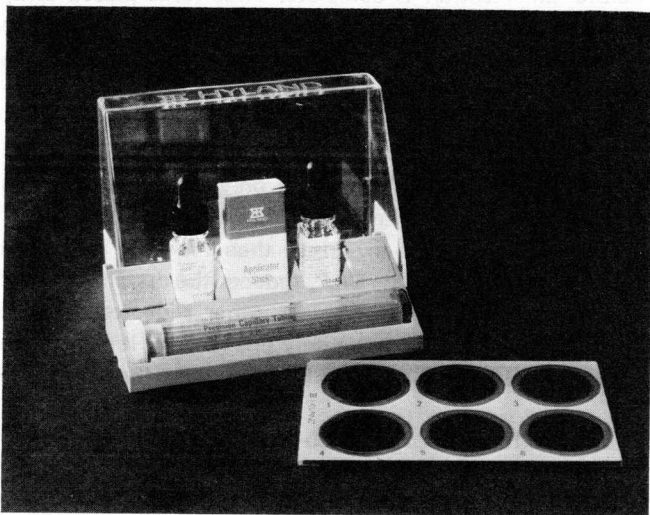
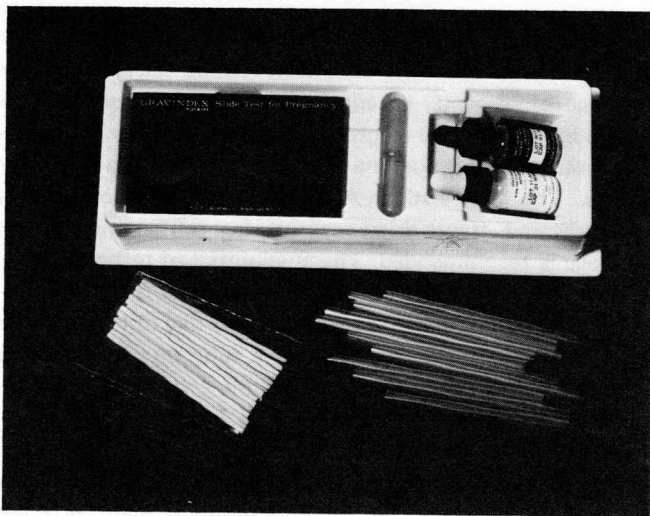


Fig. 9. Immunological slide tests for pregnancy include the Gravindex, Pregnosticon, Pregna-Chek and UCG tests. All of the test kits include the necessary reagents, a reusable slide, pipettes or capillary tubes for adding urine to the slide, and stirrers or applicator sticks for mixing the reagents and urine on the slide. (Test kits furnished by manufacturers.)

sorbed on latex particles, so that HCG in either urine or blood samples will result in agglutination. Unlike the other slide tests, agglutination indicates a positive pregnancy test.

Diagnostic Capability

Repeated comparisons and evaluations have demonstrated that the immunoassays are as reliable in the diagnosis of pregnancy as the most sensitive bioassays (15,20,24,42,60, 81).

The tube tests are generally more sensitive (able to detect lower concentrations of HCG) than the slide tests, due to a clearer endpoint from longer reaction time and to different antibody-HCG concentrations than in the slide tests.

The tube tests are generally at least as specific as many slide tests; that is, they are capable of distinguishing HCG in samples which may contain interfering substances such as large amounts of protein or drugs.

The more sensitive tube tests are capable of detecting urinary HCG at levels of .75-1.0 IU/ml of urine, and thus capable of diagnosing pregnancy as early as 4-7 days following a missed period. Tietz found that the hemagglutina-

tion tube tests were most accurate beginning the eighth day following a missed period. Prior to that, the tests detected only 77 percent of pregnancies (81). Others have substantiated these results, indicating that tube tests are indeed very reliable beginning the second week after a missed period (15,20,24,37,42,60) (see Table 2).

The more rapid slide tests are also very accurate although they are less sensitive to low levels of HCG and therefore less reliable in diagnosing early pregnancy. Slide tests vary in sensitivity from 1-5 IU of HCG/ml. Generally, they are not reliable until at least two weeks following a missed period (15,20,21,24,37,42,60) (see Table 3).

Because of the limited sensitivity of the immunoassays in detecting early pregnancy, they are associated with a high incidence of false-negatives. Thus a woman with a negative test result during this period is frequently advised to return for a repeat test one week later. Although the tube tests are more reliable than the slide tests in early pregnancy, neither type of immunologic test now commercially available can consistently detect early pregnancy or ectopic pregnancies which excrete abnormally low amounts of HCG (5,24,37, 42,60).

On the other hand, with both the tube and slide tests false-positives are rare. Courses of action, such as the decision to perform menstrual regulation, can therefore be taken when the test result is positive.

Although the immunologic tests may fail to detect ectopic pregnancies with HCG levels below the sensitivity limitations of the tests, both tube and latex slide tests can be used to measure high levels of HCG activity, thus facilitating the identification and treatment of trophoblastic diseases.

A number of the tube tests—UCG Tube and Pregnosticon Accuspheres and Tube Test—and several of the slide tests include instructions for quantifying HCG activity. Serial dilutions of a patient's urine are prepared and tested to determine the highest dilution which continues to give a positive result, thus narrowing the quantitative estimate of HCG to an identifiable range, depending on the dilutions used and the sensitivity of the test.

The UCG Titration test kit (Wampole Laboratories, Stamford, Conn.) was designed specifically for quantifying HCG activity, and includes diluents and tube tests for that purpose. The Pregnosticon Accuspheres and Tube Test kits include urine diluent in capsule form so that the clinician can prepare serial dilutions for quantitative estimates of HCG. The producers of Gravindex market a "Decaslide" marked with ten rings for comparative interpretation of results with successively greater dilutions of the patient's urine.

Convenience and Cost

All of the immunologic tube tests require refrigeration for storage of reagents, except for the UCG-Lyphotest which provides freeze-dried reagents which according to the manufacturer are stable for one year when stored in a cool, dry place. The Pregnosticon-Accuspheres Tube also includes premeasured, freeze-dried reagents, but refrigeration during storage is recommended. The possibilities of technical error in preparation are minimized in both tests, since the premeasured pellets—one of antiserum and one of HCG-coated red blood cells—are contained in a tube provided for the test. Buffer solution is included in the test kits to reconstitute the dried reagents.

Most of the tube tests are subject to vibration effects which may produce false or inconclusive results if they are jarred before the reaction is complete. Thus racks placed away from areas of vibration or on sponges are required. The latex tube test Placentex is less subject to interference from vibration, due to chemical bonding of HCG to latex particles (34,55).

The slide tests provide results in only 1-2 minutes, much quicker than the tube tests, and they are less bulky, easier to transport, and less expensive than the tube tests. The direct agglutination test, DAP, reduces the opportunity for technical error since, unlike the other slide tests, it is a one-step operation requiring only one reagent; it does, however, require prior filtration of urine, unlike the other slide

Table 2—Selected Studies of Immunologic Tube Tests for Pregnancy, 1965-1973*

Author and Date	Ref. No.	Test	Performance Time	Sensitivity IU of HCG/ml	Cost per Test**	Diagnostic Capability	Special Requirements and Features
Horwitz, 1973	34	Placentex (Roche Diagnostics)	90 min.	1.0	\$1.14	Accuracy reported as 77.8% from 31-40 days LMP; 96.8 true positive from 2-4 weeks after missed period.	Due to covalent bonding of HCG to latex particles, is less susceptible to vibration effects. Requires 37°C (98.6° F) heating block or both for incubation period.
Cabrera, 1969	15	Pregnosticon (Organon, Inc.)	2 hrs.	.7-1.5	1.90	High accuracy beginning 1-2 weeks following missed period.	
Fitzgerald, 1972	24						
Hobson, 1969	32						
Horwitz, 1970	37						
Kerber, 1970	42						
Mayo, 1965	60						
Tietz, 1965	81						
Kerber, 1970	42	Pregnosticon Accuspheres (Organon, Inc.)	2 hrs.	.75-1.0	1.46	High accuracy beginning 1-2 weeks following missed period.	Freeze-dried reagents contained in individual, disposable tubes.
Lamb, 1972	50						
Cabrera, 1969	15	UCG (Wampole Laboratories)	2 hrs.	1.0	1.19	High accuracy beginning 1-2 weeks following missed period.	
Horwitz, 1970	37						
Kerber, 1970	42						
Lamb, 1972	50						
Mayo, 1965	60						
McIver, 1969	62						
Tietz, 1965	81						

*Immunoassays, like the bioassays, diagnose pregnancy by detecting HCG. All of the above tube tests are based on agglutination inhibition principles (see Fig. 5). The tube tests generally require washing facilities for reusable tubes; test tube racks; water for performance; and good light for interpretation. All are suitable for laboratory or office use and require trained personnel for performance and interpretation.

**Manufacturer's wholesale price (US dollars) (6).

Table 3—Selected Studies of Immunologic Slide Tests for Pregnancy, 1965-74*

Author and Date	Ref. No.	Test	Performance Time (min.)	Sensitivity (IU of HCG/ml)	Cost per Test**	Diagnostic Capability	Special Features
Fitzgerald, 1972 Kerber, 1970 Mclver, 1969	24 42 62	DAP (Wampole)	1-2	2.0-3.0	\$.84	High accuracy beginning 2-3 weeks following a missed period.	The only direct agglutination test, DAP may be performed on urine (filtered) or blood.
Cabrera, 1969 Calder, 1968 Driscoll, 1971 Fitzgerald, 1972 Hobson, 1969 Horwitz, 1970 Kerber, 1970 Mayo, 1965 Spadoni, 1964 Tietz, 1965	15 16 20 24 32 37 42 60 78 81	Gravindex (Ortho)	2-3	3.0-5.0	.84	High accuracy beginning 3 weeks following a missed period.	
Driscoll, 1971 Horwitz, 1974	20 35	Pregnosis (Roche)	2	1.5-2.5	.90	High accuracy beginning 2 weeks following a missed period.	Covalent bonding of HCG reagent to latex particles produces clear endpoint and is less susceptible to interference from urinary proteins.
Edelman, 1974 Horwitz, 1972 Lamb, 1972	21 38 50	Pregnosticon Dri-Dot (Organon)	2	1.0-2.0	1.10	High accuracy beginning 2 weeks following a missed period.	Dri-Dot is the only test which does not require refrigeration. Disposable cardboard slides containing dried reagents are individually wrapped.
Cabrera, 1969 Horwitz, 1972 Kerber, 1970 Lamb, 1972	15 38 42 50	Pregnosticon Slide (Organon)	2	1.0-2.0	.90	High accuracy beginning 1-2 weeks following a missed period.	

*All of the immunologic slide tests are based on agglutination inhibition principles, except for DAP, where direct agglutination indicates a positive test. The slide tests are suitable for laboratory or office use. All of the tests except for Dri-Dot (see Fig. 10) require refrigeration and facilities for washing reusable slides. Minimal training for performance and interpretation is necessary.

**Manufacturer's wholesale price (US dollars) (6).

tests (24,62). Filter paper is included in the test kit. Except for the Pregnosticon Dri-Dot (Organon, Inc., West Orange, N.J.), all of the slide tests require refrigeration for storage and facilities for washing the reusable slides.

The most convenient pregnancy test currently available, with the least possibility of technical error in performance, is the Pregnosticon Dri-Dot. Dri-Dot is the only slide test which does not require refrigeration. The manufacturer states that it is stable at room temperature for at least 18 months and probably up to two years. This advantage makes Dri-Dot especially useful for developing countries, even in their remote rural areas. The reagents are incorporated onto a disposable cardboard slide, each individually sealed in foil. Technical error due to improper mixing of the reagents is therefore minimized, and no washing facilities for reusable slides are required (see Fig. 10). After a drop of tap water dissolves the reagents, a drop of the woman's urine is added. Results are read under good light in two minutes. Although the cost per test is higher than that of other slides (see Table 4), the advantages in respect to transportation, storage, and facilities required for processing make the Dri-Dot a relatively inexpensive pregnancy test.

As with the other immunologic slide tests, Dri-Dot is most reliable in pregnancy diagnosis beginning two weeks follow-

ing a missed period. Prior to that time it is likely to produce a high number of false-negative results (21,44,57,63).

Modifications of the slide tests have been tested in an attempt to further reduce the cost of the reagents. In Indonesia Kosasih and Tann have reported on "micro" methods of the Gravindex and Pregnosticon Planotest slide tests which reduce the amount of reagents required to approximately one-fifth of the original amount. Although reported as only 2.9 percent less accurate than the standard tests, the micro methods involve increased risk of technical error in preparing the reagents and require microscopic examination for accurate reading (48).

RADIOIMMUNOASSAYS

Radioimmunoassays of HCG detect the extremely low levels of HCG activity in very early pregnancy, and thus are capable of diagnosing pregnancy prior to or at the time of the first missed menses.

Two basic types of RIAs have been developed—one which utilizes an antibody against the whole HCG molecule and one which employs an antibody against only the beta subunits of HCG molecules (11,46,47,55,84).

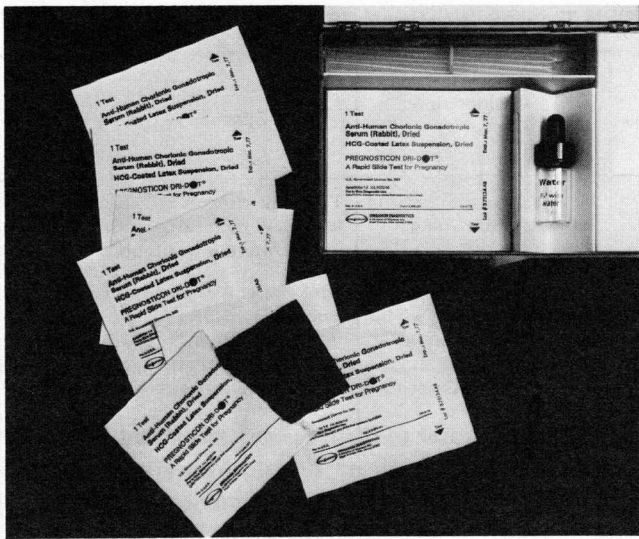


Fig. 10. The Pregnosticon Dri-Dot test (Organon, Inc., West Orange, N.J.) is a popular pregnancy test because it is convenient, easy to perform, and relatively inexpensive. Disposable slides are individually wrapped and the reagents are dried onto the slide itself. A drop of water and a drop of the woman's urine are mixed with the reagents on the slide and results—agglutination or agglutination inhibition—are read in two minutes. Dri-Dot is the only slide test which does not require refrigeration and thus is suitable for use in rural and developing areas which lack laboratory facilities. As with the other slide tests, it is most reliable beginning two weeks following a missed period.

The HCG molecule consists of alpha and beta subunits. The alpha subunits of the gonadotropins are similar but the beta subunits are different and apparently give the molecules their individual characteristics (55,84). Due to the similarity of their alpha subunits, however, HCG may cross-react with luteinizing hormone (LH—a pituitary hormone concerned with ovulation and corpus luteum formation) at the extremely low levels of HCG activity detected by RIAs. RIAs, used prior to a missed period when LH is still high, may therefore produce false-positives because of the combined measurement of HCG and LH (18,47,55,84). The

likelihood of cross-reactions and false results declines rapidly following ovulation because LH values remain low while HCG levels continue to rise. Thus assays—including all of the available immunological tests—which measure HCG at the time of or following a missed period are unlikely to be affected by cross-reaction between HCG and LH.

RIA of HCG-LH Activity

The first RIA tests required incubation periods of 24-170 hours, but in 1972 Goldstein et al. reported the development of an RIA which can be completed in only five hours and is capable of detecting HCG prior to a missed menses—6-8 days following probable implantation (28). This accurate measurement of HCG-LH activity has been used to detect pregnancy soon after implantation in patients undergoing ovulation-induction therapy. The RIA has also been used for early diagnosis of suspected ectopic pregnancy and in following physiological response to treatment of trophoblastic tumors (14,28).

RIA of the HCG Beta Subunit

Since the beta subunits of HCG and LH are different, assays directed against only the beta subunit of HCG can selectively measure HCG in the presence of LH. An RIA using antisera specific to the beta subunit of HCG was reported in 1972 by Vaitukaitis et al. at the National Institutes of Health (USA), and in 1973 by Kosasa et al. at Harvard University. The beta subunit assay, which requires 36 hours to complete, rules out the possibility of false-positives due to HCG-LH cross-reaction at low levels during very early pregnancy. With this method, HCG has been detected as early as nine days following probable ovulation. As with the RIA for HCG-LH, the beta subunit test has been used for diagnosis of possible ectopic pregnancy and for following the course of the disease among patients undergoing chemotherapy for HCG-secreting tumors. The assay has not been used for routine diagnosis of pregnancy because it is time-

Table 4—Comparison of Costs, Sensitivity, Time Required and Stability in Refrigerator of Selected Immunologic Pregnancy Tests, 1975

	Cost per Test (Tests in Kit)	Sensitivity*	Time Required	Stability in Refrigerator
Tube Tests				
Pregnosticon Accuspheres (Organon)	\$1.46 (100)	750	2 hr.	1 yr.
Pregnosticon Tube (Organon)	1.90 (20)	750	2 hr.	1 yr.
Placentex (Roche)	1.14 (100)	1000	90 min.	1 yr.
UCG-Lyphotest Tube (Wampole)	1.42 (50)	1000	2 hr.	1 yr. (cool, dry)
UCG Test (Wampole)	1.19 (25)	1000	2 hr.	18 mo.
Slide Tests				
Pregnosticon Dri-Dot Slide (Organon)	1.10 (100)	1500	2 min.	2 yr. (shelf)
Pregnosis Slide (Roche)	.90 (50)	1500	2 min.	1 yr.
Pregnosticon Slide (Organon)	1.00 (50)	1500	2 min.	1 yr.
UCG Slide (Wampole)	.85 (100)	2000	2 min.	1 yr.
DAP-Test-Macro (Wampole)	.84 (50)	2000	1 min.	1 yr.
Gest-State (Lederle)	.88 (50)	3000	2 min.	1 yr.
Gravindex Slide (Ortho)	.84 (60)	3500	2 min.	1 yr.

*International units of human chorionic gonadotropin per liter of urine.

SOURCE: Used with permission from The Medical Letter on Drugs and Therapeutics, January 17, 1975.

consuming, expensive, and requires sophisticated equipment and trained personnel for performance (45,46,47,84, 89).

Radioreceptorassay

A more rapid RIA, the radioreceptorassay (RRA), was reported in 1974 by Brij Saxena and Robert Landesman of the Cornell Medical Center. In the RRA, HCG tagged with a radioisotope is made to react with HCG receptors from membranes of ovaries of pregnant cows. When blood plasma from a woman is mixed with this preparation, any HCG present will compete with the radioactively tagged HCG already in the preparation, and the reduction in radioactivity that results can be used as a measure of the HCG in the woman's blood (52,74,75).

The RRA can be completed in only one hour and has been reported to be nearly 100 percent accurate in diagnosing pregnancy at the time of a missed menstrual period (5). The assay detects HCG as early as six days following ovulation, but because it does not distinguish between HCG and LH, it may produce false results at that early stage. At the time of the first missed period, however, HCG levels are 50 times higher than LH levels, so that diagnosis at this time is unaffected by LH activity (5).

Because the RRA is not only accurate but also faster than other RIA tests, it may have great potential for diagnosis of ectopic pregnancies and trophoblastic tumors, as well as for routine diagnosis and management of early pregnancy (5, 52,74,75) (see Fig. 11).

The reagents needed for the RRA will be marketed in early 1976 for commercial sale by Princeton Laboratories. The assay in its present form, however, is unlikely to be adopted widely outside urban areas and developed countries due to the expense of special facilities and the need for trained personnel to perform the complicated analysis procedures. Research to develop a less complicated RRA which does not require sophisticated and expensive laboratory facilities is underway at Cornell University (51).

DO-IT-YOURSELF TESTS

There is an obvious attraction in a do-it-yourself pregnancy test suitable for home use. Nearly all family planning program physicians agree that women have a basic right to information about their own fertility as simply and confidentially as possible and, of course, a do-it-yourself kit provides this. However, such a test must also be simple and reliable. It is on the latter issue that opinion is most divided. No tests are now licensed for sale in the USA, but some are available in Canada and Western Europe.

Critics question the reliability of test results interpreted by inexperienced persons and point out that false results due to technical error in performance, or to sensitivity limitations of the tests may not only mislead the user but may also cause her to take inappropriate and sometimes potentially dangerous actions (29). Test instructions included with Confidelle (Denver Laboratories, Canada, Ltd.), a hemagglutination inhibition tube test sold in Canada for \$6.00 (Canadian), advise a woman to consult her doctor if

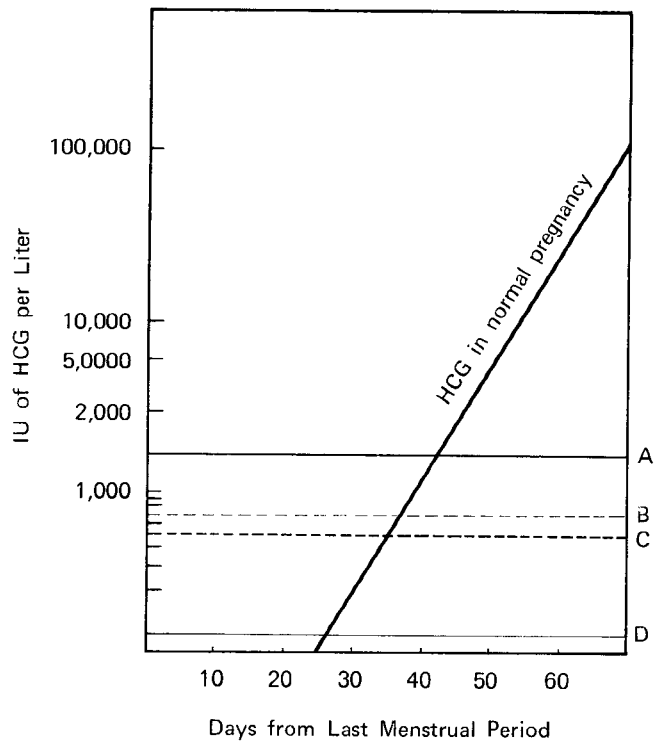


Fig. 11. The diagonal line represents the lower limit of HCG in a normally progressing pregnancy (adapted from Kosasa et al., 45). The horizontal line at A is the approximate area where most immunologic slide tests become positive; B where most immunologic tube tests become positive; C where capillary tests reportedly become positive; D where radioimmunoassays reportedly become positive.

the test is positive, or regardless of test results if she has missed her period. According to the manufacturers the test is intended as an initial screening to encourage medical consultation (see Fig. 12).

The USFDA has prohibited over-the-counter sale of pregnancy tests because of lack of evidence concerning their reliability. Recently, however, a federal judge denied FDA's right to restrict the sale of a do-it-yourself home pregnancy test, Ova II, manufactured by Faraday Laboratories, Inc. (Hillside, N.J.), stating that FDA's regulatory authority includes only drugs sold to cure diseases, whereas a test for pregnancy is merely a test for "news". The decision will be appealed, pending congressional legislation to broaden FDA regulatory authority to include medical devices (1).

A chemical test for pregnancy, Ova II is currently being sold in Europe. A woman adds a few drops of urine to each of two vials—a control vial containing two different reagents and a test vial containing a single chemical reagent. After shaking the test vial, the woman compares the two for results. If the two mixtures are different in color, pregnancy is indicated. The test is based on the increased excretion during pregnancy of hormones, including estrogens, which react chemically with the reagents to produce the color change. The manufacturer claims that the test can reliably diagnose pregnancy beginning two weeks following a missed period. The USFDA, however, determined that there was a lack of objective data to support the claims and prohibited sale of the test.

Each test kit, which includes two separate tests, is intended to sell for \$4.95 (US). The reagents do not require refrigera-



Fig. 12. Do-it-yourself pregnancy test kits are currently marketed in Canada and Western Europe. Confidelle (Denver Laboratories, Canada, Ltd.) is an immunologic tube test which includes all necessary equipment and instructions for home use. Manufacturer's suggested retail price is \$6.00 (Canadian).

PHOTOGRAPH: Courtesy of Denver Laboratories, Toronto, Canada

tion. Manufacturer's instructions advise that the test be repeated two weeks later regardless of results, and urge the woman to consult her doctor if results are positive or if she has missed her period. As with Confidelle, Ova II is described as an initial screening to encourage medical consultation (manufacturer's communication).

Do-it-yourself tests are convenient for the user, and may be less expensive than laboratory tests in developed countries. Currently available tests, however, present shipping and storage problems, require a relatively high literacy level to follow detailed instructions for performance and interpretation of results, and are too costly for widespread use in developing countries. Manufacturers claim that test sensitivity compares to that of the immunologic slide tests—both are generally not reliable until at least two weeks following a missed period—but these claims have not yet been verified by independent test data. Further research is required concerning the potential value and use of such tests.

RESEARCH

Dr. Lorrin Lau of the Johns Hopkins University (USA) is currently at work on a pregnancy test capable of early diagnosis which would be suitable for use in areas which lack transportation, refrigeration, and medical facilities. Dr. Lau's research, sponsored by USAID, has focused on a new immunoassay which appears to have significant advantages in terms of production and user's cost, convenience, and ease of performance.

His first objective was to develop a test with greater sensitivity than the currently available immunoassays, which would be capable of earlier diagnosis of pregnancy. Based upon agglutination inhibition methods for detection of HCG, the test uses a capillary tube containing premeasured, freeze-dried reagents. In preliminary trials the capillary test has been reported as detecting levels of HCG as low as .5 IU/ml, compared to a sensitivity of .7 and above for other immunoassays. No test data have yet been published (56). According to Dr. Lau, the increased sensitivity of the capillary test may permit greater use for medical care at or around the time of the missed period.

Since menstrual regulation (MR)—the simplest, safest and least expensive termination procedure—should be performed for optimal results within two weeks following a missed period, the capillary test, by offering early diagnosis, could be especially valuable for family planning programs. It could mean that only those women who are pregnant will undergo MR. Although capillary tests are less sensitive than radioimmunoassays (see Fig. 11), they may also be capable of detecting lower levels of HCG, associated with ectopic pregnancy, than the other commercially available immunoassays.

To facilitate its use in developing countries, the capillary test was designed for convenience in shipping and storage and for simplicity of performance. The capillary tube is itself a self-contained pregnancy testing kit. Reagents are freeze-dried inside the tube and are reconstituted by drawing urine into the tube. The tube is rocked for 3-5 minutes before results—agglutination or the lack of it—can be seen. It can be performed by personnel with minimal training and experience.

Each tube is only 5 inches long and 1 millimeter in diameter; 100 separate tests can be held in one hand (see Fig. 13). The reagents are designed to be stable at room temperature and to require no refrigeration. Thus the tests can be easily transported to rural areas, stored with no special facilities, and carried for field use (56).

Although the capillary test is not yet commercially available, it promises to be much less expensive than the least expensive test now available, and has been reported in field tests to cost less than \$.10 (US) per test (56,90). It should therefore be valuable for routine diagnosis of pregnancy, for early detection which will permit early termination if indicated, and for diagnosis of pregnancy-related disorders, such as ectopic pregnancy.

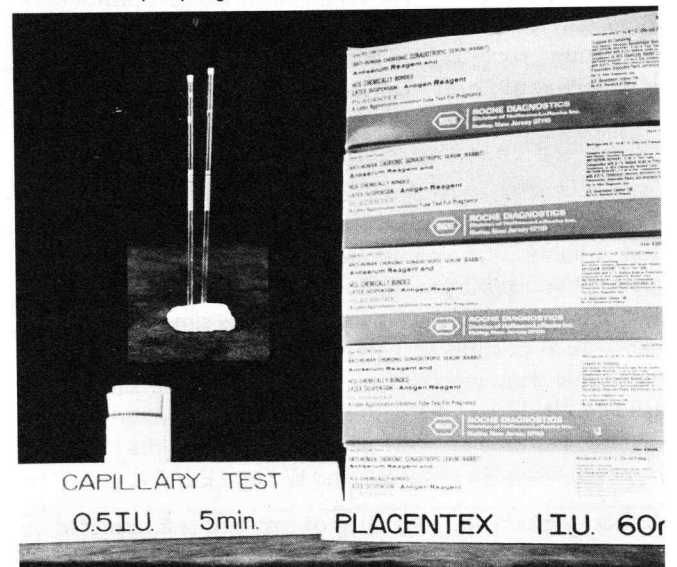


Fig. 13. Packaging and storage of pregnancy tests are important factors in developing countries, particularly in rural areas where transportation facilities may be poor and refrigeration equipment non-existent. In this respect, the capillary test may be more adaptable than other tests with similar sensitivity. On the left are 500 separate capillary tests packaged for storage (at room temperature); on the right, 500 latex agglutination inhibition tube tests. The inset (upper left) pictures two individual capillary tests, each approximately 5 inches in length and 1 millimeter in diameter.

BIBLIOGRAPHY

1. ANONYMOUS. Court prohibits FDA's pregnancy kit confiscation. *Intercom* 3(8): 4. August-September 1975.
2. ANONYMOUS. Do you have any reports of birth defects or other adverse effects associated with the use of oral contraceptives or other sex-hormone preparations during pregnancy? *New York State Journal of Medicine* 74(3): 535. March 1974.
3. ANONYMOUS. New pregnancy test hailed. *EMKO Newsletter* 1974: 1. April 1974.
4. ANONYMOUS. Pinpointing pregnancy—even earlier. *Medical World News*, June 25, 1971. p. 44A.
5. ANONYMOUS. Pregnancy detected after single missed period by HCG test. *Ob. Gyn. News* 10(14): 9. July 15, 1975.
6. ANONYMOUS. Urine pregnancy tests. *Medical Letter on Drugs and Therapeutics* 17(2): 6-7. January 17, 1975.
7. ALAMI, S. Y. Immunological tests for the diagnosis of pregnancy with special reference to a rapid simple slide test. *Journal of the Oklahoma State Medical Association*. March 1966. p. 116-123.
8. ARKIN, C. and NOTO, T. A. A false positive immunologic pregnancy test with tubo-ovarian abscess. *American Journal of Clinical Pathology* 58: 314-316. September 1972.
9. BARNETT, R. N. Comparison of an immunologic and a toad test for pregnancy. *American Journal of Clinical Pathology* 39(4): 436-438. April 1963.
10. BERMES, E. W. and ISAACS, J. H. Evaluation of a direct agglutination latex particle test for human chorionic gonadotropin. *American Journal of Obstetrics and Gynecology* 104(6): 865-870. July 15, 1969.
11. BLOCH, S. K. Use of serum luteinizing hormone in the clinical management of short-term amenorrhea. *American Journal of Obstetrics and Gynecology* 121(8): 1021-1023. April 15, 1975.
12. BRENNER, W. E., EDELMAN, D. A., DAVIS, G. L. R., KESSEL, E. Suction curettage for "menstrual regulation." In: Lewit, S., ed., *Advances in Planned Parenthood, Volume 9. (Proceedings of the Eleventh Annual Meeting of the Association of Planned Parenthood Physicians, Houston, Texas, April 12-13, 1973.)* Amsterdam, Excerpta Medica, 1974. p. 15-21.
13. BROTHERTON, J. and CRAFT, I. L. A clinical and pathologic survey of 91 cases of spontaneous abortion. *Fertility and Sterility* 23(4): 289-294. April 1972.
14. BUSTER, J. E. and ABRAHAM, E. E. The applications of steroid hormone radioimmunoassays to clinical obstetrics. *Obstetrics and Gynecology* 46(4): 489-499. October 1975.
15. CABRERA, H. A. A comprehensive evaluation of pregnancy tests. *American Journal of Obstetrics and Gynecology* 103(1): 32-38. January 1, 1969.
16. CALDER, I. M. and CHALMERS, D. G. An immunological test for pregnancy. *Clinical Trials Journal* 5(1): 911-914. February 1968.
17. CONNELL, E. B. The pill revisited. *Family Planning Perspectives* 7(2): 62-72. March/April 1975.
18. DAWOOD, M. Y. Evaluation of serum progesterone during treatment of malignant trophoblastic disease. *American Journal of Obstetrics and Gynecology* 123(3): 291-298. October 1, 1975.
19. DORLAND'S ILLUSTRATED MEDICAL DICTIONARY. 24th edition. Philadelphia, W. B. Saunders, 1965. p. 1518-1562.
20. DRISCOLL, S. G., STRAUSS, W. F., ALBA, M., ALTSCHUL, H. S., HAGER, H. J. Evaluation of a new slide test for pregnancy. *American Journal of Obstetrics and Gynecology* 110(8): 1083-1090. August 15, 1971.
21. EDELMAN, D. A., BRENNER, W. E., DAVIS, G. L. R., CHILD, P. An evaluation of the Pregnosticon Dri-Dot test in early pregnancy. *American Journal of Obstetrics and Gynecology* 119(4): 521-524. June 15, 1974.
22. FALLON, R. J. and HOBSON, B. M. A source of error in pregnancy tests. *Lancet* i(7814): 1243. June 2, 1973.
23. FISHBEIN, M. Mail-order pregnancy tests. *Medical World News* 12(39): 75. October 22, 1971.
24. FITZGERALD, T. C. Comparison of five immunological tests for pregnancy. *Journal of American Medical Technologists* 34(5): 425-431. September-October 1972.
25. FRIEDMAN, H. L. Psychosocial research on family planning in Europe. *Professional Psychology* 5(3): 326-334. August 1974.
26. GAL, I. Risks and benefits of the use of hormonal pregnancy test tablets. *Nature* 240: 241-242. November 24, 1972.
27. GAL, I., KIRMAN, B., STERN, J. Hormonal pregnancy tests and congenital malformation. *Nature* 216:83. October 7, 1967.
28. GOLDSTEIN, D. P., MIYATA, J., TAYMOR, M. L., LEVESQUE, L. A rapid solid-phase radioimmunoassay for the measurement of serum luteinizing hormone-human chorionic gonadotropin (LH-HCG) activity in very early pregnancy. *Fertility and Sterility* 23(11): 817-822. November 1972.
29. HARDWICK, D. F., BRENT, R., BURKE, M. D., COHEN, H., FALKOWSKI, F., HORWITZ, C. A., LAZO-WASEM, E., PERRIN, E. V., POLAND, B. J., REISS, A. M., SCHENKEL, B., TOWELL, M. E., VORHERR, H. Early diagnosis of pregnancy: an invitational symposium. *Journal of Reproductive Medicine* 12(1): 1-25. January 1974.
30. HARLAP, S., PRYWES, R., DAVIES, A. M. Birth defects and oestrogens and progesterones in pregnancy. *Lancet* i(7908): 682-683. March 22, 1975.
31. HELLMAN, L. M. Choices and challenges for the Federal family planning program. *American Journal of Obstetrics and Gynecology* 117(6): 782-793. November 15, 1973.
32. HOBSON, B. M. An evaluation of immunological tests for pregnancy. *The Practitioner* 202: 388-392. March 1969.
33. HOBSON, B. M. The excretion of chorionic gonadotropin by men with testicular tumours. *Acta Endocrinologica* 49(3): 337-348. July 1965.
34. HORWITZ, C. A., JEROME, E., DIAMOND, R., WARD, P. C. J. Evaluation of a latex tube agglutination-inhibition pregnancy test. *American Journal of Obstetrics and Gynecology* 116(5): 626-632. July 1, 1973.
35. HORWITZ, C. A., JEROME, E., PAULSON, C., STEARNS, J., ROSS, R., WARD, P. C. J. Evaluation of a latex agglutination-inhibition slide pregnancy test (Pregnosis). *Obstetrics and Gynecology* 43(5): 693-696. May 1974.
36. HORWITZ, C. A., MASLANSKY, R., WALDINGER, R., CABRERA, H., WARD, P. C. J. Effect of methadone on human pregnancy tests. *Journal of Reproduction and Fertility* 33(3): 489-493. June 1973.
37. HORWITZ, C. A. and SINYKIN, M. Laboratory medicine—pregnancy tests. *Minnesota Medicine* 53: 311-314. March 1970.
38. HORWITZ, C. A., SINYKIN, M., HILL, D., JEROME, E., BURKE, M. D. Evaluation of a new pregnancy test. *Obstetrics and Gynecology* 40(4): 563-566. October 1972.
39. HYTTEN, E. and LIND, T. Diagnostic indices in pregnancy. Basel, CIBA-GEIGY, 1973. p. 63-76.
40. ISLAMI, A. S., FISHER, L. M., KUPTER, H. G. Rapid slide test for pregnancy. *American Journal of Obstetrics and Gynecology* 89(5): 586-589. July 1, 1964.
41. KANITKAR, S. D., PARIKH, I., TASKAR, V., WALVEKER, V., BERNARD, R. P. Early experiences of menstrual regulation services at a teaching hospital and in a community health clinic in Bombay. (Paper presented at the Conference on Menstrual Regulation, University of Hawaii, Honolulu, December 18, 1973.) Bombay, India, Indian Fertility Research Programme, [1973]. 15 p.
42. KERBER, I. J., INCLAN, A. P., FOWLER, E. A., DAVIS, K., FISH, S. A. Immunologic tests for pregnancy. *Obstetrics and Gynecology* 36(1): 37-43. July 1970.
43. KESSEL, E. Estimated incidence of pregnancy by days amenorrhea. *Advances in Planned Parenthood* 9(3-4): 16-22. 1975.
44. KESSEL, E. Menstrual regulation in fertility control. In: Dawn, C. S., ed. *Menstrual regulation—a new procedure for fertility control*. Calcutta, Dawn, 1975. p. 106-124.
45. KOSASA, T. S., LEVESQUE, L. A., GOLDSTEIN, D. P., TAYMOR, M. L. Clinical use of a solid-phase radioimmunoassay specific for human chorionic gonadotropin. *American Journal of Obstetrics and Gynecology* 119(6): 784-791. July 15, 1974.
46. KOSASA, T., LEVESQUE, L. A., GOLDSTEIN, D. P., TAYMOR, M. L. Early detection of implantation using a radioimmunoassay specific for human chorionic gonadotropin. *Journal of Clinical Endocrinology and Metabolism* 36: 622-624. March 1973.
47. KOSASA, T. S., TAYMOR, M. L., GOLDSTEIN, D. P., LEVESQUE, L. A. Use of a radioimmunoassay specific for human chorionic gonadotropin in the diagnosis of early ectopic pregnancy. *Obstetrics and Gynecology* 42(6): 868-871. December 1973.

48. KOSASIH, E. N., and TANN, G. Modification of the Pregnosticon Planotest (micro method). In: Hudono, S. T. and Saifuddin, A. B., eds., Fifth Asian Congress of Obstetrics and Gynecology, Jakarta, Indonesia, October 8-15, 1971. Jakarta, Indonesia, Fifth Asian Congress of Obstetrics and Gynecology, 1971. p. 361-364.
49. KUTNER, S. J. A survey of fear of pregnancy and depression. *The Journal of Psychology* 79: 263-272. 1971.
50. LAMB, E. J. Immunologic pregnancy tests—evaluation of Pregnosticon Dri-Dot and Pregnosticon Accuspheres. *Obstetrics and Gynecology* 39(5): 665-672. May 1972.
51. LANDESMAN, R. Personal communication. October 29, 1975. 1 p.
52. LANDESMAN, R. and SAXENA, B. B. Results of the first 1,000 radioreceptorassays for the determination of HCG: a new, rapid and sensitive test for pregnancy. Paper presented at the Annual Meeting of the American Fertility Society, Los Angeles, April 3, 1975. 20 p. (To be published in *Fertility and Sterility*).
53. LANG, L. A rapid immunological test for pregnancy. *American Journal of Medical Technology* 30(5): 345-351. September-October 1964.
54. LANG, L. Interfering substances of menopausal urine for red cells coated with human chorionic gonadotropin hormone. *American Journal of Medical Technology* 31(5): 373-375. September-October 1965.
55. LAU, H. L. Testing for pregnancy. In: Race, G. J., ed., *Practice of medicine*, Volume II. Hagerstown, Maryland, Harper and Row, 1975. Chapter 29, 13 p.
56. LAU, H. L. Personal communication. September 4, 1975. 1 p.
57. LEAN, T. H., VENGADASALAM, D., EDELMAN, D. Initial experience with menstrual regulation at Kandang Kerbau Hospital, Singapore. Paper presented at the Menstrual Regulation Conference, University of Hawaii, Honolulu, December 17-19, 1973. 10 p.
58. LEE, L. T. and PAXMAN, J. M. Legal aspects of menstrual regulation. Medford, Massachusetts, Tufts University, Fletcher School of Law and Diplomacy, 1974. (Law and Population Monograph Series Number 19) 49 p.
59. LEHFELDT, H. Choice of ethinyl estradiol as a postcoital pill. *American Journal of Obstetrics and Gynecology* 116(6): 892-893. July 15, 1973.
60. MAYO, R. W. and THOMPSON, R. B. Comparison of pregnancy tests. *Obstetrics and Gynecology* 25(5): 699-704. May 1965.
61. McDONALD, C. I. R. Oral contraceptives. *Medical Journal of Australia* 1: 730. April 23, 1966.
62. McIVER, D. D. and BARNETT, R. N. The Direct Agglutination Pregnancy (DAP) Test. *Connecticut Medicine* 33(10): 635-636. October 1969.
63. MILLER, E. R. and FORTNEY, J. A. Minimizing menstrual regulation procedures in non-pregnant women. (Presented at the Japanese Society on Fertility and Sterility 20th General Meeting, Sendai City, Japan, October 1-4, 1975.) Chapel Hill, North Carolina, International Fertility Research Program, [1975]. (Menstrual Regulation Series Number 25) 19 p.
64. MISHALL, D. R. Immunologic assay of chorionic gonadotropin in pregnancy. *American Journal of Obstetrics and Gynecology* 96(2): 231-239. 1966.
65. MONIF, G. Rubella vaccination—an evolving problem for obstetrics and gynecology. *Obstetrics and Gynecology* 39: 304-306. July 1971.
66. NATIONAL WOMEN'S HEALTH COALITION. Early abortion. New York, 1974. 1 p.
67. NORA, J. J. and NORA, A. H. Birth defects and oral contraception. *Lancet* 1(7809): 941-942. April 28, 1973.
68. NORA, J. J. and NORA, A. H. Oral contraceptives and birth defects: preliminary evidence for a possible association. *Pediatric Research* 7(4): 321. 1973.
69. NORTH-COOMBES, D., SEEDAT, Y. K., REDDY, J. False positive pregnancy test in uraemia. *British Medical Journal* 4(5947): 410-411. November 16, 1974.
70. NOTO, T. A., MIALE, J. B., RIEKERS, H. Quantitation of human chorionic gonadotropin in urine using the slide immunologic test for pregnancy. *American Journal of Obstetrics and Gynecology* 90(7): 859-866.
71. OAKLEY, G. P., and FLYNT, J. W. Hormonal pregnancy tests and congenital malformations. *Lancet* 2(7823): 256-257. August 4, 1973.
72. POTTS, M. Personal communication. October 11, 1975. 1 p.
73. RAJAWATANA, D., and KOETSAWANG, S. The use of oral contraceptives as hormonal-withdrawal pregnancy test. *Journal of the Medical Association of Thailand* 55(9): 533-536. September 1972.
74. SAXENA, B. B., HASAN, S. H., HAOUR, F., SCHMIDT-GOLLWITZER, M. Radioreceptor assay of HCG: detection of early pregnancy. *Science* 184(4138): 793-795. May 17, 1974.
75. SAXENA, B. B. and LANDESMAN, R. The use of a radioreceptorassay of human chorionic gonadotropin for the diagnosis and management of ectopic pregnancy. *Fertility and Sterility* 26(5): 397-404. May 1975.
76. SLOTH, M. and STAKEMANN, G. Urinary excretion of chorionic gonadotropins after induced abortion. *Acta Obstetrica Gynecologica Scandinavica* 50: 227-228. 1971.
77. SPADONI, L. R., HORST, H., BRAY, R. E., HERMANN, W. L. Urinary chorionic gonadotropin (HCG) in normal and abnormal pregnancies. *Obstetrics and Gynecology* 28(6): 830-835. December 1966.
78. SPADONI, L., McLEAN, R. B., HERMANN, W. L. A rapid immunologic test for the detection of early pregnancy. *Western Journal of Surgery, Obstetrics and Gynecology*, 72: 92-97. March-April 1964.
79. STRINGER, J. "Menstrual regulation" conference in Hawaii. *IPPF Medical Bulletin* 8(2): 3-4. April 1974.
80. TANN, G., KOSASIH, E. N., WILARAS, A., SUTEDJO, S. An immunologic pregnancy test applied on serum. In: Hudono, S. T., and Saifuddin, A. B., eds. Fifth Asian Congress of Obstetrics and Gynaecology, Jakarta, Indonesia, October 8-15, 1971. Jakarta, Indonesia, Fifth Asian Congress of Obstetrics and Gynecology, 1971. p. 359-360.
81. TIETZ, N. W. Comparative study of immunologic and biologic pregnancy tests in early pregnancy. *Obstetrics and Gynecology* 25(2): 197-200. February 1965.
82. UDRY, J. R., KEOVICHIT, S., BURNRIGHT, R., COGILL, D. O., MORRIS, N. M., YAMARAT, C. Pregnancy testing as a fertility measurement technique: A preliminary report on field results. *American Journal of Public Health* 61(2): 344-352. February 1971.
83. UNITED STATES. FOOD AND DRUG ADMINISTRATION. Medroxyprogesterone acetate; norethindrone; norethindrone acetate; progesterone; dydrogesterone; and hydroxyprogesterone caproate. *Federal Register* 38(195): 27947-27949. October 10, 1973.
84. VAITUKAITIS, J. L., BRAUNSTEIN, G. D., ROSS, G. T. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. *American Journal of Obstetrics and Gynecology* 113(6): 751-758. July 15, 1972.
85. VAN DER VLUGT, T. H. History, present status and applications of menstrual regulation. Paper presented at the Menstrual Regulation Conference, Honolulu, Hawaii, December 16-19, 1973. 11 p.
86. VAN DER VLUGT, T. H. and PIOTROW, P. T. Menstrual regulation—what is it? *Population Reports*, Series F, Number 2, Washington, D.C., George Washington University Medical Center, Population Information Program, April 1973. 15 p.
87. VAN DER VLUGT, T. H. and PIOTROW, P. T. Menstrual regulation update. *Population Reports*, Series F, Number 4, Washington, D.C., George Washington University Medical Center, Population Information Program, May 1974. 16 p.
88. VENGADASALAM, D., LEAN, T. H., KESSEL, E., BERGER, G. S., MILLER, E. R. Estrogen-progesterone "withdrawal bleeding" in diagnosis of pregnancy. (To be presented at the Nineteenth All India Congress in Obstetrics and Gynaecology, Jamshedpur, India, December 27-30, 1975.) Chapel Hill, North Carolina, International Fertility Research Program, [1975]. (Menstrual Regulation Series Number 24) 11 p.
89. WIDE, L. Early diagnosis of pregnancy. *Lancet* 2(7626): 863-864. October 25, 1969.
90. WILEY, A. T. Personal communication. September 3, 1975. 1 p.
91. YAHIA, C. and TAYMOR, M. L. A 3-min. immunologic pregnancy test. *Obstetrics and Gynecology* 23(1): 37-40. January 1964.
92. ZONDEK, B. Simplified hormonal treatment of amenorrhea. *Journal of the American Medical Association* 118(9): 705-708. February 28, 1942.